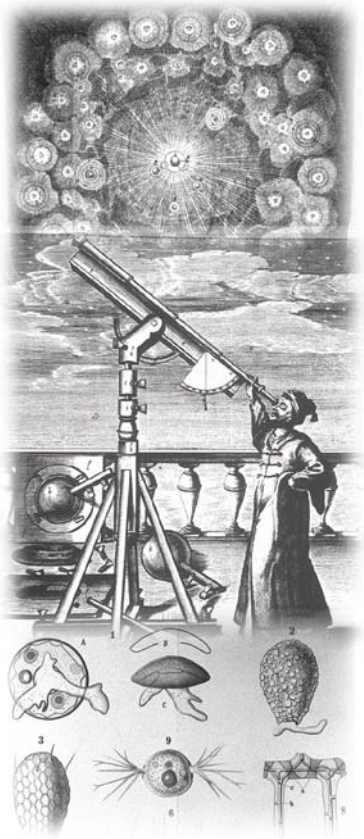
NASA Astrobiology Institute

A Compendium of the Science of Its Newest Teams: Year 2003



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A Compendium of the Science of its Newest Teams: Year 2003

Preface

The NASA Astrobiology Institute is proud to introduce the twelve Teams selected from proposals submitted in response to the 2003 Cooperative Agreement Notice:

Ames Research Center
Carnegie Institution of Washington
Indiana University
Goddard Space Flight Center
Marine Biological Laboratory
Pennsylvania State University
SETI Institute
University of Arizona
University of California, Berkeley
University of California, Los Angeles
University of Colorado, Boulder
University of Hawaii, Manoa

These Teams join those chosen in 2001 (Michigan State University, University of Rhode Island, University of Washington and the Virtual Planetary Laboratory) to comprise the NASA Astrobiology Institute (NAI). The NAI makes available in this volume the research plans of the 12 selected proposals as a way to familiarize each Team with the research being conducted by the NAI as a whole, and to stimulate interdisciplinary collaboration amongst its Members, and the broader scientific community. The present volume is the third in a series, with previous volumes produced following the selection of NAI Teams in 1998 and 2001.

The NAI released its third Cooperative Agreement Notice on December 6th, 2002. In response, 44 Notices of Intent were received and, by the due date of March 6, 35 complete proposals had been submitted. Approximately 700 individual researchers were associated with the proposals, representing 237 institutions. An external Review Panel was assembled to review the proposals, which represented scientific expertise across the full scope of astrobiology. The 40-member Review Panel was Chaired by Dr. Antonio Lazcano. The entire Panel met June 3-6, 2003 in Washington D.C. to discuss and review the proposals. A smaller Executive Committee, composed of members of the Review Panel, met subsequently to develop a series of selection scenarios which were then sent forth as recommendations to the NAI and the NASA Selecting Official Dr. Edward J. Weiler, Associate Administrator for Space Science.

The three selection criteria used were 1) Science and technical merit, 2) Plans for strengthening the Astrobiology community, and 3) Relevance to the Astrobiology Roadmap. The research emphasis of each new Team is indicated in Figure 1, where the individual projects of each Team are assigned to particular Astrobiology Roadmap Goals. Please do use this as a guide, as we invite you to explore the exciting research of the Third Generation NASA Astrobiology Institute.

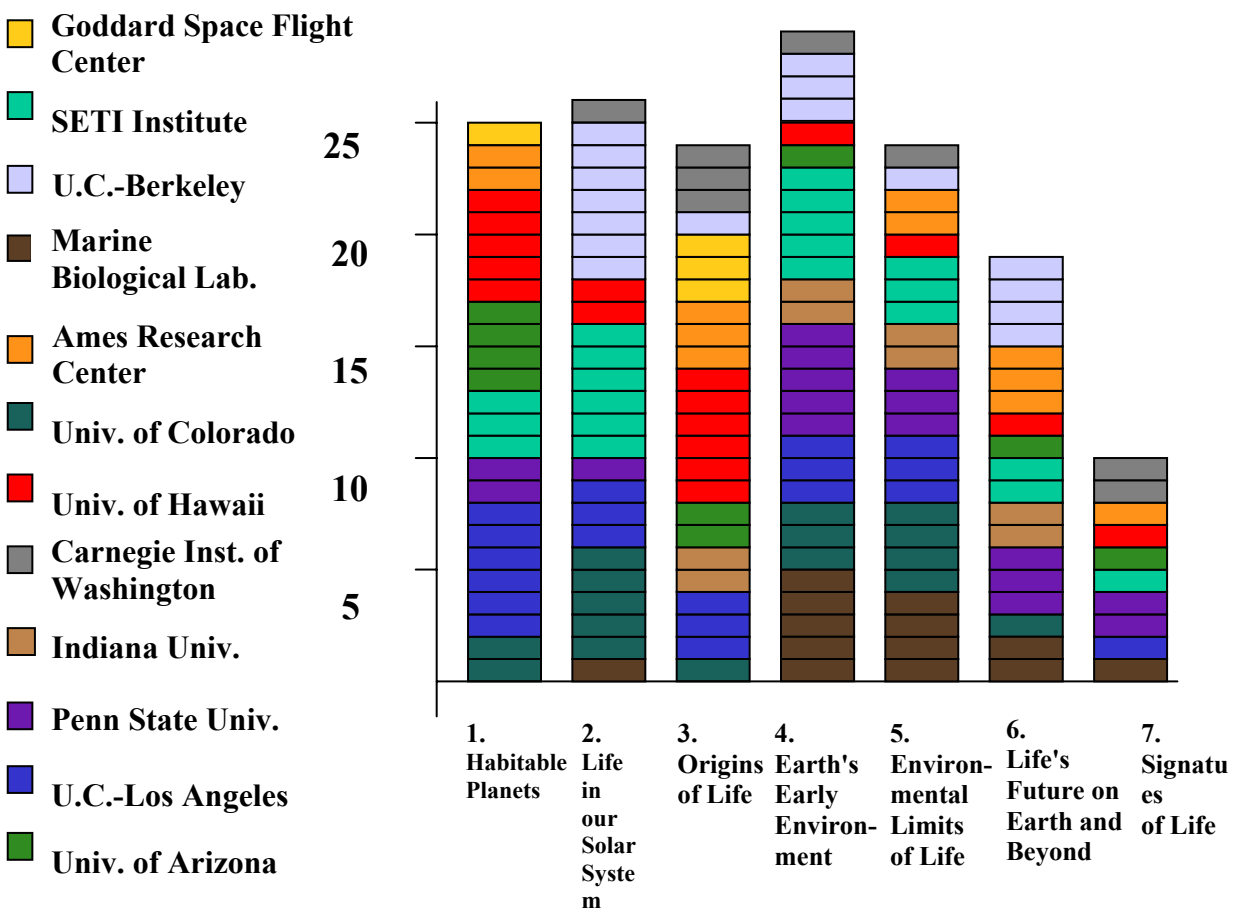

Figure 1. Summary of the research projects being conducted by the twelve newest Teams of the NASA Astrobiology Institute, categorized by Astrobiology Roadmap Goal.

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Linking Our Origins to Our Future

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LINKING OUR ORIGINS TO OUR FUTURE

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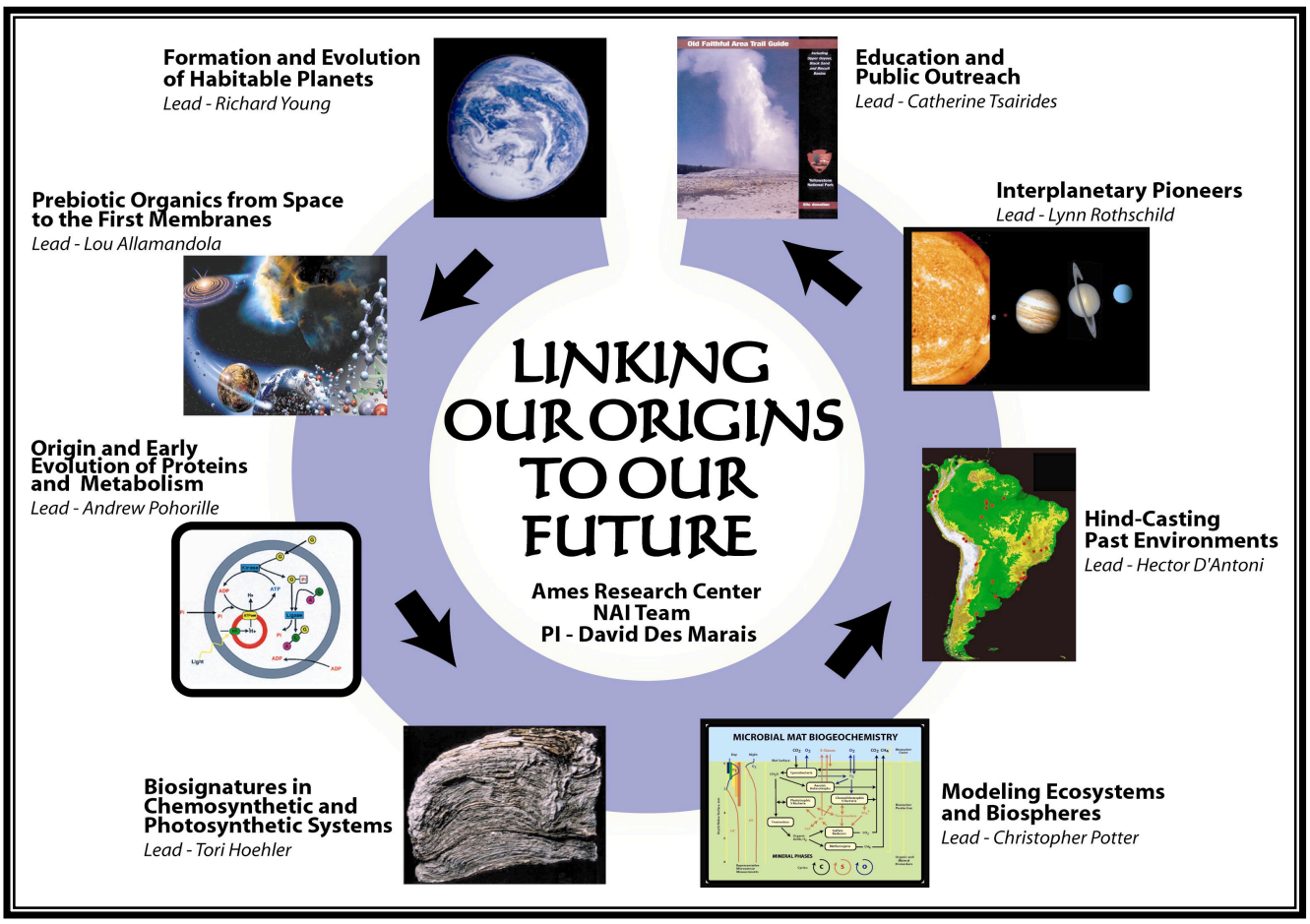

Co-Investigators:

Daniel Albert - University of North Carolina, Chapel Hill
Louis Allamandola - NASA Ames Research Center
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David Blake - NASA Ames Research Center
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Allan Treiman - Lunar and Planetary Institute
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David Ward - Montana State University
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1.0 EXECUTIVE SUMMARY

Ames Research Center proposes a coordinated program that integrates a broad, interdisciplinary investigation of the origins, evolution, and future of habitable environments and life, with a parallel, high-impact, education and public outreach effort. Tracing a path from interstellar materials to inhabited worlds and beyond, our research will link together investigations of the formation, evolution, and climates of habitable planets; the roles of interstellar chemistry in supplying potential biological precursors to these worlds; the origins and nature of metabolism in the first cells; the impact of established biospheres on planetary chemistry and climate, emphasizing the formation of detectable biosignatures; the response of vegetation to regional climate change; and the potential for life to transcend planetary boundaries through transfer between habitable worlds. Our program for education and public outreach captures these themes and builds around the expertise and enthusiasm of the Ames team to develop an engaging and informative package that will be disseminated to national- and international-scale audiences. This will be achieved through partnerships with the California Academy of Sciences, Yellowstone National Park, New York Hall of Science, and several K-14 educational organizations. Strong conceptual and functional links to multiple NASA missions provide context, motivation, and funding leverage for our research component, along with resource- and audience-sharing opportunities for our education and public outreach component. The proposed program is highly relevant to the goals of the NASA Astrobiology Institute in terms of basic science, mission support, training, education, and public outreach as specified by the goals and objectives of the recently updated Astrobiology Roadmap and NASA Strategic Plan.

The proposed research organizes multiple disciplines into complementary lines of investigation designed to understand the *context for life*, the *origins of life and its impact on the planetary environment*, and the *future of life in changing environments*. The research is broad-based, addressing *all seven goals of the new Astrobiology Roadmap,* and is formulated to address specific near-term objectives in the Roadmap in ways that link these objectives and help to unify astrobiology.

We begin with a multifaceted investigation of the formation, evolution, and climatology of habitable planets. Because extrasolar planets that host surface biospheres are the most likely to be detected by remote spectroscopic search, we focus on terrestrial (rocky) planets where liquid water is stable at the surface, and examine a critical subset of the processes that affect planetary habitability. The research objectives are to understand: how protoplanetary disks evolve and form terrestrial planets; what kinds of planetary systems are likely to harbor terrestrial planets; how volatiles are delivered to terrestrial planets by impacting planetesimals, and how impacts affect the climatology of terrestrial planets; the particular evolutionary paths of terrestrial planets that result in habitability; and how external characteristics, such as orbital eccentricity, and internal factors, such as atmospheric circulation, affect the habitability of terrestrial planets.

We will link these studies of habitability to two major initiatives in prebiotic organic cosmochemistry. Building on our previous NAI-sponsored work that demonstrated the formation of complex and potentially protobiological organics under simulated interstellar conditions, we propose to trace, spectroscopically and chemically, the cosmic evolution of organic molecules from the interstellar medium to protoplanetary disks, planetesimals, and finally onto habitable bodies. We also propose to examine the abiotic mechanisms of primitive membrane formation under the primordial conditions of a habitable planet. Both initiatives will couple spectral and chemical studies of laboratory simulations with astronomical observations and analyses of meteorites and comet dust returned by the Stardust mission.

A third facet of our investigation will address the origin of metabolism in the earliest ancestors of cells by testing the hypothesis that proteins might have arisen and initially evolved in the absence of a genome. In our prior NAI-sponsored research, we selected for the first time a functional protein from a library of random amino acid sequences using a novel *in vitro* evolution technique. We propose to evolve several proteins capable of performing functions that might have been important for early metabolism, such as synthesis of biopolymers and transport of ions across membranous cell walls. We will also estimate the frequency of finding a functional prebiotic protein among random protein sequences that might have formed spontaneously. On the basis of these experiments, we will examine the evolutionary potential of an ensemble of proteins through theoretical and computational modeling.

A fourth element of our study focuses on how to detect life once it has taken hold on a planet, by characterizing the major factors that govern the formation of potentially diagnostic biosignatures in microbial ecosystems. Two ecosystem types will be studied for their particular relevance to astrobiological searches for life (e.g., via Mars or Terrestrial Planet Finder (TPF) missions, respectively): rock-hosted ecosystems in ophiolite springs, as a potential analog for past or present

subsurface life within our solar system, and photosynthetic microbial mats, as the type of biosphere that could be remotely detected on extrasolar planets. Work in the springs will examine how microorganisms might leave residual biosignatures by affecting the formation of aqueous alteration minerals, and how biological energy requirements define an "energetically habitable zone" for chemotrophic life. Studies of microbial mats will focus on elucidating the pathways by which photosynthetic productivity is transformed into volatile biosignatures that could be distinguished in the atmospheres of distant planets.

We will extend the ecosystem-level studies of photosynthetic microbial mats to a planetary scale by refining and evaluating quantitative models that simulate energy relationships, biogeochemical cycling, trace gas exchange, and biodiversity in these systems. Global-scale fluxes of O_2, CO_2, CH_4, H_2S, and other reduced species will be estimated by combining algorithms for the production of trace gas biosignatures with process-level metabolic information from model simulation runs. The ability of the model to explore scenarios and assess implications of experimental findings will complement the field-based measurements, and will begin to assess the consequences of billions of years of ecological change driven by environmental forcing.

We will examine the effects of climate variability on a vegetation-rich biosphere over intermediate time scales, using South American ecosystems as a model. Our past NAI-funded work demonstrated a strong correlation between vegetation changes at 32 South American sites and variations in sea-surface temperature over a 12-year period, related to the El Niño Southern Oscillation (ENSO). We propose to continue this analysis using information from the MODIS and ETM+ sensors onboard more recent satellites, and to use it to predict backward, or hindcast, vegetation assemblages. We will incorporate fossil pollen profiles to extend this hindcasting back 15,000 years, and to assess whether ENSO has caused previously unknown changes in vegetation communities.

Finally, we will assess the potential for life to move beyond its planet of origin, as a potentially important component in the evolution of life in our own solar system. We propose to address natural transport, such as on a meteorite, where survivors must withstand radiation, desiccation, and time in transit. We will identify organisms and ecosystems that are likely to withstand such rigors, and examine the mechanisms for their survival in laboratory experiments and in a space simulator. We will fly these organisms and ecosystems in low Earth orbit (e.g., ESA's EXPOSE facility on the ISS) to test their resistance to the space environment.

The strong mission relevance of our proposal, combined with the direct participation of several team members in the planning and execution of NASA missions, places the Ames team in a position to influence strongly the astrobiology content of ongoing and future missions. Studies of planet formation and habitability will benefit the SIRTF, Kepler, Eddington and TPF missions. Studies of cosmic ices and organics will be synergistic with SOFIA, the Stardust mission, and the proposed Astrobiology Explorer (ABE). Studies of microbial biosignatures will benefit MER, future Mars missions, and TPF. Five of the investigators on this proposal serve as PI or CoIs on NASA missions that could reach fruition during the span of this proposal, and seven are involved in instrument development and observing strategies.

We will continue to serve the needs and interests of the nation's educators, students and public through a high-impact education and public outreach program. Specifically, we propose to partner with the California Academy of Sciences (CAS), Yellowstone National Park (YNP), and the New York Hall of Science to develop new astrobiology workshops, activities, exhibits, and other products for the public. CAS has chosen to utilize astrobiology to link its natural history museum, planetarium, and aquarium under the theme, "Earth and its Place in the Universe." As part of our partnership, Ames personnel will serve directly on the CAS design and exhibit development teams. Ames and CAS will also facilitate interactions between researchers and educators in order to develop inquiry-based programs and activities for K-14 students. Our partnership with YNP, which began as part of our previous NAI work, combines a large annual visitation with a highly effective venue for conveying astrobiology-related content. Specifically, the thermal springs that abound in YNP support concepts related to the early evolution of life on Earth, and to the search for evidence of Martian habitability or inhabitance. With material input from the Ames team, YNP will introduce astrobiology content into trailside interpretive signs, brochures, and the Yellowstone Resources and Issues Guide. Ultimately, astrobiology will be integrated into permanent exhibits for the major visitor centers. By harnessing the E/PO expertise and resources of these organizations, and by accessing the large and diverse audiences they draw, Ames is poised to engage a truly broad cross-section of the public in astrobiology outreach activities. We will extend this impact to the professional level by engaging graduate students and postdoctoral associates in the proposed research activities, through the teaching of undergraduate courses in astrobiology at

Stanford University and local community colleges, and through the sponsorship of workshops that will help introduce student researchers to astrobiology.

The Ames team combines a wealth of experience in astrobiology research and outreach activities with an unusually high degree of functional involvement in NASA missions, ongoing and planned collaborations in NAI focus groups, and a strong institutional backing that leave us well suited to contribute as integral and vital members of the NAI. During the previous term of funding, the Ames team made important advances in each of the research areas proposed here, generating more than 200 peer-reviewed research articles. The proposed efforts build on these successes and will thus be leveraged in a conceptual sense. Through their roles as members of NASA committees, as leaders in professional journals and societies, and as pioneers in astrobiology-related educational and training activities, Ames team members are also poised to broaden the impact of astrobiology in the professional community and to engage the next generation of researchers that are so critical to the long-tem prosperity of the field. Similarly, the national attention drawn by the success of our existing education and outreach program has enabled us to engage and partner with high visibility organizations such as CAS and YNP, which will afford an opportunity to bring astrobiology to a truly broad and diverse public audience.

Our proposed research and E/PO efforts are strongly supported by the facilities, programs, and direct contributions offered by Ames Research Center. Ames will contribute six work-years of civil service (CS) time, as well as CS travel support, to each year of this proposed work. The direct contribution of civil service salaries and travel support exceeds $4 million over the proposed performance period. In further support of this and related research, the Astrobiology and Space Research Directorate will add one permanent staff position during the proposed grant period. Major laboratory complexes for astrochemistry, biogeochemistry and electron microscopy, plus several smaller laboratories, will be provided for the research. Ames also maintains the largest computational facilities devoted to NASA research, facilities that will directly support the proposed theoretical research in astronomy, planetary science and molecular biology. Interactions with the Ames Education Office will enrich the team's education and public outreach efforts. Our program is also leveraged financially by individual commitments from Ames team members, who are variously contributing unsalaried time, resources and technical support derived from complementary funded projects, and research infrastructure developed with previous NAI and other funding.

The interconnected nature of the Ames team as a whole will benefit from a management structure and style that is informed by our previous NAI experience. The Principal Investigator will chair a steering committee comprised of the lead Co-Investigators who will supervise each of the seven investigations and the E/PO program. Monthly team meetings and periodic workshops will further integrate the effort and embrace the lessons learned during prior NAI experience. For example, monthly presentations of biological themes will be paired with those of related astrochemical or planetary themes. Also, discussions of science tasks will be paired with those related to training, education, and public outreach, thus further strengthening team collaboration. We will link to the broader research community through our proposed planetary science workshops and training opportunities.

We have participated actively in NAI focus groups, and will continue to do so in the next round of membership. For example, Investigations 1 and 2 will work with the proposed Astronomy and Astronomical Biosignatures focus groups. Investigations 4 and 5 will participate in the Mars, Ecogenomics, and proposed Astronomical Biosignatures focus groups. Investigations 6 and 7 will work with other NAI teams to develop groups addressing the future of life. Through these interactions with other NAI member institutions, we will continue our role in helping to forge the interdisciplinary connections that will enhance the overall viability and impact of the Institute.

3.0 RESEARCH AND MANAGEMENT PLAN

Introduction and Approach

Background

Ames Research Center proposes a broad interdisciplinary effort to understand life and habitable planetary environments in the context of their origins, evolution, and future. To encompass these themes fully, we must understand the functional links that draw a continuous thread through interstellar chemistry and physics, planet system formation, development of habitability, origins of metabolism, and evolution and persistence of life. The title of this proposal, "Linking our Origins to our Future", reflects our focus on elucidating these links. These links are further strengthened by conceptual connections and functional collaborations with other NAI members, involvement with other NASA research and analysis programs in Astrobiology, Exobiology, Solar System Exploration, Astrophysics, Earth Sciences, and Fundamental Biology, and strong associations with key astrobiology-related flight missions and technology development activities.

Research Overview

This section is organized around three scientific themes, dealing with the *Context for life*, the *Origins and early evolution of life and its biosignatures*, and the *Future of life in changing environments.* The research is broad, addressing *all seven goals of the new Astrobiology Roadmap,* but it is also focused: the research investigations address specific near-term objectives in the Roadmap in ways that strengthen the linkages between these objectives and thus help to unify the new discipline of astrobiology.

Context for life. The elemental building blocks that form the basis for life as we know it are common throughout the universe, but the occurrence of conditions suitable for the origins and sustenance of life is much less common and therefore might greatly limit the distribution of life. Any assessment of the universality of life must therefore include an analysis of the factors that allow life to begin and continue, hence *the context for life.* This research broadly addresses Goal 1 of the new Astrobiology Roadmap, "Understand the nature and distribution of habitable environments in the Universe." In support of Goal 3, "Understand how life emerges from cosmic and planetary precursors," this research also addresses Objective 3.1, "Characterize the cosmic and endogenous sources of matter…for potentially habitable environments..."

Investigation 1 proposes a coordinated research program on the formation, evolution, and climatology of habitable planets. We seek to understand how protoplanetary disks evolve and form terrestrial planets and the kinds of planetary systems that are likely to harbor habitable worlds. We will further examine how particular variables that shape the climates of extrasolar terrestrial planets might affect their habitability, using Mars, Venus, and Earth as model systems.

Investigation 2 proposes initiatives in prebiotic, organic cosmochemistry, work that is intimately bound with *Investigation 1*. We will trace, spectroscopically and chemically, the cosmic evolution of organic molecules from the interstellar medium to protoplanetary disks, planetesimals, and finally onto habitable bodies. We will explore primitive, abiotic membrane formation under the primordial conditions of a habitable planet. Both initiatives will rely on spectral and chemical studies in the laboratory informed by astronomical observations and extraterrestrial sample analysis of meteorites and comet dust returned by the Stardust mission.

These investigations will be coordinated in ways that are both novel and highly significant. For example, the dynamics of the protoplanetary disk are rigorously determined by the chemical composition of the gas. Much of the gas in the nebula is produced initially from the evaporation of the feedstock interstellar ice that forms the protoplanetary disk. Methanol, not methane, has been observed to be the most abundant simple carbon compound frozen in these ices, and also in the warm gas characteristic of star forming regions. This makes an enormous difference in the dynamical and chemical nature of nebulae, a feature that can be captured in the dynamical models of *Investigation 1* as the chemical simulations of *Investigation 2* progress.

Origins and early evolution of life and its biosignatures. The actual origins of life on Earth remain elusive, but assessments of potential mechanisms for creating catalytic and genetic functions may help to constrain this process. We can ultimately help to constrain the timing of life's origins on Earth, and its frequency in nearby planetary systems, by learning to recognize biosignatures that are discernable in deep time and space. Our work in these areas addresses Astrobiology Roadmap Objective 3.2, "Origins and evolution of functional biomolecules," Objective 6.1, "Environmental changes and the cycling of elements by the biota, communities, and ecosystems," and all of Goal 7, "Determine how to recognize signatures of life on other worlds and on early Earth":

Investigation 3 addresses the origins of metabolism in the earliest ancestors of cells by testing the hypothesis that proteins might have arisen and initially evolved in the absence of a genome. We will evolve several proteins capable

of performing functions that might have been important for early metabolism, such as synthesis of biopolymers and transport of ions across membranous cell walls. We will estimate the frequency of finding a functional prebiotic protein among random spontaneously formed protein sequences. We will examine the evolutionary potential of an ensemble of proteins using theoretical and computational modeling techniques. Conceptually, this investigation continues the line of research in *Investigation 2* by aiming to explain how biological precursors of life could be utilized in the formation of living systems.

Investigation 4 seeks to characterize the major factors that govern the formation of potentially diagnostic biosignatures in microbial ecosystems. We will assess the energetic factors that constrain the occurrence of microbes in rock-hosted ecosystems, and assess the potential for preserving biosignatures within them through deposition of aqueous alteration minerals. We also will elucidate the pathways by which photosynthetic productivity is transformed into potentially useful biosignatures (e.g., biogenic gases in the atmosphere) in phototrophic microbial mat communities, and understand the controls thereon.

Investigation 5 will refine and evaluate numerical models that simulate energy relationships, biogeochemical cycling, trace gas exchange, and biodiversity in the same microbial mat ecosystems. The goal is to extrapolate biosignatures from early Earth ecosystems to the scale of a planetary biosphere and, ultimately, over time scales of billions of years.

Studies of biosignature gas formation in photosynthetic ecosystems in *Investigation 4* will yield a conceptual basis and experimental data for the model developed in *Investigation 5*. In turn, the model will ultimately help to scale the ecosystem-level measurements of *Investigation 4* up to planetary scales. *Investigation 4* will also develop the concept of "energetic habitability", which may help to refine our understanding of the *context for life.*

The future of life in changing environments. The final two investigations address Roadmap Goal 6, "Understand the principles that will shape the future of life, both on Earth and beyond." Patterns observed in terrestrial ecosystems during recent millennia can offer clues regarding future ecosystem responses to change. The survival of microbial life as it moves beyond its planet of origin is relevant both to future scenarios of human exploration and to natural interplanetary transfers of life throughout solar system history.

Investigation 6 will examine fossil pollen and historical sea surface temperatures in order to define some of the environmental drivers that shaped extant ecosystems in the past, as a basis for understanding potential future changes. This study will discern whether the El Niño Southern Oscillation caused some past, previously unknown changes in plant communities. Similar changes might be anticipated in the future.

Investigation 7 considers how terrestrial life might survive in space during interplanetary transport. In space, biota will encounter environments with increased radiation and very low water potentials. *Investigation 7* proposes to study how these factors affect selected biological systems. The findings will be used to create descriptive and predictive ecological models for life under a range of extraterrestrial conditions.

3.1 Investigation 1 Formation and Evolution of Habitable Planets

3.1.1 Objectives and Significance of Research

Central to the context for life is the formation and evolution of habitable planets. Here we define habitable planet in the "classical" sense, meaning a planet with an atmosphere having liquid water on the surface. This is appropriate, because extrasolar planets on which liquid water and life are present at the surface should be observable spectroscopically in a search for evidence of life (Leger, 1993; Angel & Woolf, 1996), whereas subsurface biospheres may not be detectable.

The study of habitable planets directly addresses goals and objectives in the Astrobiology Roadmap (see Section 3.1.3), and is especially timely now. From ground based observations over 100 extrasolar planets have been discovered to date. It is now known that extrasolar planetary systems exist, as opposed to single planets. Within a few years space based missions using transit photometry, such as the Kepler Mission to be launched in 2007, will show whether terrestrial type (predominantly rocky), in addition to jovian type (gas giant), planets are ubiquitous in extrasolar planetary systems. Furthermore, for all detected planets including terrestrial-sized planets, the transit observations will allow determination of planet radius, orbital semi-major axis, and under the most optimal circumstances, orbital eccentricity. An additional important scientific objective of the Kepler Mission is to determine whether a detected planet is within the habitable zone of the parent star, where habitable zone is defined as that region of space surrounding a star

within which liquid water could exist at a planet's surface.

Well founded theoretical models now exist regarding planet formation, protoplanetary nebula evolution, and planetary system dynamics. There has also been progress in understanding the factors important for determining the habitable zone around a particular star. Thus, for the first time, there is now the opportunity to meaningfully combine observational and theoretical efforts in order to quantitatively evaluate the frequency and characteristics of extrasolar planets, and to evaluate whether or not they are habitable. A major objective of the proposed work is to conduct theoretical studies of terrestrial planets which will be directly relevant to the planning of, and scientific data interpretation for, missions such as Kepler and more advanced future missions, such as the planned Terrestrial Planet Finder Mission (TPF), which will be capable of obtaining spectral information of the atmospheres.

Habitable planets, in the sense we have defined habitable planets, must be terrestrial planets. We propose a multifaceted, interconnected research program that addresses the formation, evolution, and climatology of terrestrial type planets, including terrestrial planets in our own Solar System, since they provide some guidance for understanding extrasolar terrestrial planets. Obviously we cannot resolve all the questions in their entirety. Instead, the goal is to identify particular key questions, and address those.

Figure 3.1-1 illustrates that the unifying theme of the proposed tasks is planet habitability. There is not necessarily a direct link between each of the processes we propose to address (e.g. protoplanetary disk processes, Section 3.1.2.1, are not directly linked to terrestrial planet climatology, sections 3.1.2.3-5), however, each crucially affects planet habitability. As an example, from only a climatological perspective, solar type stars would be expected to be associated with habitable planets. However if the star formed in a stellar cluster, as many stars do, it is quite possible that the solar star's protoplanetary disk did not last long enough for planets to form at all. Since the density of suitable candidate stars in the Sun's vicinity is an important driver of the TPF mission design, considering just climatological criteria in a search strategy for habitable planets would not be prudent. Elements of the proposed research are relevant to several existing NASA and NSF R&A programs and would complement research being done under those programs. However, the research proposed here is beyond the scope of any single R&A program, and is beyond the interest of any group of R&A programs to coordinate. Hence, the research belongs within the purview of the Astrobiology Institute.

3.1.2 Research Tasks

3.1.2.1 Effects of protoplanetary disk processes on formation of habitable planets

Build On and Extend State of Knowledge

Protoplanetary disk lifetime, and the dynamical and chemical evolution of the disk, control the planetary incubation phase and planet composition, and therefore place constraints on planet formation and the delivery of key volatiles such as water. Without considering these processes, we could be misled into thinking that certain stars had a habitable zone, when in fact disk processes precluded the existence of habitable terrestrial planets.

It is critical to understand disk evaporation rates in the planet-forming region (<30 AU). The presence or absence of significant quantities of gas there has major implications for planet formation and dynamics including: (i) the ability of gas giants to accrete onto rocky cores over ‰ 10^7 years (Pollack et al. 1996; Ikoma et al. 2000); (ii) the extent of planet migration (Lin & Papaloizou 1986; Ward 1997); (iii) the evolution of orbital eccentricities of planetesimals and planets (Tanaka & Ida 1997; Kominami & Ida 2002; Chiang et al. 2002; Goldreich & Sari, 2003). It will become apparent from the discussion in following sections, especially Section 3.1.2.2 which discusses orbital

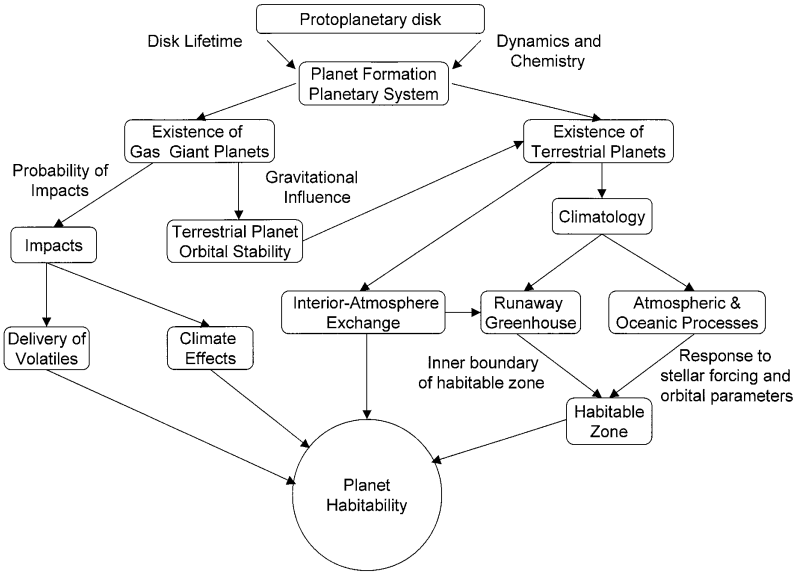


Figure 3.1-1. Illustration of how processes addressed in this proposal act to determine planet habitability. These processes are of course a subset of all processes that have to be ultimately considered, but they are critical ones.

stability and formation of terrestrial planets, and delivery of volatiles to terrestrial planets, that each of the above considerations is ultimately very important for habitable planets. While viscous accretion depletes the inner disk, protostellar winds and photoevaporation disperse the outer disk gas and dust back into the interstellar medium (ISM). Winds and photoevaporation set the ultimate time scale for disk dispersal (cf. Clarke et al. 2001). Beside planet formation, rapid disk dispersal of the outer disk, where H_2O ices form and accumulate into water rich planetesimals, will also affect delivery of volatiles to terrestrial planets (Hollenbach & Adams, 2003).

We propose to calculate the lifetime of protoplanetary disks as a function of stellar mass for stars spanning the entire initial stellar mass function (IMF) in order to evaluate the expected frequency of occurrence of habitable planetary systems. We will also study photoevaporative effects due to star formation in clusters, where most stars are born. This work will leverage from, and be complementary to, work being undertaken by one of us (D. Hollenbach) as part of a Legacy Proposal associated with the Space Infrared Telescope Facility (SIRTF) mission, currently scheduled to be launched in April 2003.

The physical state and chemical composition of forming planets is determined by dynamical and chemical evolution (especially of water) of the protoplanetary nebula. This process is always active in the inner disk and may co-exist with the photoevaporation processes discussed above (Clarke et al. 2001). Temperature and pressure conditions in the early nebula set the rate of chemical reactions, which determines the concentrations and state of water and other volatiles. If disk dynamical and chemical time constants are comparable, the dynamics and chemistry of the disk are tightly coupled to one another, forming a non-linear system that must be treated in a unified and consistent manner. In addition, dynamical accretion in the evolving disk induces spatially inhomogeneous chemical composition that can induce important radial reprocessing effects and species concentration.

Willacy et al. (1998) used a steady state disk model that essentially uncoupled the chemistry and the dynamics. (The chemical model was obtained from the widely used UMIST chemical database.) This work was extended by Willacy and Langer (2000) to include photoprocessing at the nebula margins. Aikawa et al. (1999) also used a steady state disk model and included inward (the only direction allowed by the model) accretion using a step-by-step solution of the chemical kinetic equations. Her model used a chemical database consisting of about 240 species integrated over a period of about 10^6 yrs during which the disk is assumed quiescent. Drouart et al. (1999) and Gail (2001) also investigate uncoupled chemical evolution using steady state disk dynamics. Bockelèe-Morvan et al. (2002) use a sequence of steady state models to study a related problem regarding the formation of silicates. Time dependent dynamics were considered in one recent paper (Wehrsted & Gail, 2002), but they also considered the evolution of silicate dust grains without including chemical reactions. They also used the conventional "alpha viscosity" model (Ruden & Lin, 1986, Ruden & Pollack, 1991) which is not as efficient as the recently proposed "beta viscosity" model of hydrodynamically generated turbulence (Richard & Zahn 1999, Hurè, Richard & Zahn, 2001). While all of these models have greatly contributed to a better understanding of disk dynamics and chemical evolution, none adequately treats the spatially inhomogeneous interactions between dynamics and chemistry. In this proposal, we will take advantage of new turbulence models to study coupled chemical/hydrodynamic processes. The coupled equations will be used to study the transformation of species as they migrate from grain-accreted states in the cold outer nebula to gaseous states in the inner nebula, or visa-versa if the unsteady accretion velocity changes sign.

It should be noted that the grain accreted states just mentioned are ices. These are ices whose IR spectra will be measured and characterized as described in Section 3.2, which deals with pre-biotic organics.

Technical Approach and Methodology

We propose to constrain the lifetime of protoplanetary disks focusing on stellar winds and photoevaporation. For dispersal by winds, we will apply the methods of Canto & Raga (1991) to the mass entrainment that occurs when a wind shears across the surface of disk gas. A mixing layer is created of outwardly flowing gas into which mass flux from the disk gas is inserted at some prescribed fraction of the sound speed, a number theoretically derived but constrained by laboratory experiments. We propose to perform a large parameter (gas surface density distribution, gas temperature distribution, wind mass loss rate and velocity) study to determine when, if ever, wind-stripping is important and how the wind affects the disk structure. This work includes shear physics, and is therefore much more realistic than previous work (e.g., Horedt 1982) which relied on crude energetic arguments and overestimated the dispersal.

In order to solve for photoevaporation at $r < r_g$, where r_g (about 30 AU) is the disk radius at which gas thermal speed equals escape speed from the system, we separate the hydrodynamics from the calculation of the temperature and chemical structure and iterate to achieve consistency. This

technique has been used successfully for photoevaporating clumps (Gorti & Hollenbach 2002). The gas is heated by energetic photons from the central star or a nearby massive star. We will use photodissociation region (PDR) models with both ultraviolet radiation and X-rays (e.g., Hollenbach & Tielens 1999) to calculate the chemical and thermal structure, and a 2-dimensional hydrodynamics code.

To address the dynamical and chemical evolution of the disk, we propose to develop a model that is able to fully couple the physical structure and chemical composition of the disk. We will combine recent work on nebula dynamics by Davis (2002a, 2002b, 2003) with enhanced understanding of mixing processes in the nebula by Richard and Zahn (1999) and Hurè, Richard and Zahn (2001) to produce an efficient nebula, or disk, dynamic transport model. D. Richard (currently at NASA Ames as an NRC Resident Research Associate) will be a key collaborator in developing the dynamical modeling. The chemical network will be similar to that used by Willacy et al. (1998) (see also Markwick et al. 2002). We will collaborate with S. Charnley (SETI Institute, Ames Space Sciences) and A. Markwick (at NASA Ames in 2003-2004 as an NRC Resident Research Associate) who both have extensive experience modeling cosmo- chemical processes. The chemical network will include gas phase two and three body reactions appropriate to the inner disk, and will be augmented with grain adsorption/desorption, and condensation physics models appropriate to the outer disk. A full range of reactions is necessary for an extended disk with extensive radial reprocessing. The particular chemical system we have implemented for our initial studies is a grain chemistry model originally used for molecular cloud physics (Brown & Charnley, 1990) consisting of 66 species and 705 reactions. The coupled chemistry and dynamics will be solved using methods appropriate to such "stiff" systems. In this manner we will be able to follow the coupled physical-chemical hydrodynamics over the full spatial-temporal envelope. An important component of this work will be to focus on chemical processing of water molecules.

3.1.2.2 Effects of planetary system characteristics on formation/existence of habitable planets

Build On and Extend State of Knowledge

The formation of habitable terrestrial planets, and the amount of volatile material they receive (see also Section 3.2), are mainly influenced by the nature of a star's protoplanetary disk, and the characteristics of the giant planets in the system (e.g. Chambers 2000a, 2000b; Jones et al. 2001).

Highly advanced astrobiology missions like TPF will be able to directly detect Earth-sized planets, but in order to efficiently do so, they will need to know where to look, and indeed, the density of suitable candidate stars in the Solar vicinity is a driver of the mission design.

The list of known planet-bearing stars provides an excellent source of candidates. We have unambiguous evidence that the conditions for planet formation were met in these systems, and the example of our own Solar System indicates that the formation of gas giants can be accompanied by the formation of terrestrial planets. One can then ask two interesting questions: First, how many of the known planetary systems admit habitable terrestrial planet orbits which are dynamically viable? Second, given our current understanding of terrestrial planet formation, in what fraction of the aggregate of known extrasolar planetary systems was the formation of habitable terrestrial planets possible? Definitive answers to these questions will require large-scale computation because in general, the gravitational N-body problem is analytically intractable.

To discover whether a particular planetary configuration is stable, one must integrate its equations of motion forward for a reasonable period of time (of order millions of years) and track the planetary excursions (Laughlin & Adams 1999; Rivera & Lissauer 2000; Jones et al. 2001; Marcy et al. 2002; Noble et al. 2002). When a particular system contains only one giant planet, this procedure is straightforward, albeit time-consuming. Menou and Tabachnik (2003), for example, report that time limitations prevented them from systematically evaluating the significant effects of observational uncertainty in the giant planet orbital elements.

When a system contains two or more giant planets, the evaluation of the stability of potential terrestrial planets becomes much more computationally arduous. Radial velocity observations cannot generally determine the planetary orbital inclinations, and so any evaluation of habitability must consider a wide range of mutual inclinations within each observed multiple-planet configuration. That is, for each individual system, one must build up a statistical portrait of likelihood which samples different orbital configurations for a putative habitable planet within the full range of giant planet architectures that are consistent with the observations. Laughlin et al. (2002) have computed the allowed range of mutual inclinations for the 2-planet 47 UMa system, Rivera and Lissauer (2001) have done this for the resonant pair of planets orbiting the star GJ 876, and Lissauer and Rivera (2001) and Chiang et al. (2001) have investigated the range of stable mutual inclinations which are supported by the 3-planet

Upsilon Andromedae system, but to date, the dynamical viability of habitable planets within these and all other multiple-planet systems is unknown when the possibility of mutual inclination is taken into account.

The stability of terrestrial planets in a particular exoplanetary system is a necessary but not sufficient condition for their existence. One must also evaluate whether a particular planetary architecture is likely to allow terrestrial planets to form in the first place. Terrestrial planets have mass and so they modify the strength and location of resonances associated with the giant planets, as Earth and Venus modify the θ_5 resonance in the Solar System (Namouni & Murray, 1999). During the late stages of planetary accretion, many planetary embryos with appreciable mass will be present. Embryos can gravitationally scatter one another into unstable resonance regions, and this greatly enhances the ability of resonances to remove material from a protoplanetary disk (Wetherill 1967). Chambers and Wetherill (2001) have shown that if planetary embryos formed in the asteroid belt of the Solar System, there is a two thirds probability that a combination of gravitational scattering and giant-planet resonances would have removed all embryos from the belt. Similarly, Laughlin et al. (2002) have shown that terrestrial planet formation is extremely unlikely within the habitable zone of the 47 UMa system, even though test-particle orbits within that system are highly stable. We propose to investigate these stability and formation questions using a large set of computations.

Considering now the delivery of volatiles to a planet, current models for protoplanetary disks and planetary accretion indicate that the inner planets of the Solar System formed in an environment that was initially very hot and dry (e.g. Bell et al. 1997). Observed water fractions suggest that solid materials forming in the protoplanetary nebula contained progressively less water with decreasing distance from the Sun. Apparently temperatures were never cold enough for water to condense in the terrestrial planet region and inner asteroid belt.

The two most likely external sources for Earth's water are comets and water-bearing bodies from the asteroid belt (such as the parent bodies of carbonaceous chondrites). Comets are unlikely to have contributed more than a small fraction of Earth's water for two reasons. First, the dynamical collision probability of a comet with Earth is too small, roughly 10^{-6} (Zahnle et al. 1998; Hartmann et al. 2000; Morbidelli et al. 2000). Second, cometary water has a D/H ratio that differs from terrestrial ocean water by a factor of 2 (cf. Lunine et al. 2000).

Conversely, water-bearing asteroids have much higher collision probabilities with Earth, together with a similar D/H ratio to terrestrial water. This suggests that material from the asteroid region was a more likely source of most of Earth's water than comets. Morbidelli et al. (2000) found there is a high probability that Earth could have accreted all its water in one or more collisions with planetary embryos from the asteroid region. Furthermore, Chambers and Wetherill (2001) have shown that the presence of planet embryos in the asteroid region provides a successful mechanism to explain the substantial mass depletion observed in the modern asteroid belt compared to other regions in the Solar System. The success of this model adds weight to the idea that most of Earth's water was delivered in the form of planetary embryos from the asteroid belt. The mass of volatile materials delivered to Earth depends sensitively on the giant planets' orbital eccentricities (Chambers & Cassen 2002), since these control the width and strength of the unstable resonances in the asteroid region. We propose to investigate this dependence in detail, building on the models developed by Morbidelli et al. (2000), Chambers and Wetherill (2001) and Chambers and Cassen (2002).

Technical Approach and Methodology

We propose to address the question of how many of the known planetary systems admit habitable planet orbits which are dynamically stable, as well as to address the question concerning in which systems terrestrial planets are likely to form. The evaluation of terrestrial planet formation and habitability are daunting computational tasks, but they have the advantage of being completely and immediately parallelizable. Vast numbers of individual configurations need to be separately and independently tested: these individual computations can be performed on individual computers. The proposed effort would be analogous to the SETI@Home Project, through which the University of California, Berkeley, SETI team has developed a successful program whereby an individual's home computer, when not in use by the individual, is utilized to analyze immense data sets obtained daily from the Arecibo radio telescope. We propose a similar sort of public program to run the computer codes. Individual simulations (either to evaluate dynamical stability or to test terrestrial planet accretion) involve extremely modest amounts of memory, and only a limited amount of bandwidth is required for transferring data. For both classes of simulations, we can use the public domain code "mercury.f" (Chambers 1999). This code uses the Wisdom-Holman symplectic map when planets or planetesimals are far apart (which allows very fast integration) and incorporates conventional integration to advance the perturbation terms of the Hamiltonian when bodies are close together. This code is very well tested and optimized.

The major task required to implement this project would be to compile mercury.f on various

architectures (i.e. Windows, Linux and the Macintosh OS). Users would then download the executable file appropriate to their own system. This mode of operation would be in the spirit of the SETI@Home Project.

For users who are interested in more interaction, we envision having a website (which could be linked to, or be an outgrowth of the www.transitsearch.org website) in which the integration properties of mercury.f are incorporated into a java code that also contains a detailed user interface. This interface would allow users to design their own habitable-planet configurations within the known planetary system architectures and test them for stability. The java code would likely be slower in performance, but would compensate with a great public outreach functionality.

With regard to volatile inventories of terrestrial planets, we propose to study how widely different the volatile inventories of terrestrial planets will be depending on the characteristics of giant planets in planetary systems. We will concentrate on cases where the giant planets orbit beyond the habitable zone, as in the Solar System. The experience of our own system shows that delivery of volatiles to terrestrial planets is most efficient if volatile bearing bodies exist on orbits interior to the giant planets. Hence, for planets in the habitable zone to receive significant amounts of water and other volatiles, the innermost giant probably needs to have periastron lying beyond 3 AU for a solar mass star, and certainly further out than dynamical-stability considerations alone would suggest. At present there are few if any good candidate extrasolar systems which satisfy this constraint. (Note that orbital eccentricities are not well determined in many cases, so more observations will be required to determine whether some systems could permit life-sustaining planets to form.) However, this situation is likely to change in the next few years, assuming such systems exist, because the time baseline of telescopic surveys for extrasolar planets will be extended.

The proposed work will follow two avenues, each consisting of numerical simulations of the formation of terrestrial planets in extrasolar systems. In each case, the rationale is to assume that giant planets with particular characteristics form in a system, and to then model the likely formation, stability and composition of terrestrial planets in that system, and the amount of volatile material that will be delivered to them. The first approach will make use of an N-body accretion code currently being developed by Chambers for use in a funded project to model the late stages of planetary accretion in the Solar System. A second approach will be based on the Öpik-Arnold scheme pioneered by Wetherill (1967) to model the accretion of the inner planets and asteroids in the Solar System. In the longer term, we propose to further develop the Öpik-Arnold scheme to provide a more realistic treatment of giant-planet perturbations and a crude treatment of secular interactions between planetary embryos (Chambers and Wetherill 1998 have shown that these can play a significant role during planetary accretion). This new code will straddle the gap between the modified Öpik-Arnold scheme and true N-body integrations, providing a more realistic, but fast, treatment of the dynamical evolution of planetary embryos.

Furthermore, we plan to incorporate advances in models for nebula chemistry and collisional fragmentation into our simulations. As discussed previously, understanding the thermal and chemical evolution of the nebula is vital since these determine the composition of planetesimals as a function of location in the nebula.

One of us (D. Hollenbach) is the director of the Center for Star Formation Studies, a very successful NASA-funded group since 1985 of astrophysicists from the University of California at Berkeley, the University of California at Santa Cruz, and NASA Ames. The Center studies star and planet formation. We propose to initiate a series of one-week biennial summer workshops focused on the research of our Ames astrobiology group. These workshops would bring together the diverse elements of the Ames group, the astrophysicists from the Center, and outside experts in the focused research areas of the Ames group. Both the Ames astrobiology group and the Center astrophysicists would benefit from this mutual interaction, which would stimulate new collaborations, more coherent interdisciplinary work, and cross fertilization of ideas.

3.1.2.3 Atmospheric and oceanic processes and habitable zone

Build On and Extend State of Knowledge

The circulation of, and physical process within, a planetary atmosphere (and ocean if there is one) can have considerable effect on a planet's climatology, and hence influence the extent of the habitable zone. Planets orbiting around cool (M type) stars provide an example where 3-dimensional (3-D) global atmospheric circulation enables a habitable zone to exist where 1-D models predict it would not (Joshi et al. 1997). This example illustrates the fact that a planet whose global mean surface temperature is below freezing might have regions where more clement conditions prevail that could support a regional habitat because of atmospheric circulation.

Furthermore, habitable zone studies have thus far been carried out using 1-D globally-averaged radiative-convective climate models (e.g., Kasting et al. 1993). The inner edge of the habitable zone is determined to a large extent by the loss of water

through photolysis and hydrogen escape. In 1-D models the relative humidity and lapse rates must be specified. In reality, however, the relative humidity, lapse rate, and other quantities are spatially varying and depend on dynamical processes that cannot be modeled in one dimension. We will mention such a situation in connection with the modeling of meridional heat transport as part of the study of potential habitability of planets in Venus-like orbits around stars similar to the Sun in Section 3.1.2.5.

Major advancement in our understanding of internal factors that determine the width of the habitable zone, such as the interaction of cloud microphysics and radiation with circulation, and understanding of external factors, such as parent stellar type and planet orbital characteristics (e.g., orbital eccentricity), will come from full 3-D general circulation models (GCMs) that incorporate important interactions. Computers, modeling algorithms, and analysis tools have advanced to the state where it makes sense to carry out simulations without many of the parameterizations and assumptions that have been used in the past.

Technical Approach and Methodology

We propose to apply a small suite of general circulation models (some available publicly for general scientific research, and one that has been developed on site at the NASA Ames Research Center) that are either simplified (although still 3-D) or fully complex, in order to investigate the habitability of planets. The choice of a particular model will depend on which is better suited for a given application. Members of the team have extensive experience and familiarity with the full suite of models. Our focus here will be on predominantly CO_2/H_2O atmospheres.

The most complex model is based on a new flexible version of the Ames Mars general circulation model (MGCM) (Pollack et al. 1990; Haberle et al. 2003) that has been vastly improved, and now includes a cloud microphysics scheme for both CO_2 and water clouds (Toon et al. 1988), and an updated and more generalized radiation code that can handle cloud multiple scattering in the presence of gaseous absorption for both solar and infrared parts of the spectrum (Toon et al. 1989). This model, for example, will be applied to the study of the climatological effects of impacts on Mars (see Section 3.1.2.4). The second model, which can be run at coarser spatial resolution and somewhat faster time integration because it is a spectral model, is the National Center for Atmospheric Research (NCAR) Community Climate System Model (CCSM) (Kiehl et al. 1996). The third model, ECBILT, (Haarsma et al. 1998) is a highly simplified and adaptive general circulation model that includes a realistic three-layer atmosphere, twelve-layer ocean and a sea-ice model, all with realistic heating in the solar and infrared, and heat exchanges between atmosphere and the underlying surface. The model, because of its simplified parameterizations and time splitting algorithms for the atmosphere and ocean, is very efficient for adaptation to investigate the climate system on long time scales.

Taken together, the models will complement each other, in that the CCSM and ECBILT models can be used relatively quickly to investigate a particular set of realistic climate configurations, while the modified Ames MGCM can be run at higher resolution with a more reasonable and complete physics package, corresponding to an extrasolar terrestrial-like atmosphere that can include, for example, radiative-cloud microphysical interactions which may turn out to be important. It is well known that clouds and their atmospheric radiative interactions are fundamental in understanding terrestrial climatology, but many aspects of cloud microphysics would be more parameterized in the CCSM. However in the MGCM, a detailed treatment of cloud microphysics could be run to corroborate the results of the CCSM, or to suggest how results might be modified.

We have conducted initial numerical experiments using the CCSM in which we have altered the planetary orbital setup (i.e., increased the eccentricity) for a present-day Earth in order to explore the range of seasonal/annual near-surface temperature extremes. Among the possible simulations, it is planned to continue such calculations using more realistic ocean models (our initial calculations used a present-day prescribed sea surface temperature (SST) climatology) so that realistic oceanic heat transport may occur. We also plan to investigate climate extremes using more simplified land-surface prescriptions (e.g., an aqua-planet or an aqua-planet with equatorially confined continents) to assess near-surface temperature extremes for different orbital configurations.

3.1.2.4 Role of impacts in modifying climate of terrestrial, and potentially habitable, planets

Build On and Extend State of Knowledge

Impacts of primitive bodies play an important role in delivering volatiles, such as water, to a planet which are required to make a terrestrial planet habitable. But impacts can also modify a planet's climate in other ways. It is well known that impacts have played a major role in affecting life on Earth, from the moon forming impact more than 4.5 billion years ago, to mass extinctions. Impacts must be considered as a process affecting the habitability of a planet.

As a case study, we propose to investigate the role of impacts in modifying the climate of another terrestrial planet in our solar system, Mars. Recent studies (Segura et al., 2002) of the effects of impacts on Martian climate indicate that, instead of a long lasting wet and warm climate, which has been presumed to have enabled the formation of Martian valley river networks, Mars was instead a cold and dry planet for which impacts were ultimately responsible for forming the observed surface river networks. In this case impacts bring life sustaining volatiles to a planet, but make it difficult for life to exist on the surface.

We envision two mechanisms for river formation. Due to the global distribution of the debris layers, we do not expect that rivers should be located near the craters of the objects that created the rivers. The first mechanism is rainout of the directly injected water from the cooling atmosphere. Global average precipitation rates will exceed 2 m/year depending on impactor size. Flooding and mudslides will reshape the landscape with eroded rivers containing high sediment loads as solids are broken apart by rushing water. The second mechanism is ground water release during the long periods of time the subsurface is above 273 K. Thermal heating from the hot ejecta layer will mobilize ground ice. During the long periods between impacts, vapor diffusion will recharge the groundwater aquifers. It has been suggested that most of the observed valleys were eroded by groundwater, but the absence of a recharge mechanism has frustrated this hypothesis. Impacts induce abundant precipitation that may recharge the aquifers.

Technical Approach and Methodology

The hypothesis that transient rains following impacts formed the Martian river valleys, rather than processes associated with a wet warm ancient Martian climate, has huge implications for life on Mars. The study described in Segura et al. (2002) has several aspects that deserve further work. An observable feature of repeated collisions of large objects should be the multiple ejecta blankets they may have emplaced. Numerous debris layers, contemporaneous with and intermixed with the valley networks, having an apparently subaerial mode of deposition have been identified by Mars Global Surveyor. These layers are precisely what would be expected from impacts of large enough size to generate the valleys through the mechanism we propose. Hence a test of our hypothesis would be careful comparisons of the ages of thick ejecta blankets and the initial formation times of the rivers.

We propose to better determine the origins of the layers of sub-aerial sediments on Mars. Are these from impacts as we hypothesize, or something else? We plan to work with Jen Heldman, a geology graduate student at University of Colorado on this issue. The goal of this project is relatively simple. We will expand on the work of Malin and Edgett (2000) using similar procedures. They reported extensive sub-aerial layers that were placed down at the same time as the Martian river valleys. We suspect these layers are the debris layers from the impacts that formed the craters visible on Mars today. If so, these layers should be present over much of the planet, and not be restricted to basins as would be water deposited layers. In addition the layer thickness should not be random, but instead should be related to the size of the crater and hence follow the impact crater frequency curves. Hence we will trace the layers over a wide range, and use their thickness to show they are derived from cratering.

Second, A. Colaprete and R. Haberle at NASA Ames are funded through the Mars Fundamental Research Program to simulate the aftermath of a large Martian impact with the Ames Mars General Circulation Model (MGCM) (see Section 3.1.2.3). We will collaborate with them, and provide quantitative estimates of the forcing mechanisms, i.e., atmospheric temperature fields, water vapor distribution, and surface temperature distribution as a result of the impact, after solid debris has fallen from the atmosphere. The issue that needs exploration with the MGCM is to understand the hydrological cycle in the aftermath of a large impact. Segura et al. (2002) used a one-dimensional model. Such a model is not adept at removing water vapor from the atmosphere, which is inherently a three-dimensional problem. Hence the one-dimensional model may not properly simulate the greenhouse warming by the water vapor, its latitudinal variations, and the cycling of water in the liquid phase over the planet. However at short times following the impact, horizontal variations in atmospheric properties are not likely to be great, and therefore the 1-D model can be used to provide starting conditions for the MGCM, and that is the procedure we will follow.

3.1.2.5 Runaway greenhouse and inner boundary of habitable zone

Build On and Extend State of Knowledge

The inner boundary of the habitable zone around a star effectively translates into the boundary, interior to which, a planet would experience a runaway greenhouse. This is a process in which there is sufficient positive feedback between planet surface temperature and radiative atmospheric opacity caused by water vapor abundance to produce very high surface temperatures. As more water vapor evaporates into the atmosphere because of increased surface temperature, atmospheric opacity increases, causing still higher surface temperatures, and so on. The surface temperature ultimately "runs away" to such extremely high temperatures that all surface liquid

water evaporates into the atmosphere, with subsequent loss of water from the planet. There is observational evidence for such an event on the terrestrial planet Venus (cf. Donahue et al., 1997). Kasting and Pollack (1983) showed that water could be lost from a planet when the stratosphere of the planet becomes saturated with respect to water vapor, which event occurs at stellar insolations smaller than required for the runaway greenhouse. However, such a planet could still have liquid water on the surface over a geologically significant time, and hence be considered habitable. We propose to study aspects of the runaway greenhouse effect that should give insight into this process in general by considering particular examples of the terrestrial planets in our Solar System.

One of the unexplored issues with Martian impacts, and with planetary formation, is the time scale over which runaway greenhouse atmospheres can occur. The energy input to Mars following a large impact is enough to put it into the runaway energy zone calculated with simple steady state models. Such models have been used to study the accretion of planets. However, Mars does not stay long in this state. How does the planet respond to such impulses? Clearly the planet will begin to runaway, but how long does the impulse have to last for the runaway to go to completion and does it ever sustain itself? The process was not allowed to begin in the simulations conducted by Segura et al. (2002), since the hydrological cycle was suppressed. Indeed the atmosphere plays little role in those calculations. We propose here to include the hydrological cycle in further calculations and to consider time dependent runaway conditions.

Satellite observations over the Earth's oceans may provide useful information on how clouds and atmospheric dynamics influence the greenhouse effect, and hence influence the extent of the habitable zone. Satellite observations over particular locales of the Pacific Ocean show that upwelling long-wave radiative flux at the top of the atmosphere initially increases with sea surface temperature (SST) (Rabbette et al. 2003 and references therein). However, at a certain SST the emitted long-wave flux reaches a maximum and then decreases sharply, coupled with a sharp cutoff to the maximum observed SST (Rabbette et al. 2003). The failure of the emitted long-wave flux to continually increase as SST increases is analogous to the signature of the runaway greenhouse (cf. Ingersoll, 1969; Nakajima et al. 1992; Kasting 1988). We have modeled this phenomenon, and have been able to show how increased atmospheric water vapor over the Pacific Ocean leads to the signature of the runaway greenhouse locally (Rabbette et al. 2003). We propose to further model and investigate this phenomenon in order to better understand the conditions under which a runaway greenhouse occurs, and to identify possible limiting mechanism that might exist, e.g. clouds. Ramanathan and Collins (1991) argue that formation of cirrus clouds is the limiting mechanism, but this view is not universally accepted (cf. Pierrehumbert, 1995).

Venus now lies outside the conventional habitable zone. If Earth were moved to the location of Venus it would experience the runaway greenhouse effect. The surface would become hot enough that carbonate rocks decrepitate and the atmosphere fills with CO_2. Eventually the hydrogen in the water vapor would escape to space, and a planet like Venus would result. It is useful to ask whether any planet suffering Venusian levels of insolation is in fact inevitably uninhabitable.

Venus at present has a surface temperature of 750 K, and is very dry. Yet its albedo is 78% and its effective temperature is actually lower than Earth's. The surface is hot because it is under 90 bars of CO_2. What is curious about the 90 bars is that the CO_2 partial pressure is not very far from equilibrium with carbonate rocks at the surface. $CO_2 + CaSiO_3 <-> CaCO_3 + SiO_2$.

This "Urey" reaction is crucial to regulating CO_2 on Earth. The equilibrium is temperature sensitive, such that at lower temperatures the carbonate rock is favored. If there were substantially less than 90 bars of CO_2 on Venus, the surface today would be cooler and carbonate rocks would be stable, hence most of the CO_2 would be stored as carbonate rock, and there would be a thin mostly nitrogen atmosphere.

Would liquid water be stable at the surface under these conditions? Quite possibly, yes. The runaway greenhouse threshold presumes that the atmosphere is saturated with water above oceans. If there is very little water, and the bulk of the water is cold-trapped in polar caps, it is unclear that the runaway greenhouse threshold should apply. The atmosphere would be dry if there is little open water. The generally very low relative humidity invalidates the assumption that the atmosphere's temperature is controlled by water condensation, and it also limits hydrogen escape. Such a planet would be habitable. We propose to investigate whether a planet in Venus' orbit could become habitable, even if the planet evolved through a runaway greenhouse period.

Technical Approach and Methodology

In order to model the runaway greenhouse associated with Martian impacts, we will use the one-dimensional model of Segura et al. (2002) for these simulations. We will allow clouds to form as water is slowly removed from the atmosphere. We will then allow water which is present on the ground to be recycled into the atmosphere. Much

more water should be placed into the air than is currently allowed in the model in which only impacted injected water is considered. Temperatures should then be hotter, and stay warm longer than in the current model.

With regard to the signature of the runaway greenhouse in the Pacific Ocean, we propose to include the radiative effects of clouds and aerosols in our radiative transfer model to assess how they affect the whole phenomenon, and specifically to assess whether clouds can act to limit the sea surface temperature (SST), as suggested by Ramanathan and Collins (1991). Another study will involve a set of computations to investigate the influence of CO_2 on the magnitude of SST near the SST where outgoing radiative flux at the top of the atmosphere is a maximum. Part of the motivation comes from the observation that maximum SST during the late Cretaceous and Eocene epochs appears to have been several degrees C higher than at present (Pearson et al. 2001), which Pearson et al. suggest may be related to increased levels of CO_2. And of course, CO_2 is now the predominant gas in the atmospheres of Venus and Mars. These studies can be accomplished using appropriate modifications to the radiative transfer code used in Rabbette et al. (2003).

Our proposed research regarding whether Venus might be habitable under different conditions is to investigate four questions. The first part is to understand how relative humidity affects the runaway greenhouse, since all previous studies assumed 100% relative humidity in the lower atmosphere. In this we will build on Nakajima et al. (1992), taking account of the above results of Rabbette et al. (2003) in which vertical distribution of water vapor at less than 100% relative humidity produces the runaway greenhouse signature. The second part is to link the planet's poles to the tropics and consider meridional heat transport. This will be a function of how thick the atmosphere is. At first we will use the simplest model possible to describe meridional transport of heat from the equator to the poles (cf. Gierasch & Toon, 1973). Later, we will make use of the habitable planet general circulation models discussed in Section 3.1.2.3, suitably adjusted to this case, to more accurately describe meridional heat transport. A third issue we will address is the cycling of CO_2 between the mantle and the surface — does mantle CO_2 tend to buffer the atmosphere near the Urey point? Can the mantle store excess CO_2, or must the planet be CO_2-poor from the beginning? In this we will expand on our own previous work (Sleep & Zahnle 2001). A fourth issue to address is the stability of hydrous minerals, and how these affect the budget of surface water, and whether cycling with the mantle is important for the water budget as well. We will then devise evolutionary trajectories for these planets.

3.1.3 Relevance of Proposed Work

The proposed research is directly relevant to Goal 1 given in the CAN and Astrobiology Roadmap, namely to understand habitable planets in the Universe. The proposed research addresses several of the issues described in the Roadmap, specifically, modeling the formation, evolution, and stability of planetary systems that might harbor habitable planets; the delivery of key volatiles, such as water, to potentially habitable planets; planetary processes which affect habitable conditions; the role of impacts on habitability. Portions of the proposed work dealing with the composition of the early atmospheres of Earth and an Earth-like Venus are also relevant for understanding spectra of extrasolar planets which will be obtained by advanced astrobiology missions such as Terrestrial Planet Finder (TPF).

The proposed research will benefit and enhance planned space based astronomical detection and observational astrobiology missions, such as Kepler, Eddington, and TPF, all of which have as goals the detection and characterization of terrestrial type extrasolar planets, and their classification as to habitability. Kepler and Eddinton are scheduled to be launched during the time period covered by this proposal, so the work proposed and its relevance to these missions is very timely.

3.1.4 Key Milestones

Year 1 – Theoretical estimates of disk masses around young stars, mass loss rates, wind velocities and ultraviolet fluxes (Hollenbach); work on developing dynamical and radiative transfer code that will be coupled to nebula chemistry (Davis, Richard); establish public computer network for orbital evolution simulations (Laughlin); begin comparison of ages of Martian ejecta blankets and river networks, and provide post impact initial conditions to climate models (Toon, Zahnle, Colaprete); model effects of relative humidity on runaway greenhouse (Rabbette, McKay, Young, Zahnle); apply CCSM and ECBILT models to study effects of planet orbital parameters (Hollingsworth).

Year 2 - Photoevaporation timescale of circumstellar disks; estimates of viscous accretion timescales and dispersal timescale by protostellar wind (Hollenbach); establish set of chemical reactions to be used in nebula dynamical-chemistry code (Davis, Charnley); implement "mercury.f" orbital dynamics code on public computer network and begin simulations of planet formation and orbital stability (Laughlin); apply Öpik-Arnold scheme to simulations of delivery of volatiles by planetesimals (Chambers, Lissauer); continue

comparison of ages of Martian ejecta blankets and river networks (Toon); incorporate and apply simple models of meridional heat transport to Venus-like planet simulations (Zahnle, Sleep, Toon); begin runaway greenhouse study of post impact conditions on Mars (Toon); investigate effects of clouds on local runaway greenhouse signature over Pacific Ocean (Rabbette, McKay, Young); continue application of CCSM and ECBILT models to study effects of planet orbital parameters (Hollingsworth).

Year 3 - Compute photoevaporation of disks as function of stellar mass and type for single stars spanning the entire initial stellar mass function (IMF) (Hollenbach); begin incorporating the sets of chemical reactions identified in year 2 into the dynamic/radiative code developed in year 1 (Davis, Richard, Charnley); continue simulations of terrestrial planet formation and orbital stability on public computer network established in year 1 (Laughlin); modify and improve Öpik-Arnold scheme to provide a more realistic treatment of giant-planet perturbations and a crude treatment of secular interactions between planetary embryos (Chambers, Lissauer); complete comparison of ages of Martian ejecta blankets and river networks, and continue runaway greenhouse simulations following Martian impacts (Toon, Zahnle); investigate effects of carbon dioxide levels on local runaway greenhouse signature over Pacific Ocean (Rabbette, McKay, Young); refine meridional heat transport calculations for Venus-like planet and begin CO_2 cycling study between mantle and surface (Zahnle, Sleep); compare MGCM with CCSM and ECBILT results for particular cases to assess importance and validity of model parameterizations (Hollingsworth, Young).

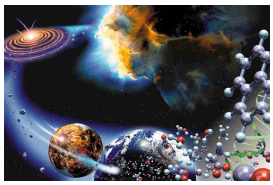
Year 4 - Calculate probability of stars forming in clusters, and probability of occurrence of high mass stars which emit large amounts of UV (Hollenbach); initiate application of coupled dynamical-chemical model to nebula simulations (Davis, Richard, Charnley); continue simulations of terrestrial planet formation and orbital stability on public computer network as number of observed planetary systems increases, and integrate results with Kepler Mission science team activities (Laughlin); apply improved Öpik-Arnold scheme to treatment of giant-planet perturbations and treatment of secular interactions between planetary embryos, and begin to incorporate results from coupled dynamic-chemistry nebula model into simulation of delivery of volatiles (Chambers, Lissauer, Davis); compute hydrogen escape rate for methane rich atmosphere in conjunction with evolution of early Earth's atmosphere (Toon, Zahnle); initiate study of what limits SST in Pacific Ocean near maximum of top of the atmosphere outgoing long-wave radiative flux (Rabbette, McKay, Young); on Venus-like planets investigate stability of hydrous minerals, and how these affect the budget of surface water, and whether cycling with the mantle is important for the water budget (Zahnle, Sleep); study effects of stellar type and orbital parameters on habitable zone (Hollingworth, Young).

Year 5 - Compute lifetime of protoplanetary disks in stellar clusters (Hollenbach); apply coupled dynamical-chemical model to nebula simulations (Davis, Richard, Charnley); continue to integrate results of coupled dynamic-chemistry nebula model to simulations of delivery of volatiles to terrestrial planets (Davis, Chambers, Lissauer); assess habitability of planet in Venus-like orbit after passing through runaway greenhouse phase (Zahnle, Sleep); assessment of habitable zone based on results from all studies (all); joint workshops with Kepler Mission Science Team.

3.1.5 Management Structure and Statements of Contribution

R. Young's (Lead CoI, Ames) responsibility will be to ensure that the research tasks are completed in a timely manner, and contribute his dynamical and climatological expertise to the investigations regarding Mars, Venus, and the Earth.

CoIs **J. Chambers** (SETI Inst.), **J. Lissauer** (Ames), and **G. Laughlin** (UC Santa Cruz) will direct and carry out the proposed simulations of orbit stability for planetary systems and delivery of water to the inner planets by planetary embryos from the asteroid belt. CoI **D. Hollenbach** (Ames) will be principally responsible for carrying out the studies of protoplanetary disk lifetime. CoI **S. Davis** (Ames) will collaborate with **D. Richard** (NRC Research Assoc.) on dynamics and A. J. Markwick (NRC Research Assoc.) and Collaborator S. Charnley (SETI Inst.) to produce a coupled dynamical-chemical model of the solar nebula. CoI **O.B. Toon** (University of Colorado) and CoI **K. Zahnle** (Ames) will be responsible for the studies involving whether impacts created the Martian valley river networks and the runaway greenhouse studies for Mars and Venus-like planets. CoI **M. Rabbette** (Bay Area Environmental Research Inst) will lead the study of the runaway greenhouse effect over the Pacific Ocean, collaborating with R.E. Young and Collaborator C.P. McKay. CoI **N. Sleep** (Stanford University will work with Kevin Zahnle on investigating the potential for habitability of Earth-like planets in Venus like orbits, and will collaborate with O.B. Toon and Kevin Zahnle on the consequences of impacts for Mars. CoI **J. Hollingsworth** will, together with CoI Richard Young and Collaborator Anthony Colaprete, be responsible for carrying out 3-D simulations of the climatology of potentially habitable planets.



3.2 Investigation 2 Prebiotic Organics, From Space to the First Membranes

How did the Earth and life arise? Is there life elsewhere? We hope to bring NASA closer to its goals of answering such questions by using molecules as historical records of the conditions in space when our Solar System formed, and as signs of life beyond Earth. Using organic chemistry as our unifying theme, this investigation spans the cosmochemical context for life, from the origin of molecules in space, through their delivery to planets by meteorites and cometary and asteroidal dust (IDPs), to the self assembly of the first membranes on the early Earth.

We intend to elucidate the role of compounds in space and prebiotic chemistry through a combination of lab work that includes *measuring spectra for comparison to astronomical observations, studying chemical reactions under low temperature space conditions, and exploring the biophysics of simple meteoritic molecules that may have made up the first membranes*. Our lab spectra will allow the identification of species in astronomical objects, and our chemical studies will advance our ability to detect potential biomarkers in extraterrestrial samples or on other planets and judge their significance.

Carbon forms in stars and is ejected at the end of the star's life. Organic compounds arise as a by-product of this stellar death. Following ejection, carbon-containing material disperses into the surrounding diffuse interstellar medium (ISM), where it is modified by different physical and chemical processes. Eventually, much of this material becomes concentrated in dense molecular clouds where, as described in the previous investigation, new stars and planetary systems form. In these dense molecular clouds molecules are modified and new organic compounds form, some of which are of potential prebiotic interest (Sandford 1996; Ehrenfreund & Charnley 2000). Thus, to study the species in these dense clouds is to examine the basic molecules from which planetary systems are made. Through lab spectra we can interpret astronomical observations and identify materials from which planets and perhaps life itself was constructed.

The production of prebiotic molecules in the interstellar medium is of little consequence to the origin of, and search for, life unless they can be delivered intact to habitable planets. This requires that they survive the transition from the dense cloud to protostellar nebula and subsequent incorporation into planetesimals, followed by delivery to a planetary surface (See Fig. 3.2-1). Deuterium isotopic measurements demonstrate that some interstellar organic species do, in fact, survive planetary accretion (Sandford, Bernstein, & Dworkin, 2001 and refs therein).

Meteorites and IDPs bring ~1×10^6 kg/yr organics to Earth (Love & Brownlee 1993), and probably provided orders of magnitude more to the early Earth (Chyba & Sagan 1992). This suggests that this "exogenous" delivery helped to make the early Earth (and other planets) habitable by contributing to our prebiotic chemistry (Oro *et al.* 1990; Chyba & McDonald 1995; Thomas *et al.* 1997).

Thus, the early Earth swept up vast quantities of extraterrestrial organic matter, some of which was created in the interstellar medium. These exogenous molecules include many of prebiotic importance, including functionalized polycyclic aromatic hydrocarbons (PAHs) and amino acids (Cronin & Chang 1993), as well as simple membrane forming compounds (Deamer 1985). Perhaps exogenous materials did more than simply deliver carbon as a starting ingredient for the primordial soup. Rather, the specific molecules may matter, having properties that were of relevance to the rise of life on Earth. We outline below a series of lab experiments to explore this possibility.

First, we propose lab studies of ice spectroscopy and chemistry that allow us to *identify and describe the chemistry of these pre-biotic organic compounds where they form in the cold environments in space.* This will lead to a better understanding of the origin, distribution, and significance of these molecules in meteorites and

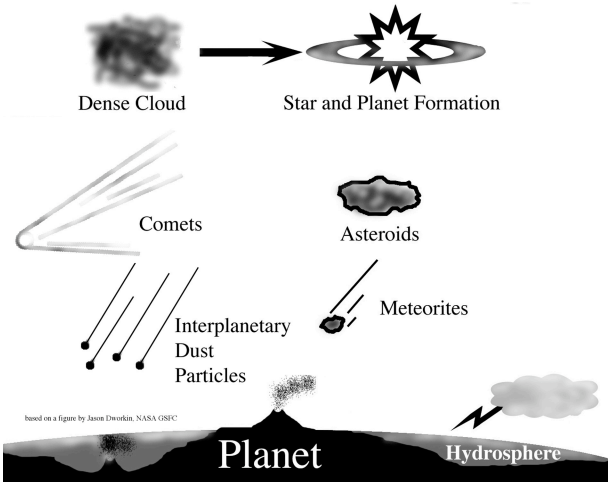


Fig. 3.2-1. Investigation 2 traces our chemical heritage, from the interstellar cloud that made the Solar System to the start of life on Earth. The molecules from which planets and life are composed originate in the interstellar medium. They are modified on incorporation into the solar nebula, and delivered to the surface of planets by meteorites and dust from asteroids and comets.

on other habitable worlds, and will help in the interpretation of potential biomarkers. Second, we propose experiments on synthetic, meteoritic, and model compounds to help us *understand how the first membranes may have arisen.* Membranes are a prerequisite to the maintainance of a chemical gradient, and perhaps the development of self or mutually replicating informational molecules (Szabo et al. 2002).

We are well suited to study extraterrestrial and prebiotic organic compounds, being pioneers in the analysis of mid-IR spectra of interstellar material, having participated in astronomical observations and developed lab techniques for comparison. We have identified interstellar ice constituents, established the ubiquity of PAHs in space (Allamandola et al. 1989, 1999), and constrained the nature of the refractory organic component of the diffuse ISM (Sandford et al. 1995; Pendleton & Allamandola, 2002).

3.2.1 Objectives and Significance of Research

1. Acquire lab spectra of relevant ice molecules under protostellar and outer Solar System conditions so as to improve the identification and our understanding of cosmic ice chemistry. Our work on protostars will inform the chemical and dynamical modeling of Investigation 1, task 1. Our spectra (and optical constants) will be used to fit spectra from ground-based, airborne, and space borne infrared (IR) observatories. This work directly supports NASA missions such as Stratospheric Observatory for Infrared Astronomy (SOFIA), the Space Infrared Telescope Facility (SIRTF), and the Astrobiology Explorer (ABE), and addresses Astrobiology Roadmap Goal 2, directing NASA to "Explore for past or present habitable environments, prebiotic chemistry and signs of life elsewhere in our Solar System."

2. Perform lab experiments of the radiation processing of PAHs (and amino acid precursors) in interstellar and outer Solar System ice analogs, and characterize the products formed. This will test the potential of ice chemistry to produce meteoritic organic compounds that could help set the chemical stage for the development of life. This work on the formation and survival of prebiotic organics falls under Astrobiology Roadmap Goal 1, which includes study of "The processes by which crucial biologically useful chemicals are carried to a planet and change its level of habitability…" In some cases these molecules could be confused as biomarkers if they are detected in a meteorite or on an extraterrestrial body. The potential of abiotic organics that form on the surface of icy bodies such as Europa to be mistaken as biomarkers correlates to Goal 7 to "Determine how to recognize signatures of life on other worlds."

3. Explore the behavior of pre-biotic compounds that could have served as the first components of membranes. We will analyze molecules from meteorites, our ice processing experiments, and perform studies on off-the-shelf model compounds to put constraints on the earliest membrane systems. We will assess how vesicles made from mixtures of linear acids and PAHs (both simple meteoritic molecules) withstand variation in pH and other parameters. This task is directed at Astrobiology Roadmap Goal 3, which includes studies of "sources of organic compounds for the origin of life" and "origins of cellularity and protobiological systems."

3.2.2. Research Tasks

3.2.2.1 Task 1 - Characterize interstellar, protostellar, and outer Solar System ices.

We propose to measure spectral fingerprints of organic compounds in realistic, lab analogs of interstellar, protostellar, and outer Solar System ices to support numerous NASA projects and missions motivated by a desire to understand the chemical context in which planetary systems and life arise. By facilitating the analysis of the original material in protostellar systems from which planetary systems are made this task feeds into Investigation 1, on nebular processes and habitable planet formation,

Build On and Extend State of Knowledge

Dense interstellar clouds, from which all stars and planetary systems form, attenuate the interstellar radiation, permitting the survival of organic compounds. Mixtures of molecules condense at 10-50 K to form ice mantles on the surfaces of refractory dust grains where they participate in additional chemical reactions. When a new star forms in such a cloud it is called a protostar. Most of what is known about protostars is based on observations of those more massive than our Sun, because they are bright enough to measure. Much less is known about small protostars, and quiescent regions of dense interstellar clouds. By comparing IR spectra of lab ices with absorption spectra of bright protostars, we and others have been able to show that ices around these stars are composed of H_2O, mixed with several percent of simple molecules such as CO, CO_2, CH_3OH, and NH_3 (Gibb et al. 2000; Ehrenfreund & Charnley 2000; Gibb & Whittet 2002). The Astrochemistry Lab Group at Ames has been one of the world's leaders in establishing this field, both observing and identifying the main components of these protostellar ices. Dr. Allamandola, with his collaborators at Leiden University, developed the lab techniques to provide the first refractive indices (*n*'s and *k*'s) of amorphous ices. This approach was applied to realistic mixtures (Hudgins 1993) and that data has been used by us and others to quantify the

abundance of compounds in extraterrestrial ices. Past observations from ground based observatories, the Kuiper Airborne Observatory, and the European Infrared Space Observatory (ISO) led to the detection of the major components of bright protostars, with many minor components and fainter objects left uncharacterized. But this is about to change.

We are approaching an astronomical golden age, with missions directly addressing key astrobiological issues soon to be launched. NASA's IR observatory SIRTF and the airborne IR telescope SOFIA will be flying during the duration of this proposal. The Astrobiology Explorer Mission (ABE), an orbiting IR telescope devoted exclusively to characterizing the evolution of organics in space by measuring their IR spectra to higher levels of sensitivity and resolution than ever before, is now under review. Dr. Sandford, a CoI on this proposal is PI of the ABE mission; Dr. Allamandola is an ABE CoI.

With greater sensitivity and spectral resolution, these missions will make possible observations of new objects and trace species not accessible before. For example, past observations were limited to bright protostars – those producing stars much larger than the Sun. Preliminary data suggest that composition varies with size, and the greater sensitivity of ABE, SOFIA and SIRTF will allow faint objects to be analyzed thoroughly for the first time. For the first time we will directly be able to analyze protostellar environments like the one from which our Solar System arose. These analyses will provide crucial input for the models of planetary system formation described in Investigation 1.

This greater sensitivity also means that new compounds will be detected in the ice. Similarly, better spectra of quiescent regions will reveal the least-processed, coldest, environments. The determination of spectroscopic data described below is essential to maximize NASA's scientific return on its investments in IR missions such as ABE, SOFIA and SIRTF.

We are active CoIs with groups reducing the ISO Spectral database. ISO's archive of mid-IR spectra of the brightest interstellar and extragalactic objects was opened to the public during this past Astrobiology funding cycle. Supported by other funds, we are analyzing this ISO data with our lab spectra, and the results will inform our future work.

Furthermore, longer wavelength (to 5 μm) spectra now achievable from ground-based observatories such as NIRSPEC (a near-infrared echelle spectrograph at Keck) and SpeX (a Medium-Resolution Spectrograph on IRTF) have created new opportunities in the analysis of Solar System ices. Observations of dim Solar System objects have revealed NH_3 on Charon (Brown & Calvin 2000) and CH_3OH on Pholus (Cruikshank 1998). We have a collaboration with Eliot Young of the Southwest Research Institute on an NSF funded project on the interpretation of amino acid precursors (such as HCN, CH_2O, and NH_3) in outer Solar System ices. Our experimental work on these systems will indirectly support NIMS (Galileo) and VIMS (Cassini) missions by providing lab data for comparison, although we are not CoIs on these missions.

All of this new interstellar, protostellar, and outer Solar System data will bring a flood of astronomical spectra of molecules under unusual conditions demanding new reference spectra for interpretation. We propose to extend our historically successful lab spectroscopy program to accommodate this need.

Technical Approach and Methodology

Interpreting astronomical observations requires lab experiments to determine the spectral properties of relevant materials under appropriate conditions, especially refractive indices for light scattering models. We will vapor deposit mixtures of simple ice components (i.e. H_2O, CH_3OH, CO_2 exact components and proportions based on observations) onto a cold substrate. We will measure spectra with an IR spectrometer and ice thickness with a HeNe laser. *From these we will determine refractive indices (n's, k's) of the ice components as a function of concentration and temperature*. The properties of molecules in the ice vary greatly with the conditions; in the solid phase interactions between molecules can be quite strong, causing spectral changes. New spectra are needed each time observations indicate changes, since even if the components are the same, simply changing concentration or temperature can change band profile and strength.

We will also assess effects of radiation processing on the ices. The UV photolysis of CH_3OH in water ice forms HCO, H_2CO, CH_4, and CO_2 and often the spectral appearance of such species formed *in-situ* differs from those vapor deposited. We, and others, have used this behavior to better fit infrared spectra of protostellar objects. By identifying the new photoproducts we made correct predictions and thus discovered new molecules in space. As new more sensitive telescopes produce better spectra of low-mass protostars and quiescent regions we will use the same techniques, exposing to radiation the new mixtures implied by the latest observations, perhaps leading to new astronomical observations. In addition, such lab experiments can constrain chemical models, i.e., by monitoring the photo-destruction rate of the amino acid glycine we were

able to estimate its lifetime in interstellar environments (Ehrenfreund et al. 2001).

Similarly, we will employ lab experiments to determine spectral properties of ices germane to icy outer Solar System bodies to fit reflection spectra. These are primarily ices dominated by water, but the components, types of radiation, and temperatures can be quite different from protostellar ices. For our compositional studies we will focus on objects and spectral regions where there are unidentified features. For example, The NIMS spectra of Callisto and Ganymede display absorptions at 4.57 μm (McCord et al. 1997; 1998) which have been attributed to an organic nitrile (a compound bearing a -C■N), but the carrier has yet to be identified. Nitriles are a longstanding interest of ours, and we have noted how the IR peak positions vary with the molecular environment (Bernstein et al. 1997; 2000). As above we propose to measure the effects of radiation processing in model Solar System ices i.e., the formation of CO_2 from degradation of exogenous organics on a satellite's icy surface. This portion of the proposed work has recently been partially funded through a grant (from NASA's PG&G program) to study the radiation processing of ices on the icy Galilean satellites.

3.2.2.2 Task 2 - The Connection Between Ice Chemistry and Pre-Biotic Organics Delivered to Earth (and Elsewhere).

We propose to further elucidate the connection between meteoritic pre-biotic compounds and interstellar and protostellar ice chemistry. Understanding the origin of these species is relevant to the first Goal of the Astrobiology Roadmap, which deals with the delivery of prebiotic molecules and their connection to habitability. In addition, inasmuch as some of these molecules are very similar to and can be confused with biomarkers, this work also relates to the second roadmap Goal as well.

Build on and Extend State of Knowledge

In early photochemical studies of interstellar and Solar System ice analogs it was noted that a film of non-volatile organic material sometimes formed (Hagen et al. 1979; Sagan & Khare 1979). It has since become clear that energetic processing of ices at low temperature can create many complex organic molecules from simple starting materials (Bernstein et al. 1995; Gerakines et al. 2000; Khare et al. 2002). The suite of species produced under interstellar ice conditions resembles those found in meteorites. These chemical correspondences, combined with isotopic enrichments typical of low temperature reactions, suggest that some of the complex organic compounds in meteorites may have derived from energetic processing of interstellar or protostellar ice. This source of meteoritic organics from ice complements the paradigmatic pathway to them via reactions in liquid water on the parent asteroid or comet from which the meteorite came (Peltzer et al. 1984). Whereas parent body reactions in liquid water require conditions that are somewhat local, dense molecular cloud ice chemistry is universal, since the material from which all planetary systems are made comes from such clouds. Prebiotic molecules that derive from the ice are ubiquitous, making them relevant to the habitability and prebiotic chemistry of, and recognition of signatures of life on, all solar system bodies.

In order to evaluate the connection between ice photochemistry and the complex organic compounds in carbonaceous meteorites, we have been pursuing two paths in the lab: a reductionist approach studying in great detail the reactions of a single compound in ice at low temperature, and a synthetic method in which we approximate the complexity of extraterrestrial ices with realistic mixtures of several components, expose them to radiation and see what forms. Over the course of our previous NAI funding period, the reductionist and synthetic techniques both yielded significant advances in our understanding of chemical structures and patterns of deuterium enrichment suggestive of ice processing. The former resulted in a good explanation for the chemical groups that decorate meteoritic aromatics (and 6 papers including one in *Science*). The latter produced two papers on ice photochemical syntheses of important pre-biological meteoritic molecules: a paper last year in *Nature* on meteoritic amino acids (Bernstein et al. 2002a), and a paper in *Proc. Nat. Acad. Sci* on simple amphiphiles - membrane forming compounds (Dworkin et al. 2001). The work on amphiphiles especially improved our appreciation of the remarkable complexity that can arise from simple interstellar starting materials. The results from, and proposed future work for, both approaches will now be reviewed in greater detail.

Technical Approach and Methodology

Reactions of Aromatics in Ice

PAHs are observed in emission throughout the universe (Allamandola et al. 1999; Cox & Kessler 1999), and are seen in absorption towards cold dense clouds (Sellgren et al. 1995; Brooke et al. 1999; Chiar et al. 2000; Bregman et al. 2000; Bregman & Temi 2001) where they should condense into ices. PAHs are probably present in cometary ices (Moreels et al. 1994), and are ubiquitous in meteorites and IDPs, so they should be delivered to planetary polar caps and the surfaces of icy bodies in the outer Solar System (such as the Galilean satellites). In all of these environments PAHs would be exposed to UV and cosmic rays while frozen in the ice, but little was known about their reactions in ice at the time we proposed to NAI five years ago.

Given that the D-enrichment of PAHs present in meteorites and comet and asteroidal dust suggest low temperature chemistry (Sandford et al. 2000; Sephton & Gilmour 2000; Sandford, et al. 2001), we proposed and carried out lab experiments on the UV photo-chemistry and MeV proton irradiation of PAHs in ice (Bernstein et al. 1999; 2001; 2002b; 2003). We found that exposure of PAHs to moderate doses of UV radiation in solid H_2O at 10 K, leads to oxidation (alcohol, ketone and ether formation) and hydrogen atom addition (see Fig 3.2-2).

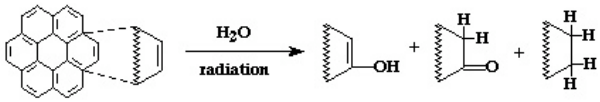


Fig. 3.2-2. A plain PAH, like the kind made at the death of a carbon rich star, is quickly converted to its oxidized and reduced forms when exposed to UV in H_2O ice under interstellar conditions. We proposed that interstellar ice chemistry is a source of these molecules in meteorites. If so, these carbon molecules should be universal, since these dense interstellar clouds are the source of every planetary system.

To explore the link between ice processes and the molecules in meteorites, we examined the UV-induced oxidation of naphthalene (a PAH). We separated isomeric alcohols and napthoquinones using HPLC (Bernstein et al. 2001) and described its regiochemistry (what ends up where). In addition, since deuterium enrichments of extraterrestrial molecules are often taken as the strongest indication of an interstellar heritage, isotopic labeling is an important test of lab experiments. We have used deuterium labeling in PAH-ice experiments in the lab and published papers making specific predictions of where deuterium should be found on meteoritic molecules (Sandford et al. 2000, 2001). *We propose to extend this work to study the regiochemistry of anthracene*, the oxidized variants of which have been observed in the Murchison meteorite (Krishnamurthy et al. 1992).

We have also studied the UV processing of PAHs with common interstellar compounds like NH_3, HCN, CH_4, CH_3OH, and CO_2 in the ice. In addition to the products in Fig 3.2-2 we found the addition of amino, cyano, methyl, methoxy, and carboxylic acid groups (see Fig. 3.2-3).

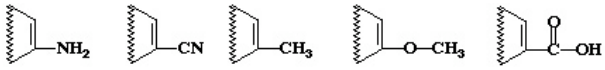


Fig. 3.2-3. Simple interstellar ice compounds add to PAHs to produce a range of functional groups. This is important because it explains a meteoritic molecules. Given that substituted aromatics have been invoked as biomarkers, understanding all sources of such compounds is important for the search for chemical signs of life.

Substituted aromatic species are common in meteorites including ketone (>C=O; Krishnamurthy et al. 1992), acid (-COOH; Sephton et al. 2001), and alkyl (-CH3; Cronin et al. 1988) groups. Evidence from degradation analyses (Hayatsu et al. 1980; Komiya et al. 1993; Sephton et al. 1999) and NMR studies (Gardinier et al. 2000; Cody et al. 2002) suggest meteoritic macromolecular material also contain such functional groups. Similarly, two-step laser mass spectra of IDPs are consistent with alkyl and nitrogen-containing functional groups attached to aromatics (Clemett et al. 1993).

In conjunction with our collaborators at NASA Goddard we have begun to explore simple oxidation of and acid formation (-COOH Fig. 3.2-3) on PAHs by MeV proton irradiation in ice (Bernstein et al. 2003). Comparisons of UV and MeV proton experiments indicate differences between the conditions and mechanisms that give rise to acid formation, but this is not yet understood. We plan to continue this collaboration and work on PAH MeV proton chemistry in the presence of the other common interstellar ice components. *We also propose to study these reactions under conditions germane to Galilean satellites*, investigating the effects of higher temperatures, and low energy (<10 Kev) electrons.

Understanding the chemistry of PAHs bearing side groups is important for astrobiology for a number of reasons. First, to the extent that we understand their formation these molecules have potential as tracers of interstellar chemistry in Solar System materials. Second, given that substituted aromatics not unlike those above were invoked as biomarkers in a Martian meteorite (McKay 1996) understanding non-biological processes that produce them must bear on issues of finding signatures of life.

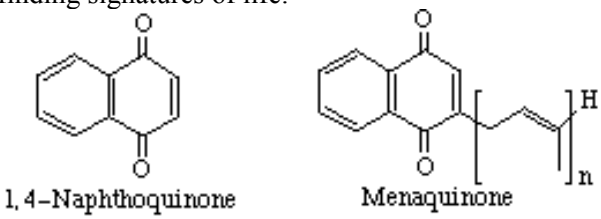


Fig. 3.2-4. The molecule on the left was formed in one of our interstellar ice simulations, the one on the right is a biomarker. The striking similarity of an ice photoproduct to a ubiquitous biochemical suggests that searching for chemical biomarkers on places like Europa will be difficult if we fail to fully understand the non-biotic chemistry first.

Finally, some of the other functionalized PAHs that we have generated look so much like those in living systems that, as compounds exogenously delivered to the early Earth, they may played a role in the evolution of life. For example, 1,4-

napthoquinone is a significant product in our naphthalene/H_2O photolysis experiments. Substituted naphthoquinones, such as menaquinones (see Fig. 3.2-4), perform key biochemical functions (Suttie, 1979), helping or acting as electron transporters and oxidative phosphorylation co-enzymes, even in "primitive" organisms that are often studied by astrobiologists. For example, the Archaea Thermoproteus tenax use menaquinones in the reduction of elemental sulfur to H_2S as their main energy source (Thurl et al. 1985). Perhaps extraterrestrial quinones, which spontaneously partition into model membrane systems (see 3.2.2.3, below) first afforded an evolutionary advantage to early organisms by providing some protection against UV radiation on the early Earth (Wynn-Williams and Edwards 2002), and later were exploited for their capacity to pump protons across bilayers (Deamer 1985).

Synthesis of Complex (Pre-Biotic) Organics

Our lab experiments simulating interstellar environments support the notion that dense molecular clouds permit the synthesis of species far more complex than the simple starting molecules seen in interstellar spectra. In particular, our ISM ice experiments have yielded two classes of compounds that are also present in meteorites and are of interest to astrobiology because of the potential relevance to the origin of life: amino acids (Bernstein et al. 2002a) and amphiphiles (Dworkin et al. 2001).

Many indigenous amino acids have been detected in a number of meteorites, (Cronin & Pizzarello 1983; Cronin & Chang 1993). It has been generally assumed that the amino acids in meteorites formed in *liquid* water (Peltzer 1984) on an asteroid or comet parent-body. However, the water in the Murchison meteorite, for example, was depleted of deuterium (Robert and Epstein 1982), making the distribution of deuterium in organic acids in Murchison difficult to explain (Lerner 1995; 1997). Similarly, occasional but consistent meteoritic excesses of left handed (L), non-terrestrial, amino acids (Cronin & Pizzarello 1997) are difficult to rationalize by liquid water parent-body reactions. Rather this bias towards L has been postulated to arise in space from circularly polarized radiation (Rubenstein et al. 1983; Cronin & Pizzarello 1997; Rubenstein et al. 1999) – essentially, handed molecules from handed light. But the formation, under interstellar conditions, of any kind of amino acids, let alone handed ones, had not been demonstrated.

As part of our previous NAI grant we reported the first interstellar ice photo-synthesis of amino acids (glycine, alanine, and serine) and other meteoritic molecules (Bernstein et al. 2002a; Muñoz-Caro et al. 2002). Careful controls, chirality, and isotopes demonstrate they are not contaminants. We propose to employ partial ^{13}C and ^{15}N isotopic labeling experiments to uncover the mechanism by which these molecules form.

Furthermore, amino acids are of great biological significance, since proteins are made of amino acids. This aspect of our work goes hand in hand with the research presented in Investigation 3 on the polymerization of amino acids leading to enzymes and proteins. Since living systems are made of L amino acids, understanding the origin of the excesses of L amino acids in meteorites relates to astrobiology both in terms of the use of handedness as a sign of life on other planets (Rodier 2002; Brinton et al. 2002) and as a way in which life on Earth was potentially predisposed towards L (Greenberg 1997). *We propose to employ circularly polarized radiation to test whether the meteoritic bias towards L can indeed be explained by a photo-synthetic process from "handed" light.*

While amino acids formed in our interstellar ice synthesis experiments are more complex than the simple one-carbon starting materials, they still are relatively small molecules (75-100 amu). A mass spectrum of trace material from the UV photolysis of the realistic interstellar ice analog H_2O:CH_3OH:CO:NH_3 (100:10:1:1) at 15 K gives an indication of the complexity of the product mixture (see Figure 3.2-5).

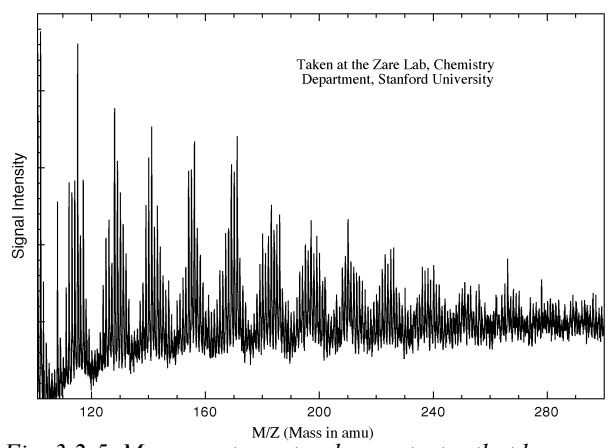


Fig. 3.2-5. Mass spectrometry demonstrates that large molecules can be made from a realistic ice mixture of simple interstellar molecules of no more than one carbon atom each. If the products contain carboxylic acids (as our results below suggest) then these spectra are consistent with organic acids of up to 18 carbon atoms!

These ices begin with only a few simple species but larger ones are produced, with molecules of almost 300 amu made from compounds of only one carbon atom each. Careful control and isotopic labeling experiments demonstrate that these are not contaminants (Dworkin et al. 2001). Little is known about how product formation depends on

conditions or the mechanism. We propose to determine the range of extraterrestrial conditions that can give rise to these compounds by exploring the effects of temperature and composition on the mass spectra. But even more remarkable than the large masses and diversity of these compounds is the capacity of these mixtures to form vesicles (bi-layer spherical structures like membranes). This is also a property shared by chloroform extracts of the carbon-rich Murchison meteorite (Deamer 1985).

3.2.2.3 Task 3 - The First Membranes: Meteoritic and Model Systems that Self-Assemble

We propose to study the structure, stability, permeability and dynamics of vesicles (bi-layer spherical structures like membranes) made of pre-biotic compounds, to understand roles they may have played in forming the first cell membranes. We will use primarily off-the-shelf model molecules, but we will also continue to test the amphiphilic capacity of the compounds created in our ice simulations (above). Furthermore, we will closely examine the meteoritic extracts that form these compounds, separating out and testing components for vesicle formation and deuterium enrichment.

Build on and Extend the State of Knowledge

Membranous boundary structures define all life today, and were probably essential for the rise of life (Deamer et al. 2002). Not only are barriers needed to maintain a chemical gradient, but recent work suggests that the isolation they provide was a prerequisite for the development of self-replicating informational molecules, i.e., DNA or RNA (Szabo et al. 2002). In fact, rather than focusing on a single, self-replicating, informational compound, some suggest the origin of life involved the self-assembly of molecular systems within cell-sized environments (Morowitz 1992; Segré et al. 2001).

Amphiphiles - compounds that can self-assemble to form bi-layer structures (see Fig. 3.2-6) - are present in the Murchison meteorite (Deamer 1985) and are produced in our interstellar ice simulation experiments (Dworkin et al. 2001). Whether exogenous or endogenous (or most likely a mixture of the two), such self-assembling molecules probably played a key role in the emergence of life because of the unique advantages they can provide. For example, enclosed vesicular structures are capable of concentrating molecules, overcoming dilution and facilitating interactions that develop or maintain macromolecules. Moreover, since the aromatic components of the prebiotic organic inventory include non-polar pigments (i.e. naphthoquinone, Fig. 3.2-4), they have the potential to capture light energy and screen the interior from damaging UV light when partitioned into the hydrophobic phase of a bi-layer. This emergent set of biophysical properties can only arise from amphiphilic molecules that have the ability to self-assemble into more complex vesicular structures.

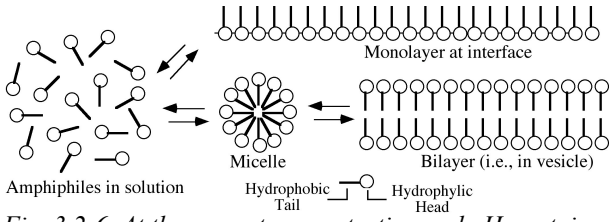


Fig. 3.2-6. At the correct concentration and pH, certain compounds spontaneously self-assemble into bi-layer structures known as vesicles. Mixtures of molecules from the Murchison meteorite and from our ice simulations have this capacity. We suspect long chain linear carboxylic acids are the active molecules in these cases, but little is known about the "prebio"-physics of the vesicles formed by these prebiotic molecules.

Technical Approach and Methodology

We plan a three-pronged approach to advance our understanding of prebiotic amphiphiles. First, we will extend our successful lab work on reproducing the ice chemistry of space environments to assess under what conditions amphiphiles form. Second, we will extract, separate by HPLC, and structurally characterize meteoritic amphiphilic compounds, looking for deuterium enrichments. Of course, meteorites and prebiotic soups have mixtures of compounds, and this leads to potential complications, whereas traditional studies on amphiphiles have been performed on individual (pure) compounds. In attempting to approach realism by using mixtures, a synergistic effect was recently established: the bilayer structures made from mixtures are far more stable than pure compounds, at least for poor amphiphiles (Apel et al, 2002).

These vesicles were made from monocarboxylic acids, which are found in carbonaceous chondrites. Nonanoic acid forms vesicles only at concentrations above 85 mM at pH 6.9 (the pKa of the acid in membranes). However, upon the addition of 10% nonanol, only 20 mM of total acid is necessary to form vesicles, and they are stable up to pH 11 (Apel et al. 2002). Thus, the addition of a small amount of alcohol to the acid creates membranous structures that are stable over a far wider range of conditions, even at a quarter the concentration.

Meteoritic PAHs and their polar derivatives (Mautner et al. 1995) have been shown to partition between membrane bi-layers. In certain lab settings aromatics (such as naphthoquinones Fig. 3.2-4) can generate protons and establish transmembrane pH gradients (Klein & Pilpel 1973; Deamer 1985). However, PAHs have never been studied with prebiotic amphiphiles, like a

carboxylic acids. With their multiple rings and flat structure, PAHs may change the properties of these vesicles, as cholesterol stabilizes biological membranes. We will explore the effect of adding PAHs to prebiotic vesicles, with a special emphasis on changes in stability and permeability to pH gradients. These were presumably the important parameters if such structures were employed by the first organisms.

3.2.3 Description of Instruments and Hardware

The instrumentation and hardware needed to carry the proposed lab work are present at the Astrochemistry Lab at NASA Ames, or our collaborator's labs at UC Santa Cruz, and Stanford. For a detailed description of our equipment see Section 5.2.5.

3.2.4 Relevance of Proposed Work

The work in this module squarely addresses the following Astrobiology Roadmap Goals: 1- including the study of "The processes by which crucial biologically useful chemicals are carried to a planet and change its level of habitability…", 2- "prebiotic chemistry and signs of life elsewhere in our Solar System," 3- "sources of organic compounds for the origin of life and origins of cellularity and protobiological systems," and 7- "Determine how to recognize signatures of life on other worlds." Moreover, our lab spectral data will help fit spectra from NASA's ground-based, airborne, and spaceborne IR observatories. As a result, this work will directly support missions such as the SOFIA, SIRTF, and ABE.

3.2.5 Key Milestones

Year 1 - Use Existing IR database to interpret ISO spectra. Perform IR spectroscopy on outer Solar System ices and a series of naphthalene-H_2O mixtures of known concentration for generation of n's and k's. Begin amino acid ^{13}C partial labeling experiments.

Year 2 - Continue ISO data analysis program. Perform UV photolysis experiments on anthracene-H_2O mixtures and analyze using HPLC. Continue IR spectroscopy on outer Solar System ices for n's and k's. Begin studies of amphiphilic model compounds, mixtures of PAHs and long chain (>9) acids. Continue amino acid ^{13}C partial labeling experiments and begin ^{15}N amino acid partial labeling experiments.

Year 3 - Use lab spectra to interpret data from SIRTF and direct SOFIA mid-IR instrument requirements and first observations. Continue studies of amphiphilic model compounds exploring pH stability. Perform UV photolysis experiments on PAH-H_2O mixtures and other ice components at higher (50-100 K) range. Complete amino acid partial labeling experiments and explore effects of temperature.

Years 4 and 5 – Continue astronomical observations extending to other galaxies. Perform irradiation experiments on PAH-ice mixtures using electrons as radiation source. Test the permeability of the most stable nonanoic acid PAH vesicles, and explore the effects of UV radiation. Perform amino acid experiments with circularly polarized radiation to attempt to generate an enatiomeric excess, this will require exploring different wavelengths.

3.2.6 Management Structure and Statements of Contribution

CoI Dr. **Lou Allamandola** (NASA/Ames) has extensive experience in all the aspects of lab astrophysics and IR observations relevant to this proposal and will participate fully in those areas. He will supervise the overall program.

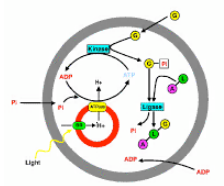
CoI Dr. **Max Bernstein** (SETI and NASA/Ames) is responsible for performing ice processing experiments and analysis of product molecules. He will aid NRC post-doc Charles Apel on the self-organizing behavior of amphiphiles extracted from meteorites and produced synthetically in the lab.

CoI Dr. **Scott Sandford** (NASA/Ames) will aid in the interpretation of the results in the context of comparisons with meteorites and IDPs, especially regarding isotopes. He will also coordinate the lab IR studies with those required for ABE, the proposed MIDEX mission for which he is PI.

Collaborator Prof. **David Deamer** (UC, Santa Cruz) will provide Murchison samples, the use of microscopes, and his expertise for the studies of meteoritic amphiphiles.

Collaborator Prof. **Richard Zare** (Stanford University) and his group will continue to measure mass spectra of molecules made in PAH-ice processing experiments.

Collaborator Dr. **Jason Dworkin** (NASA Goddard), will perform MeV proton irradiations of PAH-ice mixtures and some chromatographic analyses of amino acids.



3.3 Investigation 3 The Origin and Early Evolution of Proteins and Metabolism

3.3.1 Objectives and Significance of Research.

Hypotheses. We propose to study the origin and early evolution of the peptides and proteins that putatively carried out metabolic functions in the ancestors of contemporary cells. There are two competing models that describe the possible

origins of these molecules. According to one model, catalysis of biochemical reactions was initially carried out by RNA enzymes (ribozymes). The transition from "the RNA World" to the modern, protein-dominated biological world was bridged by the evolution of ribozymes that synthesized peptides in a non-coded fashion. This was followed by the coded evolution of short peptides, and then of longer proteins. An alternative model begins with an autocatalytic network of peptides which evolved to a state of increasing metabolic complexity prior to the evolution of RNA-coded information. Although these two models are quite different they share a common feature – both assume the existence of proteins not coded by nucleic acids. To evaluate the validity of this assumption and to distinguish between the two models, it is critical to establish the probability that random peptides can perform protocellular functions, especially those that RNA is unlikely to perform, such as the transport of specific ions across membranes. In order to understand the subsequent evolution towards modern proteins, it is also essential to determine the structure of the first functional peptides and collective properties of networks of chemical reactions catalyzed by these peptides.

Specific Objectives. The proposed work builds on our accomplishments in the previous NAI granting period. For the first time, we were able to select, from a very large population of random-sequence proteins, four families of small peptides with novel amino acid sequences that efficiently bind adenosine triphosphate (ATP) (Keefe & Szostak 2001). From this study, it appears that the emergence of a functional protein from a pool of random sequences is approximately as likely as the emergence of a functional RNA molecule from random RNA sequences (Ellington & Szostak 1990, Bartel & Szostak 1993). This research direction will be continued with the following specific objectives:

1. To obtain the 3-dimensional structure of the ATP-binding protein. This will allow us to determine if the peptide, which has an amino acid sequence unrelated to any known proteins, has a novel structure or, alternatively is folded into an already known structure. As discussed in the next section, the evolutionary implications of these two possibilities are both important and quite different.
2. To reconstruct in the laboratory the emergence of early catalytic functions by constructing for the first time models of protobiologically ubiquitous protein enzymes. We will identify their structures, properties, relationships to known proteins performing similar or different functions, and the ability to evolve to proteins with altered functions.
3. To select peptides that assemble into transmembrane channels capable of transporting ions across membranous cell walls which was, most likely, one of the earliest cellular functions. This function is very difficult (and perhaps impossible) to achieve using RNA molecules because they do not partition into membranes.
4. To examine the evolutionary potential of a collection of proteins in the absence of self-replicating mechanisms. We will determine under what conditions evolution could progress and assess whether these conditions were protobiologically plausible.

The first three goals will be pursued mostly experimentally. At the center of this effort will be *in vitro* selection approaches similar to that applied before to obtain ATP-binding proteins. The last goal will be pursued through theoretical and computational modeling. The experimental and theoretical approaches complement each other. Experiments provide estimates of efficiencies with which non-coded proteins can catalyze biochemical reactions. These efficiencies are critical parameters for modeling non-genomic evolution. Computer modeling will aid interpretation of experimental results in terms of protein structure, evolution and mechanisms of action. Finally, the proposed modeling effort can guide future experiments aimed at demonstrating the emergence of coordination between different metabolic processes using the suite of protein enzymes created in the laboratory.

Significance. If successful, the proposed studies will yield novel, essential information about the origin and evolutionary potential of the earliest biopolymers that facilitated the chemical reactions supporting life. Successful selection of novel enzymes that are not derived from biological proteins could shed new light on the potential of life beyond Earth and open new avenues for biotechnology. Furthermore, the ability to evolve small, proteins with high affinity and specificity for binding small molecules could have applications in biosensor development.

Conceptually, the proposed research is a continuation of Investigation 2, which addresses the origin of the building blocks of both proteins and membranes. It also complements Investigation 1, which deals with physical constraints to habitability, by providing potential biological constraints.

3.3.2 Building on and Extending State of Knowledge

The identity of the earliest biopolymers. In modern organisms, most functions are performed by proteins, which are, in turn, synthesized on an RNA template. The discovery that RNA possesses catalytic capabilities led to a suggestion that the present world of nucleic acids and proteins was preceded by the "RNA World", wherein RNA molecules alone acted as both catalysts of

biochemical reactions and as information storage systems (Gilbert 1986; Gestland et al. 1999). This model is supported by the fact that a variety of RNA enzymes have been created in the laboratory using *in vitro* selection (Wilson & Szostak 1999), and by the recent discovery that the decoding and peptidyl transferase centers of ribosomes are composed entirely of RNA.

The major difficulty with this model is that no efficient, prebiotically relevant means of RNA synthesis has been found. Furthermore, RNA cannot be readily incorporated into membranes to perform functions, which, in modern cells, include the vital functions of energy transduction and transport.

In an alternative view, the first biological macromolecules were proteins. They do not suffer from the same problems as RNA. Specifically, both terrestrial and extraterrestrial sources of protein building blocks - amino acids - have been identified (Chang 1993; Cronin & Chang 1993). A protobiologically plausible mechanism to convert simple molecular precursors that might have been abundant on the early earth into amino acids has been proposed and tested by Weber (1998, 2001). Furthermore, the condensation of amino acids into peptides was demonstrated in a liposome model system (Luisi et al. 2000). In contrast to nucleic acids, simple proteins insert spontaneously into membranes and aggregate into functional assemblies.

The "protein-first" concept, however, encounters serious difficulties of its own. In particular, with only a few exceptions (Severin et al. 1997a; Yao et al. 1998), attempts to create simple, protobiologically relevant proteins possessing catalytic activity have not been successful. Furthermore, since amino acids cannot form base-pairs like nucleic acids it is not clear that peptides alone could transfer information between generations and evolve. Resolving such difficulties will advance our understanding of how protocellular metabolism emerged.

Experimental basis for the "protein-first" concept. Several recent studies demonstrated the novel catalytic and evolutionary potential of simple peptide systems. Ghadiri and co-workers have shown that two activated peptide fragments can be joined (ligated) on a template of a third, longer peptide with a rate enhancement of 10-fold to 4000-fold over the background (Severin et al. 1997a). The system exhibits not only sequence specificity but also selectivity between diastereo-isomers (Saghatelian et al. 2001). In the presence of several different templates this peptide system forms autocatalytic, self-correcting networks (Lee et al. 1997). Chmielewski et al. (Yao et al. 1998) have extended this work by constructing another peptide system capable of auto- and cross-catalysis and of generating self-replicating peptides that were not present in the original mixture. Importantly, these peptide systems do not suffer from a "diversity catastrophe", in which the identity of the original replicator is lost in a sea of novelty, as was expected for systems lacking an accurate self-replicating mechanism (Eigen 1971).

Recently, we have used a novel *in vitro* selection technique (Roberts & Szostak 1997) to select ATP-binding proteins from six trillion random polypeptides (Keefe & Szostak 2001). It yielded four new protein families, each containing proteins with highly similar amino acid sequences, that were unrelated to each other or to anything found in the current protein databases. Because they were selected from a random-sequence library, these proteins can be considered as the best currently available models of protocellular proteins.

Proteins from one family have been characterized in fair detail and appear to form folded structures. The originally selected protein contained 80 amino acids but deletion studies revealed that the minimal binding unit is less than 50 amino acids long and, thus, is the smallest known ATP-binding protein. The proteins are highly selective towards ATP, as they bind neither guanosine triphosphate (GTP) nor cyclic AMP. However, their sequences do not contain any known ATP-binding motifs. To function, they require zinc ions and contain conserved cysteine residues. This suggests that their three-dimensional structures may be similar to those of zinc finger proteins**.**

The emergence of catalytic functions. The three-dimensional structures of the selected ATP-binding proteins remain unknown because they are insufficiently soluble in water for high-resolution structure determination. This information, however, is of utmost interest. If the structure resembled that of zinc fingers, which are one of the most common DNA-binding motifs found in eukaryotic transcription factors, it would mean that a potentially protocellular protein could share a folding pattern with seemingly unrelated proteins from higher organisms, rather than with functionally related proteins. This could inspire a search for a small number of "protocellular folding patterns'' shared by different, protobiological proteins. If the newly selected proteins have a novel fold, this fact may indicate that some original folding patterns were lost during subsequent evolution. This would raise doubts about our ability to elucidate the structure of protocellular proteins by analysis of proteins from contemporary cells. An alternative interpretation of such a finding might be that this folding pattern and perhaps many others were never present among proteins that started the evolutionary pathway on the early Earth. However, they could have been encountered along a different evolutionary pathway on a different habitable

planet. Clearly each possibility has important and different implications to the studies on the origin and early evolution of cellular life on Earth and beyond. The work identified in Objective 1 will advance our knowledge in this direction.

Selection of ATP-binders is only the first step on the road to identifying a suite of possible protobiological proteins that could support metabolism of the simplest protocells. Among them, and of special interest, are enzymes capable of catalyzing the formation of peptide bonds in proteins, and of phosphodiester bonds in nucleic acids. In the work proposed in Objective 2, we will focus on these two enzymes because they would have been necessary for self-reproduction of a protein-based system, and for its evolution to contemporary cells based on both proteins and nucleic acids. With a single exception (Severin et al. 1997a), there are currently no protobiologically relevant models for proteins catalyzing these reactions. One approach to generating simple enzymes with desired functions is by rational design and engineering. This approach, however, has met with little success (Corey & Corey 1996). More promising is an approach based on *in vitro* selection (Roberts & Szostak 1997) since it has already been successfully applied to create novel ATP-binders (Keefe & Szostak 2001). In the proposed work, we will extend its capabilities by generating novel catalysts.

The origin of transmembrane channels. Not all protocellular proteins essential for metabolism were enzymes. Some proteins transported ions, nutrients and waste products across cell walls, transduced environmental signals or captured energy to store in high-energy compounds. In many instances, no means of performing these essential functions that would not require proteins are known, or have even been postulated. All of these "non-enzymatic" proteins are integrated into membranes. Although they can be complex, their transmembrane regions are often remarkably simple. The most common structural motif for these regions is a bundle of α-helices (Montal 1995, Bayley 1999). Many simple, α-helical membrane peptides aggregate spontaneously and form functional complexes, exhibiting sequence-dependent specificity (Akerfeldt 1993, Bayley 1999, Bechinger 2000). These characteristics suggest that the earliest transmembrane proteins might have been built of α-helices (Popot & Engelman 2000, Pohorille et al. 2003).

Transmembrane protein domains reveal considerable variability in their amino acid sequence, even among closely related proteins (Wallin & von Heijne 1998). Such heterogeneity indicates that a highly specific sequence may not have been required for a peptide to function in a protocellular membrane. This, in turn, suggests that the likelihood of finding a transmembrane peptide among non-coded proteins may be relatively high. While this would be a desirable property in early evolution it also poses some difficulties. Structural similarity of transmembrane peptides formed from assemblies of α-helices raises questions about the origin of specificity towards different functions or substrates, which was essential for evolution. For example, channels that transported both positive and negative ions, independent of size, would have been of no utility to a protocell. Specificity must have emerged from sequence-modulated, interhelical associations. However, the number of channel-forming peptides that were ion-selective is not known, and the nature of interactions that drive associations of helices is poorly understood. *In vitro* selection experiments, proposed as Objective 3, can contribute to resolving these issues.

Protein evolution without a genome. Our recent findings that truly different, simple peptides (Keefe & Szostak 2001) can perform the same function (such as ATP binding) provide experimental support for a novel mechanism of early protobiological evolution without a genome. The central concept underlying this mechanism is that the reproduction of cellular functions, without replication of macromolecules, was sufficient for self-maintenance of protocells. The precise transfer of information between successive generations of the earliest protocells was unnecessary and, possibly, undesirable. The key requirement in the initial stage of protocellular evolution was an ability to rapidly explore a large number of protein sequences in order to "discover" a set of molecules capable of supporting self-maintenance and growth of protocells. Undoubtedly, the essential protocellular functions were carried out by molecules not nearly as efficient or as specific as contemporary proteins. Many, potentially unrelated sequences could have performed these functions at an evolutionarily acceptable level. As evolution progressed, proteins must have performed their functions with increasing efficiency and specificity. This, in turn, put additional constraints on protein sequences, and the fraction of proteins capable of performing their functions at the required level decreased. At some point, the likelihood of generating a sufficiently efficient set of proteins through non-coded synthesis was so small that any further evolution required coupling between proteins and the informational polymers. The emergence of such coupling must be postulated in any scenario of the origin of life, whether it starts with RNA or proteins.

Several theoretical studies have addressed a possibility of non-genomic evolution (Kauffman 1993; Dyson 1999; Morowitz 1992, 2000; Segrè & Lancet 1999; Bagley & Farmer 1991; New & Pohorille, 2000). Models developed by Dyson (1982, 1999), Kauffman (1986, 1993) and Lancet

(Segrè et al. 2000, 2001) describe possible transitions between initial, poorly organized catalytic networks and evolved, well-connected catalytic systems. The basic idea behind these approaches is that evolution of networks of chemical transformations does not necessarily require self-replication of biopolymers that catalyze these transformations. In the Dyson "mean field" model (Dyson 1982, 1999), molecules enclosed in a bounded environment exist in either catalytically active or inactive states. An autocatalytic phenomenon is assumed, whereby active molecules activate inactive molecules. Inactivation reactions prevent exponential explosion of active species. Kauffman (1986, 1993) demonstrated that a set of random molecules could replicate in its entirety forming an autocatalytic set, providing that there is sufficient diversity of molecular species. Segrè et al. (2000, 2001) further advanced these concepts and methodologies by introducing the graded catalysis models and demonstrating that populations of molecules with better interconnected catalytic networks emerge as a consequence of compositional mutations. A recent paper by Segrè and Lancet (1999) provides a more complete account of the long history of "metabolism-first" concepts.

Our work, defined as Objective 4, builds on these accomplishments by introducing into the model both constructive and destructive processes in a stochastic manner and focusing on global autocatalytic processes and their diversity. However, it also differs from the previous work in several important respects. Instead of assuming random reaction sets we consider only a suite of protobiologically plausible reactions, which most likely was fairly limited (Weber, 2001). Peptides are explicitly considered as protoenzymes and their catalytic efficiencies are assigned on the basis of biochemical principles and experimental estimates obtained from our current (Keefe & Szostak 2001) and future work. Finally, simulations will be carried out using a novel approach that is appropriate even for very low concentrations of reactants (Gibson & Bruck 2000, New & Pohorille 2000).

3.3.3 Technical Approach and Methodology

3.3.3.1 Determining Structure of the ATP-binding Protein

As already mentioned, our initially evolved ATP-binding protein is not sufficiently soluble at high protein concentrations to allow biophysical studies, presumably due to the low thermodynamic stability of the folded state. More stable mutants of this protein have been recently obtained by continued selection for ATP binding in the presence of guanidine (Chaput & Szostak, to be published). Guanidine is a denaturant of protein structure and, therefore, its presence in solution favors selection of proteins in stable folded states. Some of the variant proteins show increased expression as maltose binding protein (MBP)-fusion proteins in *E. coli,* and some of the purified MBP-fusion proteins show increased solubility. When cleaved with thrombin, the purified free ATP binding protein (ABP) from a subset of the selected clones also remains soluble. This is shown in Fig. 3.3-1. As expected, more of these proteins stay soluble in the presence of ATP, presumably because ATP binding stabilizes the folded state of the protein.

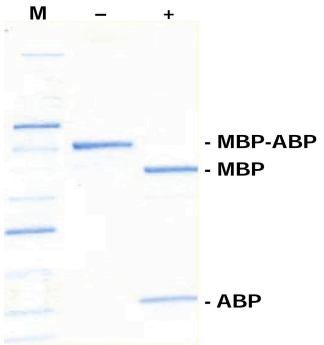


Figure 3.3-1. Purified MBP-ABP fusion protein and corresponding thrombin cleavage products; lower band is ATP binding protein. M, molecular weight markers.

We are currently testing our soluble ATP-binding proteins by size exclusion chromatography (SEC) to establish whether the protein is monomeric or aggregates into multimers. Preliminary results show that at least some of our MBP-fusion proteins exist as monomers. We are currently exploring methods for the preparation and purification of monomeric ATP-binding protein without MBP either by direct expression in *E. coli*, or by thrombin cleavage of purified MBP-ABP fusion protein.

If at least one of our sequence variants remains monomeric at high concentration we will proceed to its physical characterization, initially by recording circular dichroism melting curves in the presence and absence of ATP. These experiments, which can be carried out in samples of ~ 20 µM, will yield evidence for or against a cooperative folding transition and the presence of secondary structure elements (see *e.g.* Oakley and Kim, 1997). If we are able to prepare samples of concentrated protein (~ 0.5 µM, or ~ 5 mg/ml) that remain unaggregated we will proceed to determine the high-resolution, three-dimensional structure of the protein using NMR spectroscopy. This work will be carried out by James Carothers, a graduate student in the Szostak laboratory in collaboration with the laboratory of Gerhard Wagner at Harvard Medical School. The structure will be further

examined in the presence and absence of ATP. We will also compare this structure with other structures available in protein databases. These studies will be carried out collaboratively by the Szostak and Pohorille groups. They will allow us to determine whether the selected protein has a novel folded structure (or, alternatively, which known proteins have related structures) and the mechanism of ATP binding.

If further work is required to increase the solubility of the protein, we will remove regions of the protein that are not essential for its function but might lower its solubility. If these efforts fail to yield a well-behaved sample, we will repeat *in vitro* selection, starting from a mutagenized version of our current best clone. We will use higher concentrations of denaturants to further stabilize the folded state. To improve solubility, we will select against proteins with exposed hydrophobic residues using a hydrophobic chromatographic resin.

As a follow up (and backup) to the above studies, we will take another ATP-binding protein (family C in Keefe & Szostak 2001) through the same path. We already have a version that has been optimized for binding under our standard selection conditions (Cho & Szostak, unpublished), and we are ready to begin selection for a more stable folded state in the presence of denaturants.

From the origin of life standpoint, not only the emergence of functional proteins but also their evolutionary potentials are of interest. We have begun to address this issue by attempting to evolve the ATP-binding protein to a GTP-binding protein. Considering the evolutionary flexibility of biological proteins evidenced by the numerous examples of natural evolution of enzymes that led to alteration of substrate specificity we expected this to be a straightforward task. However, despite several attempts, we were unsuccessful. Without structural information, this negative result is difficult to interpret. Once we can examine the structure of the ATP-binding protein, we might be able to explain the apparent difficulty in accomplishing this seemingly simple evolutionary task. Equipped with this additional knowledge we should be able to carry out evolutionary experiments with greatly improved chances for success. These experiments may begin to answer the question whether some protein frameworks can evolve more easily than others.

3.3.3.2 Selection of Catalytic Proteins

Recently, we have initiated efforts to use the *in vitro* selection technique, which has been successfully used to select ATP-binding proteins, to evolve novel enzymes. However, selection for proteins that catalyze reactions is more complicated than selection for binding proteins because it is difficult to identify successful catalysts once they separate from the reaction product. To deal with this difficulty a number of approaches have been developed for evolving ribozymes. In the approach that appears to be suitable for our purposes, the reverse transcription of the RNA that is attached to the peptide is primed with an oligonucleotide bearing the potential substrate. Then the RNA will hold the substrate in close proximity to the potential catalyst. Subsequent separation of the product of the reaction from the unreacted substrate will reveal the identity of the protein catalyst encoded in the nucleic acid sequence to which it is attached.

Our initial efforts have concentrated on enzymes that catalyze Diels-Alder reactions (a 1,4-addition of a diene and a dienophile to form unsaturated six-membered rings). Since selection of protein enzymes *de novo* has never been accomplished before we have chosen this reaction for the ease and stringency of the selection process rather than for its protobiological significance. A postdoctoral fellow, Burckhard Seelig, who isolated for the first time Diels-Alderase ribozymes composed of unmodified RNA (Seelig & Jaschke 1999) will carry out this work. Furthermore, antibodies that catalyze this condensation reaction have been isolated (Romesberg et al. 1998), suggesting that selections for *de novo* enzymes might also be successful. Specifically, we are selecting for a protein that can accelerate the condensation of an anthracene moiety (the diene, coupled to an oligonucleotide that also serves as a reverse transcriptase (RT) primer) to a biotinylated maleimide (the dienophile, with the biotin serving as a selectable tag). The initial library of random-sequence proteins has been created and selection is in progress. The reaction and selection scheme is illustrated in Fig 3.3-2.

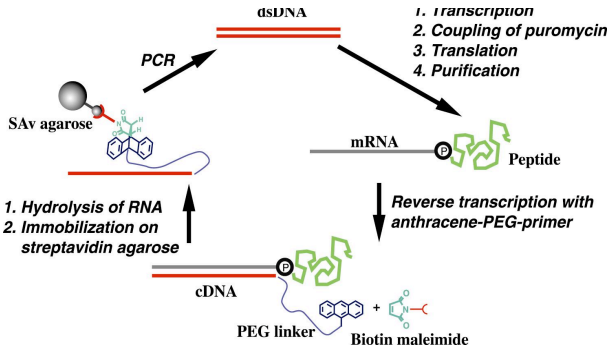


Figure 3.3-2. Selection for a Diels-Alderase Enzyme

We have also developed a plan for an alternative approach, which should allow for searching protein sequence space more effectively, thus increasing the probability of finding enzymes. This plan is based upon our observation, made during the selection of ATP-binders, that most of the functional proteins present in the initial library

have a low probability of folding into the active structure. To counter this effect, we will exploit a new emulsion-based selection technology (Tawfik & Griffiths 1998, 2003), in which DNA templates are compartmentalized into emulsion droplets, followed by transcription and translation, so that several hundred copies of each protein are expressed per template. Even if folding is inefficient, there is a good chance that some molecules will fold properly and exhibit the desired enzyme activity. Furthermore, this approach is intrinsically more suited for enzymatic selections because it allows for multiple catalytic turnover. Even if our current selections yield active Diels-Alderase proteins, we will optimize their activities using the emulsion selection approach.

Once we demonstrate selection of *de novo* enzymes by either of these approaches, we will try to isolate novel enzymes that require more involved selection schemes. We are especially interested in two activities that are relevant to the early evolution of life: a simple RNA polymerase and a simple protein ligase. Previous experience with isolating ribozymes that perform the same functions will be helpful to this end. Ribozymes with limited RNA polymerase activity (Johnston et al. 2001) have been generated by directed evolution of RNA ligase progenitors, which were in turn isolated by *in vitro* selection from large pools of random RNA sequences (Bartel & Szostak 1993). The selection methods required for RNA ligase enrichment are well worked out and are extremely efficient, yielding enrichments approaching 10^6 per round. We plan to use similar procedures in our attempts to isolate protein enzymes capable of accelerating the same chemical steps. If successful we will attempt further directed evolution to generate novel polymerases.

In preparation for this selection, we have already designed and synthesized the substrates required for a ligase selection (see Fig. 3.3-3). Successful ligation of the tag oligonucleotide to the acceptor will allow RT-PCR amplification of the encoding protein ligase sequence. The enriched pool of amplified products will then be used, as usual, as input to the next cycle of selection and amplification.

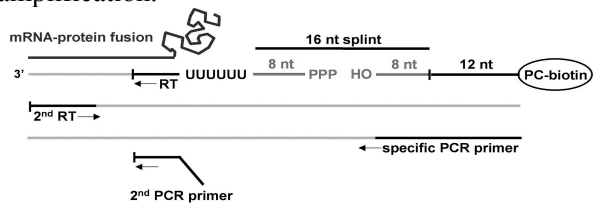


Figure 3.3-3. Selection for an RNA ligase enzyme. The splint and complementary oligonucleotides are composed of RNA; other sequences are DNA. cDNAs that become ligated to the tag oligonucleotide can be enriched both by the biotin and by specific RT-PCR.

Ribozymes capable of catalyzing acyl transferase reactions including peptide synthesis have been isolated (Lohse & Szostak 1996, Zhang & Chech 1997). We propose to isolate novel proteins that accelerate peptide ligation using a scheme analogous to that described above for RNA ligase selection, except that the complementary oligonucleotides will be replaced with peptides similar to those used by Ghadiri et al. (Severin et al. 1997, Lee et al. 1997). The peptide to be ligated onto the cDNA-peptide conjugate will contain affinity tag sequences that allow for selection. In preliminary experiments we will examine various activation chemistries, beginning with the thioester activation used by Ghadiri. However, since this procedure is limited to peptides with a N-terminal cys residue, we plan to identify more general approaches. One possibility is to retain the C-terminal thioester activation, but to select for direct attack by the N-terminal amino group of the reacting peptide, such that the new peptide bond is formed directly, instead of by trans-thioesterification followed by an intramolecular rearrangement. An alternative approach would be to supply a condensing agent, such as a water-soluble carbodiimide, and select for an enzyme that accelerates the resulting peptide ligation.

3.3.3.3 Selection of Transmembrane Ion Channels

To find amphipathic α-helices that self-assemble into channels we will construct libraries of peptides sufficiently long to span the membrane (approximately 23-27 amino acids). We will try both random sequence libraries and a library of sequences in which both hydrophobic and hydrophilic residues are distributed periodically such that they form separate "faces" if a peptide folded to an α-helix. Precise choice of periodicity determines the widths of both faces, which in turn influence how many monomers associate to form the channel. To discriminate against peptides that could act as ionophores (*i.e.* physically carry ions across the membrane) instead of channels, we will include in our sequences a short, terminal segment composed of polar and/or charged amino acids which should "anchor" them to the membrane surface.

Our approach will be similar to the microemulsion selection, described in section 3.3.3.2, but instead of microemulsions we will use vesicles. We will exploit the fact that channel-forming peptides and proteins spontaneously insert into membranes and self-assemble, as discussed in sections 3.3.2. Our first task will be to devise an efficient protocol for the encapsulation of DNA templates into phospholipid vesicles, along with a coupled transcription/translation mix. This will require the efficient and reproducible formation of relatively large vesicles of 0.5 to 2 microns in

diameter to allow expression of multiple copies of single peptides within each vesicle. In parallel, we will begin the validation of a selection method. We will prepare vesicles with or without ionophores, and use fluorescent sensors for ion detection inside the vesicle. Experiments with vesicle populations will confirm changes in ion composition and fluorescent signal output. These data will then be used to devise an effective vesicle sorting procedure by fluorescence activated cell sorting.

If the selection works we will determine the number of monomers forming functional channels. Further, we will examine the stability, specificity and mechanism of action of the selected channels by a combination of systematic mutation studies, model building (Torres *et al*., 2001) and molecular-level computer simulations (Roux 2001; Pohorille et al. 2003). The Szostak and Pohorille groups will collaborate in this effort.

3.3.3.4 Evolution of Populations of Protocellular Proteins

To examine the evolutionary potential of a non-genomic system, we have developed a simple, computationally tractable model, which is capable of capturing the essential features of the real system. In this model, protocellular walls are permeable to small molecules and amino acids but not to oligopeptides of any length. Within the protocells, chemical reactions are catalyzed by peptides, albeit possibly with low efficiency and specificity. Protocells can grow either by acquiring amphiphilic material from the environment or by producing it internally. Once a protocell reaches sufficient size it can divide, distributing its content between the two "offspring" protocells.

In our model, which is stochastic in nature, the specific identities of the amino acids forming peptides are not considered. Instead, the key quantity is the probability distribution of finding a peptide with a given efficiency of catalyzing a desired function, irrespective of its sequence. In this case, efficiency can be thought of as the inverse of the turnover rate. Biochemical considerations dictate that the efficiencies of short peptides increase only slightly with the length of the polymer. Only when peptides reach lengths sufficient for them to adopt an ordered three-dimensional structure do the average efficiencies increase markedly with length. Increasing the lengths of polymers in which catalytic centers have already been formed produces no significant improvement in catalytic properties. In the current formulation, it is assumed that the catalytic efficiencies of peptides of a given length are distributed normally. Other distributions, such as the decaying exponential or Gram-Charlier (distorted normal), can be implemented.

Considering efficiencies of proteins without explicit reference to their sequences is also motivated by practical reasons. According to the canonical view of the structure-function relationship in proteins, the sequence of amino acids determines the three-dimensional structure of a protein, which, in turn, determines its function (Creighton 1992). In principle, there is good correspondence between sequence and function, which suggests an approach where large libraries of sequences are generated on the computer and then each peptide is assigned function and efficiency based on its sequence. However, despite extensive efforts, the nature of the sequence-function relationship in proteins has not yet been unraveled.

Central to our model of protein evolution is the emergence of protoenzymes forming peptide bonds (ligases). A simple ligase has been developed experimentally by Ghadiri et al. (Severin et al. 1997) and we expect to evolve other ligases, as described in section 3.3.3.2. Most of the peptides generated in the model are disordered. Since the ability to adopt ordered structure is required for efficient catalytic activity these peptides are non-functional or only weakly functional. However, a few of the newly synthesized peptides are better ligases than the peptides that generated them. They, in turn, ligate even more peptide bonds and, by doing so, increase the repertoire of peptides in the protocellular system. As a consequence, the likelihood of finding an even better ligase increases. When two peptides are joined to form a new peptide, the catalytic properties of the product are chosen from a probability distribution contingent upon the properties of the peptides from which the new peptide was formed. This formulation captures the biochemical intuition that when two functional peptides are joined, the catalytic center of the product will be "inherited" from one of the parent peptides (although there is also a finite probability of forming a new catalytic center).

Some of the peptides generated by ligases act as proteases and hydrolyze existing peptide bonds. Peptide bonds in disordered and, therefore, non-functional molecules are more likely to be exposed to the aqueous medium than bonds in structured peptides. Since proteases require water for their function this means that they preferentially destroy non-functional peptides. This property is incorporated into our model. As in the case of ligases, the catalytic efficiencies of protein fragments cleaved by proteases are related to the efficiency of their "parent". The conditional probabilities of ligation and hydrolysis are not independent, however. In real proteins, joining two peptides and then cutting the newly formed bond reproduces the original peptides. If the amino acid sequences are not explicitly considered this property cannot be exactly captured. However, by relating the two conditional probability

distributions by Bayes' Theorem, we can preserve this relationship for *the population* of peptides.

Using Monte Carlo (MC) methods, we have already simulated in detail the behavior of a simple system composed of only ligases and proteases (New & Pohorille 2000). That paper also provides mathematical detail of our model. We found that over a fairly wide range of parameters the number, length and overall catalytic efficiency of peptides in the system increases, and eventually reaches a steady state. The increase is determined by the balance between ligating and proteolytic activities and the bias towards the destruction of unstructured peptides. These conclusions were quite robust with respect to other parameters of the model, including the shape of the probability function.

The simple, two-function model is too restricted to describe the emergence of novelty (emergent properties) in the system. To capture these features and to provide a more biochemically faithful description of possible protein evolution, the current model has to be extended in two directions. First, we assume that some of the newly produced peptides can catalyze reactions other than ligation and hydrolysis. Examples of such reactions are pathways and cycles that lead to the utilization of external energy for activating reactants with high-energy groups (*e.g.* thioesters), synthesis of amino acids, membrane-forming amphiphiles and nucleic acids, and metabolism of small molecules. Several such pathways and cycles have been postulated on experimental grounds (see e.g. Weber 1984, 1991, 1998; Morowitz 2000). They may couple constructively to peptide synthesis, allow for protocellular growth and division and provide links to systems that involve both proteins and nucleic acids.

Second, we will add to the model new features that allow longer peptides to increase not only catalytic efficiency but also specificity. Specificity toward different substrates will be determined from the set of descriptors assigned to each peptide. A similar approach is commonly taken in Qualitative Structure-Activity Relationship (QSAR). Of course, in our case the good descriptors and their relation to functions and specificities are not known, so they will have to be assigned somewhat arbitrarily. However, they could be, in principle, established experimentally. Furthermore, we will systematically investigate the robustness of our results with respect to the mapping between descriptors and specificity. During ligation and hydrolysis, values of the descriptors will propagate according to the same probabilistic rules as catalytic efficiency.

These additions to the model require extensions of the methodology. Specifically, we want to simulate time evolution of a system with many species and many reaction channels. In the most common approach, one assumes that there are sufficiently many molecules of each species that their number can be replaced by continuous variables (concentrations) that vary deterministically over time. This leads to a coupled system of differential equations that are solved numerically. This approach, however, is poorly suited to our systems. Its basic assumptions are not well supported. Furthermore, the approach involves reaction rates that are continuous variables and species that can act either as substrates, products or catalysts of reactions. For these reasons we will use, instead, the recently developed Next Reaction Method, an exact and efficient stochastic algorithm to simulate coupled chemical reactions (Gibson & Bruck 2000; Gillespie 1977). For a system in a given state, the method defines a probabilistic algorithm to answer two questions: (1) which reaction occurs next, and (2) when does it occur? The method also allows for incorporation of other cellular processes such as channel-mediated transport and cell growth and division. This approach can be seamlessly connected with our stochastic model of a protein system.

Our simulations will be aimed at determining conditions that are necessary for evolution of a population of proteins in increasingly complicated systems. Examples of issues that we will focus on are:

(1) What are the frequencies of finding functional peptides that allow for evolution of the system and how do they compare with the frequencies estimated experimentally; (2) How does the balance between constructive and destructive processes (including substrate and product inhibition and possible emergence of useless pathways) influence evolutionary potential of the system; (3) Can we observe self-organized pathways and auto-catalytic cycles and what is the degree of complexity of the system in which they emerge; (4) How compartment-alization of proteins in vesicles influence their evolution; (5) How robust are the results with respect to the change of different parameters of the model?

3.3.4 Relevance to NASA OSS Programs

The proposed work will advance our understanding of the physical and chemical principles underlying the origins of life, as outlined in Goal 3 of the Astrobiology Roadmap. Specifically, it directly addresses Objective 3.4 devoted to investigating the origins and early coordination of key cellular processes such as metabolism, energy transduction and translation. We will follow the main approach outlined in the Roadmap – to create and study artificial chemical systems that undergo natural selection in the laboratory, without regard to how life actually emerged on Earth. Our research will be a step

towards the development of a broader discipline, a "Universal Biology", as described in the Roadmap.

3.3.5 General Plan of Work and Key Milestones

Year 1 - Obtain purified, folded, functional ATP-binding protein. Generate and test the code for computer modeling of populations of peptides.

Year 2 - Characterize first newly evolved enzyme (Diels-Alderase), and begin selection for an RNA ligase enzyme.

Year 3 - Complete high-resolution structure of ATP binding protein and compare with biological protein structures. Use directed evolution to obtain optimized versions of newly selected enzymes. Complete modeling of self-organized pathways and auto-catalytic cycles without considering vesicles in the model.

Year 4 - Evolve novel protein ligase enzymes and channel forming peptides.

Year 5 - Complete structural and biochemical studies of newly evolved RNA and protein ligases, and channel forming peptides. Complete modeling of evolution of early peptides in protocellular environments.

3.3.6 Management Structure and Statement of Contribution

Lead CoI, Dr. **Andrew Pohorille** (NASA-Ames) will be responsible for theoretical work, as outlined in objective 4 and sections 3.3.3.4 and 3.3.3.1. He will share his expertise in ion channels, as needed for accomplishing Objective 3. He will also carry out management and organizational duties associated with this project.

CoI, Dr. **Jack Szostak** (Harvard Medical School) will be responsible for experimental work, as outlined in objectives 1-3 and in sections 3.3.3.1, 3.3.3.2 and 3.3.3.3.

Collaborator, Dr. **Michael Wilson** will participate in designing and analyzing computer simulations outlined in sections 3.3.3.1, 3.3.3.3 and 3.3.3.4.

3.4 Investigation 4 Biosignatures in Chemosynthetic and Photosynthetic Systems

3.4.1 Objectives and Significance of Research

The past or present occurrence of life on an extraterrestrial planet does not ensure our ability to detect it. Recognition of life or its processes requires biosignatures that are clearly discernible against a landscape of abiotic processes, which may vary widely depending on the host environment. *The overarching goal of the work proposed here is to provide astrobiological search strategies and missions with an enhanced understanding of factors that control biosignature formation in terrestrial ecosystems.* Our approach recognizes that astrobiology search targets fall predominantly into two categories: those where detailed observation and/or physical sampling is (or could be) feasible, and those where assessment of biogenicity must be made exclusively via telescopic observation.

The first category (detailed observation and sampling) includes only bodies in our solar system. Presently, Earth is the only planet on which liquid water is stable against the atmosphere, and may therefore be the only one capable of supporting a surface biosphere. The possibility of an extant biosphere on other solar system bodies is thus limited to the subsurface, where water is stable but light is unavailable as an energy source. Metabolism in such environments must limited to non-photosynthetic anaerobic processes than can be supported by the local geochemistry. In this effort, we will explore the biological and biosignature potential of possible subsurface environments on the planets via studies of similar environments on Earth.

The subsurface environments we will study are basaltic and ultramafic (olivine-rich) rocks that contain liquid water. These rock types are (and were) abundant on planetary bodies: the crusts of differentiated bodies (Earth, Mars, Venus, 4 Vesta) contain basaltic and ultramafic rock, and most undifferentiated bodies (chondritic asteroids) are composed entirely of ultramafic rock. Liquid water reacts spontaneously with rocks of this type to generate H_2 (which could fuel microbial metabolism) and the aqueous alteration mineral serpentine (which could preserve biosignatures in a resilient lithified form). Our studies will focus on a series of northern California springs that percolate through ophiolite host rocks (sections of basaltic/ultramafic ocean crust that have been obducted onto land). These springs offer perhaps the best available terrestrial analog for the early and modern Martian crust. Our preliminary studies show that these springs support a microbial community, and likely possess an aqueous chemistry capable of providing energy to non-photosynthetic microbes. *The first major task of this investigation is to examine biosignature formation in this model system, with a primary emphasis on the mineralized component.*

The primary objectives of this task are to:

(i) Determine whether the microbial life in ophiolite-hosted alkaline springs leaves a residual mineral biosignature. We emphasize the mineralized component of this process because, in the astrobiological exploration of Mars, everything

older than a few tens of millions of years (~99% of Mars history) will either be a rock or will only be interpretable in the context of the rocks that contain it.

(ii) Assess the energetic requirements of these biological systems in order to establish boundary conditions on their potential distribution in a planetary subsurface, as a guide to sampling strategies on future life-detection missions.

For bodies beyond our solar system, any assessment of inhabitance must be made telescopically, via a basic analysis of atmospheric chemistry. It is generally believed that photosynthetic biospheres offer the best possibility for telescopic detection because: (a) by harnessing starlight as an energy source, a photosynthetic biosphere can attain a level of productivity orders of magnitude greater than that possible in a non-photosynthetic one; (b) photosynthetic biospheres can drive planetary surface chemistry dramatically away from thermodynamic equilibrium (whereas non-photosynthetic life catalyzes planetary chemistry towards equilibrium), and this disequilibrium condition may itself represent a potential biosignature. While photosynthetic biology is the engine that drives the production of chemical biosignatures, the composition and magnitude of biosignature flux ultimately depend on the specific nature of interactions between photosynthetic and non-photosynthetic elements of the biosphere. These interactions govern, for example, whether photosynthetic productivity is partitioned into volatile versus non-volatile forms, or into compounds that are diagnostically biogenic versus those that are merely ambiguous. As such, they directly impact the detectability of putative extrasolar biospheres. *The second major task of this investigation focuses on examining the nature of interactions between photosynthetic and non-photosynthetic microorganisms in tightly and loosely coupled associations, in order to understand the mechanisms by which photosynthetic productivity is transformed into detectable biosignatures.*

The primary objectives of this task are to:

(i) Determine the ultimate fate of photosynthetic carbon and electrons within several model oxygenic and anoxygenic photosynthetic microbial ecosystems, with particular emphasis on potentially diagnostic and detectable biosignatures;

(ii) Determine the chain of organisms, and organism-organism interactions that are involved in transforming photosynthetic productivity into biosignatures;

(iii) Understand how the community-level mechanisms of biosignature production are influenced by changes in the physico-chemical environment, with particular emphasis on parameters that may vary substantially during planet-biosphere co-evolution.

The anticipated significance of the proposed work lies chiefly in its potential to support the design and interpretation of astrobiology missions with respect to biosignature detection, and in its relevance to understanding the co-evolution of Earth and its biosphere. As such, this module squarely addresses Astrobiology Roadmap Goals 4 and 7. The aqueous mineral alteration studies in Task One will provide a context for interpretation of Mars rover, sample return, and Mars meteorite data. Development and quantification of the energetic habitability concept will establish stringent boundary conditions that may greatly narrow the search parameters for subsurface life on Mars. Collectively, these studies will aid in the exploration of past or presently habitable zones within our solar system (Goal 2). Task Two will directly support the design and interpretation of Terrestrial Planet Finder and related missions by identifying potential chemical search targets and examining the controls on their flux to the atmosphere. In that these targets may also be radiatively- or redox-active, these studies will also address the contribution of biology to long-term chemical and climatic evolution on Earth's surface (Goal 4). The specific emphasis on elucidating and understanding microbe-microbe interactions will help to provide a foundation for exploring Earth's uncharacterized microbial diversity, much of which may be inextricably bound in such associations.

3.4.2 Research Tasks

3.4.2.1 Task 1: Biosignatures in Chemosynthetic Systems

Build On and Extend the State of Knowledge

Earth is presently the only body in the solar system with a habitable surface environment, as defined by the stability of liquid water. Any extant life elsewhere in the solar system must therefore be capable of survival in subsurface niches, where light or other exogenous sources of energy are unavailable. Metabolically, such communities are limited to low energy anaerobic chemical processes sustained by local mineral geochemistry. This limitation on possible biospheres applies to Mars during most of its history and to Earth for some periods of its history (e.g., prior to the advent of photosynthesis, during surface-sterilizing impacts and extreme climate fluctuations). The search for evidence of life in our solar system therefore hinges on our ability to (a) discriminate the biosignatures of the subsurface environment, and (b) understand and quantify the factors that constrain the habitability of these settings.

Despite the importance of chemosynthetic ecosystems, very little is understood about either the microbial ecology of such environments or

their potential to generate detectable biosignatures. There is a lack of demonstrably analogous systems, because most accessible environments on Earth are dominated by photosynthesis or its products. Among several environments proposed as possible examples of chemosynthetic ecosystems (Baross & Deming 1995; Stevens & McKinley 1995; Chapelle et al. 2002), energy is generally hypothesized to derive from the interaction of reducing rocks (e.g., unweathered basalts or ultramafics) with water. However, detailed characterization of these systems is limited by their inaccessibility.

We propose to examine ophiolite-hosted alkaline springs as model systems in which to identify possible mechanisms of biosignature formation, and to quantify the energetic constraints on chemosynthetic communities. The continental borderland of California contains several ophiolite units that are ~150 My old. Springs emanating from these units have a unique geochemistry that has not been studied in the context of microbial ecology or biosignature formation. A predominant type of rock found in ophiolites is peridotite, which is composed almost entirely of the mineral olivine [$(Mg,Fe)SiO_4$]. Olivine is unstable at conditions near the Earth's surface, and reacts with water to form the mineral serpentine along with accessory minerals such as magnetite and brucite: Olivine + H_2O → Serpentine + Magnetite + Brucite + H_2. The "serpentinization" process yields H_2, and fluids emanating from ophiolite terranes are often highly enriched in H_2 as a result (Neal & Stanger 1984). This carries significant implications in the context of mineral-hosted life, because H_2 can be used as metabolic fuel by CO_2-reducing methanogens ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) and a host of other anaerobic microorganisms. Indeed, fluids emanating from serpentinizing terranes often contain high methane concentrations. The strong kinetic inhibition of abiotic methanogenesis during serpentinization (McCollom & Seewald 2001), suggests that much of the methane is biologically-produced. Recently, much interest has been generated by the discovery of large submarine ultramafic bodies in the process of serpentinization (Kelley et al. 2001a; Kelley et al. 2001b). Remarkably, however, the much more easily accessed surface equivalents of these systems (i.e., ophiolite-hosted alkaline springs) are largely unexplored and undocumented with respect to the life that may inhabit them.

The specific mineralogy and aqueous geochemistry of ophiolite-hosted alkaline springs suggests that they represent a particularly compelling analog for potential life-bearing systems on early or modern Mars, and on the pre-photosynthetic Earth. On the early Earth, oceanic-type crust was the principal surface rock type. However, little of this early mafic/ultramafic crust remains in a form that has not been highly altered by the continual resurfacing of the Earth via tectonic processes. Although the Jurassic-Cretaceous age of most ophiolite terranes in the western U.S. post-dates the pre-photosynthetic era of Earth's biosphere, one could argue that these highly accessible pockets of exposed oceanic crust are much more representative of early terrestrial mineralogy than are the slivers of highly metamorphosed rock that survive from that period. The potential importance of rock-hosted subsurface habitats as refugia from surface-sterilizing impacts, high UV fluxes, or extreme climate fluctuations underscores the necessity for understanding their potential to harbor life, even in a solely terrestrial frame of reference.

Most evidence thus far available suggests that ophiolite terranes may also represent the best available terrestrial analog for the early and modern crustal geology of Mars (Longhi et al. 1992; Singer & McSween 1993). The Martian meteorites so far identified are either basalts or more ultramafic rocks (Fisk & Giovannoni 1999) which closely resemble the rocks present in ophiolite sequences. Mars apparently experienced little (if any) plate tectonic activity and little compositional differentiation of its primitive lithosphere. Aqueous activity and presumably aqueous alteration would thus in all likelihood involve the stabilization of mafic and ultramafic rocks, as occurs presently in terrestrial ophiolites. This hypothesis is supported by evidence from Martian meteorites, where the predominant style of aqueous alteration within the Nakhlites (the "N" of SNC Martian meteorites) is that of olivine to phyllosilicates (Treiman & Goodrich 2002; Treiman & Lindstrom 1997; Treiman et al. 1993), in complete analogy to the serpentinization of ophiolites. Due to the similarities of the minerals and processes involved, we anticipate that studies of terrestrial ophiolite-hosted alkaline cold springs may provide significant insight into the nature of aqueous geochemistry on Mars. This is particularly important given the discovery of possibly recent aqueous seeps which suggests that even present conditions might allow for a rock-hosted chemosynthetic biosphere in near-surface regions of the Martian crust. Similarly, areas of the Martian crust that exhibit a history of aqueous alteration (e.g., the "hematite feature") are among the chief targets under consideration as possible MER landing sites. For these reasons, we believe that studies of terrestrial ophiolite-hosted alkaline springs are particularly timely.

Missions designed to search for evidence of life on bodies within our own solar system will benefit materially from input in two key areas: What to look for as evidence of present or past life

(biosignatures) and where to look (habitability). We address these issues with two hypotheses, which are formulated with an emphasis on Mars mission architecture and rock-hosted ecosystems:

Hypothesis 1: The formation of secondary mineral phases during aqueous alteration of ultramafic minerals preserves biosignature information in a highly stable lithified matrix.

Hypothesis 2: The habitability of subsurface environments is constrained by the ability of the mineral matrix to deliver chemical energy (e.g., as H_2) at or above minimum rates and levels required by mechanisms of microbial energy metabolism.

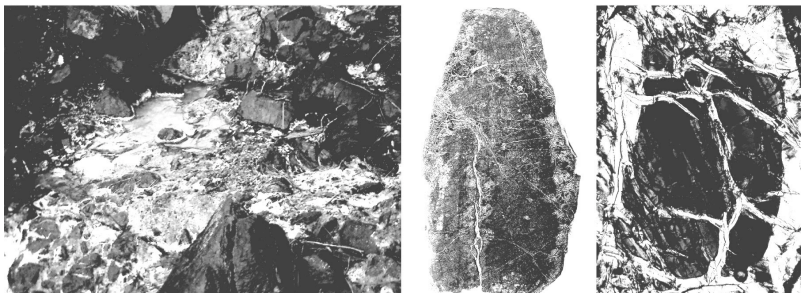


Figure 3.4-1. (a) Complexion Spring, field of view is about 2 meters; (b) hand specimen of ophiolite rock from Complexion spring showing serpentinized edges, 10 cm in width; (c) Optical micrograph of serpentinized ophiolite (crossed polars), showing single crystal of olivine complexly bisected by serpentine alteration, field of view 500 microns.

Technical Approach and Methodology

We propose to address these hypotheses with a combination of field, laboratory, and theoretical work focused on ophiolite-hosted alkaline springs. These springs will provide an excellent means for linking the work of our two hypotheses, and for tying our efforts to Mars missions, for several reasons:

(1) Ophiolite-hosted alkaline springs may be the best available terrestrial analog to the mineralogy and aqueous geochemistry of the Martian crust;

(2) Ophiolite-hosted alkaline springs combine a potential mechanism for preservation of microbial biosignatures (deposition of secondary mineral phases during aqueous alteration) with the presence of active microbial communities (which likely include metabolic types that could be supported by Mars-type aqueous geochemistry);

(3) Comparison of the aqueous geochemistry and energetics of ophiolite-hosted alkaline springs with the structure and activity of the microbial populations they support will provide real-world constraints on the magnitude of geochemical energy transduction that is required to maintain microbial energy metabolism.

Preliminary scouting trips to several locales within the Coast Range Ophiolites (northern California) indicate that Complexion Springs (Peters 1993) embodies each of these advantages, and thus represents an ideal setting in which to examine our hypotheses. Rocks collected at the site are composed primarily of the ultramafic mineral olivine, which has been extensively serpentinized along numerous fracture faces (indicating active deposition of secondary mineral phases; see Figure 3.4-1). Water emanating from this spring is enriched in hydrogen, a potential energy source for chemotrophic metabolism. A preliminary analysis of the 16S-rRNA contents of the spring water by our group indicates the presence of a variety of archaea and bacteria. Analysis of this rock-water system will allow us to simultaneously examine the influence of microbial life on the deposition of aqueous alteration minerals and correlate the community structure with the distribution of chemical potential energy within the spring. The high likelihood that Complexion Springs harbors a community of chemotrophic microorganisms (the only form of life possible in the Martian subsurface) benefits our study in several respects; however, it is noteworthy that neither aspect of the proposed research requires that the community consists exclusively of chemotrophs.

1. Serpentinization as a mechanism of biosignature preservation (addresses Hypothesis 1). We assume that robotic missions to Mars will analyze or bring back rocks and soil, not water or biological samples. We will characterize the mineral and morphological biosignatures that exist within and around ophiolite–hosted alkaline springs. The near certainty that life elsewhere in our solar system is or was exclusively microbial strongly suggests that biosignature detection efforts should be focused on developing highly discriminatory visualization and mineralogical methodologies for use at a microscopic scale. The controversy surrounding the reported presence of "life-like structures" and "mineral biomarkers" in Mars meteorite ALH84001, combined with the demonstration that similar structures and mineralogies can be found in demonstrably abiotic samples, underscores this need. The approach we propose will deploy an extensive array of chemical, mineralogical and morphological techniques (including optical petrography, electron microprobe (EMPA), field emission Scanning Electron Microscopy (FESEM), Analytical Transmission Electron Microscopy (AEM, TEM/STEM), confocal laser Raman microscopy, Atomic Force Microscopy (AFM), Scanning Tunneling Microscopy (STM), micro X-ray Diffraction/X-ray Fluorescence (μXRD/XRF) and others to a Mars-analog system where life is

known to be present, and where the deposition of secondary mineral phases is active. Detection of diagnostic biosignatures through one or more techniques would serve to suggest which particular technologies or methodologies would be most useful for flight or sample-return missions. On the other hand, equivocal or negative results with regard to biosignature detection would provide an independent and objective assessment of putative biosignatures prior to the return of data or samples from extraterrestrial sources.

Microscale characterization of rock samples from ophiolite-hosted alkaline springs. As ultramafic rocks react with water to release hydrogen, secondary sialic mineral phases (e.g., serpentines) are deposited on the active alteration front. Because these phases are precipitated along the surface, where chemotrophic microbes would most likely position themselves, the potential exists for the creation of persistent biosignatures by "entombing" microbial forms into a highly recalcitrant lithic matrix. Alternatively, some microbes actively catalyze the dissolution of substrate minerals in order to increase the energy flux they receive, which can result in the creation of definitively biogenic microscale features within the rock matrix. We will collect rocks from Complexion Springs "fixed" on-site with glutaraldehyde. Bulk mounts, fracture surfaces, thin sections and the like will be prepared. A combination of light microscopy (with DNA staining), mineralogical and micro-morphological techniques will be used to identify regions of (a) previous but not current aqueous alteration, (b) active alteration without the presence of microbes, and (c) aqueous alteration in the presence of microbes. Fracture surfaces will be prepared for FESEM using cold ion-beam deposited Ir to eliminate coating artifacts and careful analyses will be made of the exposed surfaces. Similar studies will be carried out at all scales of resolution using complementary techniques. Polished thin sections will be used for optical microscopy (with fluorescent staining) as well as petrology and Electron Microbprobe analysis. We hope to identify unique morphologies associated with the presence of microbes, as well as to characterize mineral reactants and products associated with the abiotic / microbial serpentinization processes.

Evolution of aqueous alteration fronts. Utilizing rocks collected from Complexion Springs and abiotic equivalents, we will create fresh fracture surfaces and track the evolution of secondary mineral phases in time-course aqueous alteration experiments. Bulk samples will be subjected to aqueous alteration in sealed glass vessels under a variety of conditions (see below); samples will be removed at designated intervals and analyzed through the suite of microscale characterization techniques described above. Preliminary experiments will be conducted to establish an appropriate timescale for subsampling in these time experiments. In even the most gradual weathering processes, we should be able to identify incipient alterations using high resolution imaging techniques. Initially, these time course studies will focus on the alteration process in samples that have been rendered abiotic through a combination of physical sectioning, washing, or dry autoclaving. These studies will establish a baseline for the spectrum of possible morphologies or alteration styles that can generated even in the absence of life. Subsequent experiments will examine samples that are selected specifically for the presence of active microbial populations. These experiments will be conducted under a variety of physical and chemical conditions (temperature, pH, salinity, silica concentration) that (a) represent a range of possible aqueous geochemistries that might be encountered in terrestrial or Martian settings; and (b) will likely affect rates and styles of mineral alteration.

2. Mineral-water interactions as an energy source for microbes (addresses Hypothesis 2). Habitability of a given environment is generally considered to stem from the presence of liquid water (in an appropriate temperature range) and energy that can be used in metabolism. By these criteria, much of the terrestrial and Martian subsurface might be considered habitable. However, it is less frequently considered that, in order to be useful to life, energy must be available at a certain minimum level, and must be delivered at a certain minimum rate. In surface environments, the presence of abundant energy in the form of sunlight or the products of photosynthetic metabolism means that biological energy constraints are seldom tested. Without access to sunlight or O_2, however, the metabolic processes available to subsurface microbes are frequently characterized by inherently low energy yields. For these organisms, relatively minor fluctuations in substrate concentration and flux, or in physical conditions (e.g., temperature), can render an environment energetically uninhabitable. Quantifying these microbial energy requirements, and assessing the potential of various mineral matrices to meet them, would establish further (and far more stringent) boundary conditions on the habitability of the subsurface realm. Our approach will focus on these goals, with specific emphasis on the chemistries and metabolisms that characterize ophiolite springs.

Biological Energy Quantum (BEQ) Requirement. All known mechanisms of biological energy conservation require that free energy must be available at a minimum "quantum" level, below which metabolism is not possible. The energy available for an organism to use in meeting this requirement depends directly on the concentrations

of metabolic products and reactants within the cell (Schink 1997). In some energy-limited ecosystems, the natural ordering of microorganisms into specific geometries relative to substrate sources apparently leads to the near equalization of intra- and extracellular product/reactant concentrations in cells operating at the energetic limits imposed by the BEQ (Hoehler et al. 2001a). In such systems, the magnitude of the BEQ can therefore be determined through measurement of product and reactant concentrations in the bulk extracellular fluid. We will mimic these natural conditions in a series of well-defined culture experiments that will allow us to quantify the BEQ for organisms possessing metabolisms that could be supported in subsurface environments. Our initial (and chief) focus will be on H_2-consuming methanogens, since the presumed ubiquity of both CO_2 and H_2 in the subsurface suggests this process should be considered among the leading candidate metabolisms. Briefly, the culture apparatus will supply H_2 to an active population of microbes by diffusion through a semi-permeable membrane. Colonization of the membrane by microbes (in order to gain the most direct access to substrate H_2) will continue until the flux of H_2 is reduced to levels that are minimally sufficient to meet their energy requirements. Measurement of the residual H_2 partial pressure, along with partial pressures of CO_2 and CH_4 (through periodic or continuous-monitoring GC-based techniques), will allow us to calculate the BEQ for the particular organisms in culture. These experiments will be carried out with varying temperatures, differing strains of methanogens, and ultimately, different types of (possible subsurface) metabolisms, in order to simulate a spectrum of possible subsurface environmental conditions. Where possible, these experiments will utilize microbial strains that are isolated from, or closely related to organisms present in, Complexion Spring (as determined by 16S-rRNA phylogenies).

Maintenance Energy Requirement. A second constraint on microbial metabolism is imposed by the need to obtain energy at a rate that is minimally sufficient to maintain cellular integrity (to "fix" what periodically "goes wrong" in the cell). As with the BEQ, a simple culture apparatus that mimics the natural environment will be used to quantify the maintenance energy requirement. Briefly, this apparatus will consist of small tube filled with culture medium, in which the delivery of substrates occurs solely by diffusion through opposite ends of the apparatus (e.g., H_2 through one end, CO_2 through the other). Knowing the diffusion coefficient of the medium and the flux rates of products and reactants in and out of the apparatus, the energy flux into any zone of the tube can be accurately calculated. In initial experiments, we will allow populations to grow up to a level that is at steady-state with respect to the available energy. Subsequent determination of the total biomass within the system will provide bulk estimates of the maintenance energy requirement, as a function of biomass supported. Once the methodology is well established, we will use physical sectioning or chemical microsensors to quantify energy availability through small regions within the culture, and correlate these with biomass abundance in the same region. Experiments will be conducted over a range of temperatures in order to test a hypothesized temperature dependence of the maintenance energy requirement.

Mineral H_2 Yields. A putative subsurface biosphere would depend extensively on the capacity of mineral-water interactions to deliver energy-bearing substrates (e.g., H_2) at concentrations and rates sufficient to meet the above-determined energy requirements. Minerals with high Fe^{2+} contents (e.g., ultramafic rocks) should be capable of evolving equilibrium H_2 partial pressures that far exceed the BEQ requirement. However, their capacity to support fluxes consistent with the maintenance energy requirement remains uncertain. To help quantify this capacity for mineralogies relevant to Mars exploration, we will examine rates of H_2 evolution during serpentinization of rocks from Complexion Spring. These experiments will entail creating fresh fracture surfaces on rocks collected from the springs, determining the resultant surface area of unreacted mineral, and reacting the sample with water in a sealed gas vessel. H_2 evolution over time will be observed for a variety of temperatures and fluid compositions. We will also track the alteration of the reactive surface by SEM, to determine how rapidly the capacity for H_2 evolution is diminished through deposition of sialic alteration minerals. By normalizing time-dependent H_2 evolution rates to mineral surface area, we will be able to establish the capacity of ultramafic minerals to sustain microbial metabolism under a range of conditions, as determined by their ability to meet the above-determined BEQ and maintenance energy requirements.

Complexion Springs. We will synthesize the results of our BEQ, maintenance energy, and mineral experiments to evolve a predictive capacity with respect to the potential distribution of chemotrophic organisms in rock-hosted environments. This predictive model will be correlated with and tested against the naturally-occurring distribution of chemotrophic energy sources and organisms in Complexion Springs.

We will measure the suite of physical and chemical parameters necessary for calculating the potential energy yields available to several forms of chemotrophic metabolism (e.g., H_2-based methanogenesis and sulfate reduction), using established analytical techniques that can largely be employed on-site. The overall community composition of organisms in the spring water and host rocks will be determined by constructing 16S-rRNA phylogenies through established methods. The results will be screened for the presence or absence of organisms that would appear to be allowed or disallowed on the basis of the experimentally-determined energy requirements, as a partial validation of our predictive model. For chemotrophic metabolisms whose presence is supported by both chemical (energetic) and phylogenetic analyses, we will subsequently seek to confirm the presence of actively metabolizing populations. First, we will determine bulk rates of metabolism in aqueous or mineral samples by applying previously-described radiotracer, stable isotope, or natural-abundance methodologies. Second, utilizing 16S-rRNA sequences from organisms representing the metabolisms of interest, we will design fluorescently-labeled, species-specific nucleic acid probes that will facilitate microscopic visualization of the microscale distribution of organisms in the spring system (through fluorescent in situ hybridization (FISH)). Our chief focus in this regard will be on the aqueous alteration fronts present on mineral surfaces.

3.4.2.2 Task 2: Biosignatures in Photosynthetic Systems

Build On and Extend the State of Knowledge

An astrobiological search for life in star systems other than our own offers the remarkable opportunity to observe potentially dozens of habitable worlds, where any life almost certainly emerged and developed independently from that on Earth. While such a survey could help to address some of the most fundamental questions concerning the frequency or rarity of life on habitable worlds, it will come at the cost of extreme limitations in the data sets that will form the sole basis for determination of habitation. Telescopic observation in mid-IR or visible to near-IR wavelengths can provide basic information about the size, mass, effective temperature, and atmospheric chemistry of extrasolar planets, but the collection of more detailed information falls beyond our current capacities (Des Marais et al. 2002). Characterization of atmospheric chemistry offers a potential indicator of habitation (Beichman et al. 1999), because all known life alters the chemistry of its host environment in the course of energy harvesting and biomass building. For this reason, any search for extrasolar life will depend critically on obtaining a clear understanding of the uniquely biogenic impacts of life processes on planetary chemistry. Specific examples might include gaseous compounds that are only produced by life, or levels of chemical disequilibrium (in reactive pairs such as O_2 + CH_4) that require continuous biological input to sustain.

Because the impact of biology on atmospheric chemistry must always be considered within the context of concurrent abiotic processes, planets harboring photosynthetic biospheres offer a much greater potential for telescopic detection than those with solely non-photosynthetic life (Caroff & Des Marais 2000). Photosynthetic life captures an extra-planetary energy source (starlight), and is thereby capable of greatly increased productivity relative to its non-photosynthetic counterpart (by approximately three orders of magnitude on Earth, for example (Des Marais 1997)). The result is a greatly enhanced biological "signal" relative to the "noise" of abiotic planetary chemistry. Additionally, light-aided biocatalysis can impart a degree of thermodynamic disequilibrium to planetary chemistry that might be considered uniquely biogenic, whereas non-photosynthetic life tends instead to catalyze planetary chemistry towards an equilibrium that would ultimately prevail even in the absence of life (resulting in a signal that is ambiguous with respect to biogenic character).

The capacity of a photosynthetic biosphere to contribute a flux of uniquely biogenic chemistry to the atmosphere of its host planet is constrained by: (i) the magnitude of global productivity by photosynthetic microorganisms, (ii) the specific nature of chemical or microbial processes that transform productivity into gaseous biosignatures, (iii) the sensitivity of these transformation processes to variations in physico-chemical environment. Much work has focused on understanding the global scale controls on past and present photosynthetic productivity. However, little is known about the subsequent microbial transformation of this productivity within tightly-coupled microbial ecosystems, and even less is understood about the potential response of these processes to global geochemical change, such as that occurring on the evolving Earth. Our work will seek to elucidate in detail the mechanisms involved in transforming photosynthetic productivity into potential biosignature compounds, and to understand in more basic terms the influence of a changing environment on those mechanisms.

This investigation will focus primarily on the anaerobic microbial community that is supported by photosynthesis, because these organisms are the most likely source of a distinctly biogenic flux to the atmosphere. Photosynthesizers themselves may produce the volatile and spectroscopically

detectable end-product O_2, but the potential for abiotic photochemical O_2 production makes this signal ambiguous without the contemporaneous presence of reduced volatiles, such as those produced by anaerobic microbial communities (Kasting 1997). Similarly, the primary volatile end-product of aerobic metabolism is CO_2, which yields no evidence of a biogenic origin. Anaerobic metabolism, by contrast, yields a wide array of volatile and spectroscopically-detectable products that, by themselves or in combination with other compounds, may constitute a signal diagnostic of life (Visscher 1996; Visscher et al. 2003). Indeed, any signs of life on Earth during approximately the first billion years of its habitation would have arisen exclusively from anaerobic activity. Our work will examine the liberation of reduced volatiles along with O_2 in order to address both the production of species that are uniquely biogenic in themselves, and also the creation of a "disequilibrium signature" in O_2 / reduced gas pairs.

The transformation of complex organic molecules (such as those produced by photosynthesis) in the absence of O_2 requires the coordinated activity of multiple groups of organisms (Schink 1988), often in close physical association, and the transfer through free solution of a variety of chemical intermediates (Dolfing 1988). Studies of purely anoxic systems, such as organic sediments, indicate that almost every stage of this multi-step process is strongly influenced by micro- and macro-scale variations in population structure and physico-chemical environment, such that the ultimate products of the transformation process (the prospective biosignature compounds) are subject to substantial variability (Boone & Bryant 1980; Wolin & Miller 1982; Lee & Zinder 1988; Hoehler et al. 1994). The same must be true of anaerobic transformation processes within microbial mats, the primary source of biological productivity (and therefore, of biosignature production) for nearly half the history of Earth's biosphere. Yet, the close physical association of photosynthetic and anaerobic organisms in these mats may profoundly affect the composition and flux of fixed carbon between the two groups, and dramatically alter the microchemical environment relative to organic sediments (Cohen et al. 1984; Stal & Caumette 1994). Although the nature of interaction between groups of organisms in microbial mats has been, historically speaking, perhaps the most significant influence on the biological component of Earth's atmospheric chemistry, studies in this area have just begun.

The latter stages of our previous NAI-funded research began to focus on the transformation of photosynthetic productivity, with several significant early findings. The photosynthetic members of microbial mats were shown to deliver a large flux of highly labile organic compounds and hydrogen to the anaerobic community, forming a potential chemical basis for close interactions between these populations (Albert et al. 2000; Hoehler et al. 2001b; Hoehler et al. 2002). Mat communities expressed a distinct interdependence of the microbial carbon and sulfur cycles, which represents a likely source of diagnostically-biogenic organosulfur compounds (Thamdrup et al. 2003; Visscher et al. 2003). Mats also produced appreciable quantities of methane under conditions where traditional microbial ecology principles would predict none (Hoehler et al. 2001b; Bebout et al. 2003). These processes, and the ultimate array of chemical products, exhibited marked dependence on macroenvironmental factors such as light intensity, temperature, and redox state (presence or absence of oxygen, sulfate). The proposed investigation will build on and elaborate each of these findings to create a detailed mechanistic picture of biosignature production in photosynthetic microbial communities. Quantitative mechanistic information, particularly with respect to environmental controls, will foster an ability to expand outward in the application of our findings: initially, to understanding the changing biological input to Earth's atmosphere as the planet's chemistry and solar radiation budget varied over geologic time; subsequently, to providing input for predictive, ecosystem-level models of gaseous efflux (see Investigation 5); and ultimately, to providing a highly generalized observational framework to aid in the design and interpretation of telescopic searches for life on extrasolar worlds.

Technical Approach and Methodology

Photosynthetic processes on Earth extract electrons from inorganic reductants (e.g., H_2O, H_2S, H_2, Fe^{2+}) and use them to "fix" inorganic carbon (CO_2) into organic compounds. Our overall approach is to track the fate of these electrons and fixed carbon through each stage of the transformation into reduced volatiles (e.g., organosulfur species, methane, methylhalides) or non-volatile end-products. We will characterize the major chemical pools and organisms or organism associations involved in each step of this process in order to identify specific "critical points", where the partitioning of carbon and electrons within the system are influenced by micro- and macro-environmental factors. Our work will be guided by four main hypotheses concerning the nature of these critical points:

Hypothesis 1: The production of hydrogen and highly labile organics by phototrophic members of the community creates a functional link to the anaerobic members that directly affects both the

composition and quantity of biosignature compounds that are ultimately produced.

Hypothesis 2: The flux of photosynthetic electrons into the microbial sulfur cycle strongly controls the composition of gaseous efflux from the system; rising levels of sulfate (such as characterized much of the Archaean Era) shift the flow of electrons from reduced carbon compounds towards more oxidized and often sulfur-bearing compounds.

Hypothesis 3: Conditions of low oxygen (such as prevailed during the first half of Earth history) enhance the flow of photosynthetic productivity to anaerobic microorganisms, and thereby increase the potential efflux of biosignature gases.

Hypothesis 4: The partitioning of photosynthetic productivity into the spectrum of possible end-products is directly dependent on specific physical associations between photosynthetic and non-photosynthetic organisms, and within the population of non-photosynthetic organisms.

Hypotheses will be addressed experimentally by three coordinated efforts:

1. Create a quantitative budget for the fate of photosynthetic fixed carbon and electrons, with specific emphasis on the ultimate partitioning into terminal "biosignature compounds" (addresses Hypotheses 1 and 2). A combination of chemical concentration and flux measurements, radio- and stable-isotope tracer experiments, and natural abundance stable isotope measurements will be employed to quantify the partitioning of photosynthetic resources into various end-products. We anticipate that the major steps involved in the transformation of photosynthetic productivity can be grouped broadly into four coupled processes (see Figure 3.4-2).

Productivity. Rates of carbon and electron fixation by photosynthetic microorganisms will be determined in order to (i) quantify the pool of starting materials available for the ultimate production of reduced volatiles, (ii) provide a simultaneous measure of O_2 production (for use in quantifying the creation of "disequilibrium signatures"), and (iii) provide a normalization factor that will facilitate the scaling of biogas flux measurements up to planetary scales, using published estimates of past and present global productivity. The total influx of reducing equivalents attributable to photosynthesis will be determined at several times of day via oxygen microelectrode measurements employing the dark-shift method (Revsbech et al. 1981). Our previous NAI-supported work experimentally quantified the sensitivity of these rates to light intensity and temperature, allowing us to estimate daily integrated rates of gross photosynthesis by interpolation between measured time points. The total amount of carbon fixed by photosynthetic organisms will be determined by measuring the integrated flux of CO_2 into the system by means of a flux chamber methodology (Canfield & Des Marais 1993; Des Marais 1995). Finally, the daytime accumulation of fixed carbon as storage polysaccharides (SPS) and osmoregulants will also be quantified; we hypothesize that this reservoir serves as the primary source of carbon and electrons to the associated community of anaerobes, via fermentation into highly labile forms.

Fermentation. Our previous NAI work showed that much of the carbon and electrons fixed by photosynthetic organisms during daylight hours is subsequently released at night in the form of organic acids and hydrogen – strongly suggesting that fermentation of fixed-carbon storage products is the primary pathway for mobilization of photosynthetic productivity into the chain of transformation (Albert et al. 2000; Hoehler et al. 2001b). Because these simple fermentation products can be directly utilized by many anaerobic microorganisms, their production may provide a functional link between the phototrophic and non-phototrophic populations in the system (Hoehler et al. 2001b). Fermentation rates will be

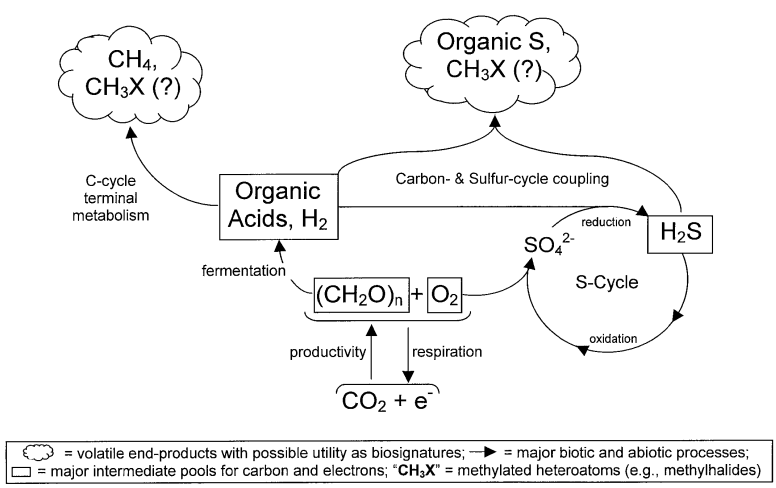


Figure 3.4-2. Major steps in the anaerobic transformation of photosynthetic productivity. Our approach will quantify the flow of carbon and electrons fixed by primary productivity through each of the major processes and reservoirs identified in this diagram. Molecular biology and organic biomarker approaches will be used to characterize the organisms that mediate the transformation process. At major "junction points", where carbon or electrons may have alternate fates (e.g., H_2/Organic Acid or H_2S pools), we will characterize the micro- and macro-environmental factors that regulate their ultimate partitioning into the spectrum of biomarker end-products.

determined by quantifying the time-dependent decrease in SPS content, and the increase in concentrations and fluxes of H_2 and C_1-C_5 organic acids. Measurements will be made under natural conditions and following the inhibition of potential consumption pathways (e.g., oxic respiration, sulfate reduction, or methanogenesis) in order to estimate total gross rates of fermentation.

Electron Transfer to Sulfur Cycle. Due to high oceanic concentrations of sulfate on the modern Earth, and the metabolic accessibility of fermentation end-products to sulfate-reducing bacteria, the sulfur cycle represents the dominant sink for photosynthetic carbon and electrons in marine or hypersaline microbial mats (Jørgensen & Cohen 1977; Canfield & Des Marais,1993). The coupling of carbon and sulfur cycles in phototropic systems carries several important implications with respect to production of biosignatures: (i) electrons that enter the sulfur cycle and are further transformed through oxidative inorganic sulfur metabolism will be rendered largely non-volatile, and therefore insignificant for atmospheric chemistry (this represents a decrease in the maximum possible contribution of photosynthesis to remotely detectable biosignatures); (ii) some of the volatile organic sulfur compounds that result from coupling of the biological sulfur and carbon cycles may be difficult to form abiotically, and might therefore provide a more robust biosignature than volatile carbon compounds (Visscher et al. 2003); (iii) through their own high levels of productivity, organisms associated with an active sulfur cycle can "recycle" energy and electrons back into the pool of storage polysaccharides; (iv) abiotic "sulfurisation" reactions involving sulfide and unsaturated organics can serve to couple carbon and sulfur isotopic information into lasting organic biomarkers, of potential utility in understanding Earth's early history; (v) oceanic sulfate concentrations are believed to have increased more than 100-fold since the Archaean, so that the coupling of the carbon and sulfur cycles (and the importance of (i) and (ii)) may have varied substantially as the Earth system evolved (Habicht & Canfield 1996; Canfield et al. 2000). The primary link between the carbon and sulfur cycles, is the biological reduction of sulfate to sulfide using fermentative electron donors. Rates of sulfate reduction will be quantified by measuring the accumulation of radio-labeled $H_2{}^{35}S$ from $^{35}SO_4^{2-}$ (Jørgensen 1978), and by advection-diffusion-reaction modeling of microelectrode-derived H_2S profiles. Subsequent production of organic sulfur compounds from H_2S will be quantified by GC and HPLC techniques (Visscher et al. 2003), and compared to experimentally-determined abiotic reaction rates for these compounds. Incorporation of H_2S into the inorganic sulfur cycle will be quantified by measuring changes in the pools of elemental sulfur, thiosulfate, and sulfate.

Terminal Metabolism. In the final stages of photosynthate transformation, carbon and/or electrons are converted into volatile compounds that can escape the system. These compounds represent the ultimate influence of the photosynthetic system on atmospheric chemistry, and the "fingerprint" by which any determination of biogenicity must be made. We anticipate that methane, organic sulfur compounds, and methylhalides will constitute the major volatile end-products of the transformation of photosynthetic productivity. Release of these compounds from the system will be measured using a flux chamber methodology, and possibly via open-system techniques (bubble flux measurements, open-path laser array system). This methodology will permit easy quantification of shifts in carbon/electron partitioning that result from imposed environmental manipulations.

Overall. The coupled effects of each of these processes will be assessed in isotope-tracing experiments that follow the fate of labeled carbon through the system as a whole. Radio- or stable isotope-labeled CO_2 will be introduced into the system during peak rates of photosynthetic carbon fixation. The techniques described above will be used to quantify the partitioning of labeled material into specific chemical pools at discrete time intervals following its introduction. These experiments will present a time-dependent, whole-system view of photosynthate transformation, and will also aid in identifying the specific groups of organisms involved in the process (see following section).

2. Determine the key organisms and organism-organism interactions that mediate the transformation of photosynthetic productivity (addresses Hypothesis 4).

Molecular methodologies and organic biomarker analyses will be combined with isotope labeling techniques to ascribe phylogenetic identity to the major groups of organisms involved in the transformation process. The application of a culture-independent approach is critical for the systems in this study, because >99% of mat associated microorganisms are phenotypically uncharacterized and have yet to be grown in culture (Amann et al. 1995). In the isotope tracing experiments described above, we will follow the incorporation of labeled compounds into lipid biomarkers (elucidating the involvement of specific functional groups) and into DNA/RNA (elucidating phylogenetic identity). By introducing the label in different chemical forms (representing different stages of the chemical transformation

process), the organisms associated with specific steps or chemical transfers will be individually targeted. Population sizes of key groups of organisms will be assessed by monitoring natural abundances of lipid biomarkers that are labeled in the isotope experiments and ascribed a phylogenetic origin through DNA-based identification methods. These findings will be linked to the rock record of recalcitrant organic biomarkers, which currently provide the best evidence of specific microbial functionality in Earth's deep past.

Using ribosomal RNA phylogenies constructed through our previous and proposed NAI work, and the genetic identities ascribed in the above experiments, we will design group- and species-specific nucleic acid probes for key organisms in the transformation process. Applying these fluorescently-labeled probes in FISH assays will facilitate microscopic visualization of organisms in three dimensions (by laser scanning confocal microscopy), allowing us to infer the specific assemblages or physical juxtapositions of organisms that are involved in chemical transfer (DeLong et al. 1989; Upton et al. 2000). Furthermore combination of FISH with secondary ion mass spectrometry (SIMS) will enable fine scale determination of the assimilation and transfer of specific stable isotope-labeled substrates, confirming which organismal associations are involved with specific chemical transfer processes (Orphan et al. 2001; Orphan et al. 2002). The structure and function of these associations can then be followed through the course of ecophysiological manipulation studies, as described in the following section.

3. Identify the physico-chemical factors that influence the partitioning of photosynthetic resources into different biosignature groups (addresses Hypotheses 2 and 3).

The multi-step nature of photosynthate transformation exposes multiple critical points, where the flow of carbon and electrons may be channeled into differing sets of products or pathways, depending on external forcing factors. Abiotic processes (e.g., in parts of the organic and inorganic sulfur cycle) depend on relative pool sizes among products and reactants, and on the wealth of physico-chemical parameters affecting the thermodynamics and kinetics of these reactions. Biological processes share these chemical sensitivities, and are also influenced by variations in gene expression, population size, and activities of partner organisms (parameters that are, themselves, controlled by physico-chemical variability). Collectively, these effects have a dramatic influence on pathways of organic transformation in purely anoxic systems, and we anticipate that the same will be true of anaerobic processing in phototrophic systems.

Using the results of our coupled biogeochemistry and molecular biology studies, we will identify the major critical points (in terms of organisms or chemical intermediates) in the transformation process. Subsequently, we will isolate these critical points through the use of simplified model systems, enrichment cultures, or FISH-probing, and determine the effects of variable environmental conditions. Our primary goal will be to determine the effects of variable oxygen and sulfate concentrations, since these likely represent the primary species through which Earth's evolving redox state has been "sensed" by biological communities over geologic time. We will examine the influence of variable sulfate concentrations on the balance of oxidizing and reducing metabolism with respect to carbon compounds (e.g., CO_2 versus CH_4 production), and on the degree of coupling between the carbon and sulfur cycles. The sensitivity of sulfur isotope fractionation in various pools to the concentration of sulfate will be determined concurrently as a way to link our studies to the Archaean sulfur isotope record. We will examine the effect of variable atmospheric oxygen levels on the flow of productivity to the anaerobic community (e.g., decreasing the productivity flux due to increased consumption by aerobic respiration or limiting the spatial distribution of anaerobes through toxicity effects). We will also determine the influence of variable O_2 levels on the partitioning of H_2S electrons into oxidative metabolism (chiefly non-volatile products, but potential "recycling" into SPS pool) versus formation of volatile organic species (potential utility as biomarkers). Each of these variables will be studied with respect to chemical cycling and organismal function (at the metabolic, gene expression, and population levels), with results providing a basis for inclusion of environmental forcing parameters in the numerical simulations of Investigation 5.

Field Sites. Our model systems for this work will be the hypersaline microbial mats of Baja California, (which are well-characterized with respect to general chemical function and microbial composition through our previous NAI work) and the thermophilic microbial mats of Yellowstone National Park (YNP). The majority of this work will be performed on samples returned from Baja and maintained in the Ames greenhouse facility, which permits substantial environmental manipulations at the community level. In addition to the primary focus on cyanobacteria-dominated mats, we will undertake parallel studies of photosynthate transformation in mats dominated by eukaryotic photosynthesis, and by anoxygenic photosynthesis. The eukaryotic system (mats of the red alga *Cyanidium* in YNP) will extend our studies to include acidic conditions (pH 0.5-3.5)

and variability in cell structure (eukaryotic versus prokaryotic). The anoxygenic system will embrace a critical period in the co-evolution of Earth and its biosphere – a time with potentially high rates of global productivity, but with insignificant levels of environmental free oxygen. These studies will be based on mats of *Chromatium* and/or *Chloroflexus* found in YNP, and on greenhouse-maintained mats in which oxygenic photosynthesis has been chemically inhibited.

3.4.3 Relevance of Proposed Work

The principal importance of our work in an astrobiology context lies in its intent to provide basic science content relevant to the design and interpretation of search-for-life missions within and outside our solar system. The focus on identifying useful biosignatures in two widely disparate biosphere types directly addresses Roadmap Goal 7. Because our studies are based on terrestrial ecosystems, they are also inherently relevant to understanding the co-evolution of Earth and its biosphere (Roadmap Goal 4). For example, microbial mats represented the principal biologic agents of global geochemical change throughout most of the Precambrian Era. Our studies of the factors that control and channel photosynthetic productivity within these systems will aid in understanding their role in the oxidation of the planet's surface, or in the liberation of climatically-important trace gases. Similarly, our studies of the factors that constrain life in the subsurface realm offer a possible insight into the nature of Earth's earliest biosphere, and of the viability of deep refugia from events that might render the surface uninhabitable. This work also addresses Goal 1 in that it may help to revise our concept of habitability to include stringent, energetic constraints on metabolism.

3.4.4 General Plan of Work and Key Milestones

Year 1 - Basic characterization of ophiolite spring geochemistry and microbial population structure; initial isotope tracing experiments to quantify carbon and electron flow in microbial mats; feed results to the numerical model of Investigation 5 (continued in years 2-5).

Year 2 - Conduct electron microscopy characterizations and experiments on ophiolite samples and begin bioenergetics experiments; lipid biomarker studies in microbial mats and ecophysiology study using mat and sediment communities.

Year 3 - Continue electron microscopy and bioenergetics experiments related to ophiolite springs; long-term manipulation studies of oxygen and sulfate availability in microbial mats.

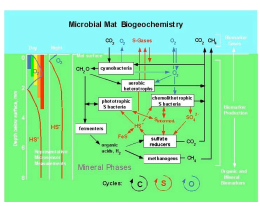
Years 4-5 - Deploy genome specific probes and perform microscopic characterization of organismal associations in both environmental settings; metabolic process measurements in ophiolite springs; continued manipulation and ecophysiology studies in microbial mats, culminating with large-scale group experiments for validating mechanistic models.

3.4.5 Management Structure and Statements of Contribution

Management: **Tori Hoehler** (Ames, Lead CoI) is responsible for overall coordination of the investigation and manages Task 2. **David Blake** (Ames, CoI) manages Task 1.

Science: **Daniel Albert** (U. North Carolina, CoI), a biogeochemist and expert in anaerobic fermentation processes; will characterize the processes related to cycling of organic acids in photosynthetic mats. **David Blake** (Ames, CoI), a leading mineralogist/geologist who has developed mineralogy instrumentation for possible deployment in Mars missions, will be responsible for electron microscopy and petrologic analysis of ophiolite samples. **Donald Canfield** (Odense University, collaborator), a leading biogeochemist who has pioneered the use of sulfur stable isotopes in studying the redox evolution of Archaean Earth, will link studies of sulfur transformations and sulfur isotope chemistry in the photosynthetic mats to the Archaean sulfur isotope record. **Richard Castenholz** (U. Oregon, collaborator), a microbiologist and leading authority on the cyanobacteria, will be responsible for the studies of acidophilic eukaryotic systems of microbial mats; **David Des Marais** (Ames, CoI) a biogeochemist and authority on stable isotope geochemistry, will be responsible for flux experiments and measurements of photosynthetic productivity. **Tori Hoehler** (Ames, Lead CoI), a biogeochemist and leader in applying thermodynamic principles to the ecology of microbial ecosystems, will be responsible for the bioenergetics studies and for measurements of H_2 cycling and terminal metabolism in springs and microbial mats. **Tom McCollom** (U. Colorado, collaborator), a geochemist and expert in the thermodynamics of aqueous geochemistry, will employ computation models to characterize the ophiolite springs with respect to free energy potential. **Linda Jahnke** (Ames, CoI), a microbiologist and expert in lipid biomarker analysis, will be responsible for the studies of lipid biomarkers in the microbial mats. **Victoria Orphan** (Ames, collaborator), a molecular biologist who has pioneered the coupling of microscale biological and geochemical analyses for use in microbial ecology, will be responsible for molecular biology and microscopy in the ophiolite springs and microbial mats. **Mitchell Schulte** (Ames, CoI), a geochemist and leader in modeling the energetics of aqueous geochemical processes, will lead the ophiolite field campaign, and will characterize the aqueous

geochemistry and energetics of these springs. **Roger Summons** (MIT, collaborator), a leader in the field of lipid biomarkers who has established the earliest evidence of oxygenic photosynthesis, will correlate studies of lipid biomarkers in photosynthetic mats with analyses from the Archaean rock record of organic fossils. **Bo Thamdrup** (Odense University, collaborator), a microbial ecologist and expert in microbial sulfur cycling, will characterize the oxidative sulfur cycle, sulfur isotopes, and ecophysiology of photosynthetic mat communities. **Allan Treiman** (LPI, CoI), an authority on Mars meteorites and aqueous alteration processes, will participate in ophiolite-related field work, and characterize samples with respect to petrology and primary and secondary mineralogy. **Pieter Visscher** (U. Conn., CoI), a microbiologist and expert in the ecology of microbial mats, will characterize the photosynthetic mat communities with respect to organic sulfur cycling, methylhalide fluxes, and ecophysiology.



3.5 Investigation 5 Modeling Ecosystems and Biospheres

3.5.1 Objectives and Significance of Research

The creation of a mathematical simulation model of photosynthetic microbial mats is an important stepping stone in our understanding of key biogeochemical cycles that may have altered the atmospheres of early Earth and of other terrestrial planets. A modeling investigation is presented here as a tool to utilize and integrate empirical results from research in Investigation 4 Photosynthetic Systems (of this proposal) into a computational system that can be used to simulate biospheric inputs of trace gases to the atmosphere.

Photosynthetic microbial mats are prokaryotic assemblages that grow from the topmost photic layers of submerged or partially submerged sediment. These microbial communities were abundant in shallow seas surrounding continents during the Precambrian, primarily during the Proterozoic era from about 2.2 billion years onward. (Brock & Madigan 1991; Castenholz 1994). Microbial mats are significant to the field of Astrobiology because of their paleontological importance and because their trace gas emissions to Earth's atmosphere, when modeled, can serve as remote indicators of life (a.k.a., biosignatures, sensu Hoehler et al. 2001b) elsewhere in the solar system. The evolution of cyanobacteria, (the major bacterial guild of these mat ecosystems) with their ability to use water as a source of reducing power, probably caused Earth's biosphere to increase productivity by 2-3 orders of magnitude (Des Marais 1997). Despite their inherently high productivity and dominant role in evolution of the biosphere, many of these cyanobacterial mats are found in environments that today could be considered extreme or stressed, at least with respect to salinity, acidity, temperature, radiation flux, and potential for desiccation.

Microbial mats residing in benthic marine and hypersaline environments may be living analogs to biological communities of early Earth. Thus, they are useful in understanding biotic-atmospheric interactions of early Earth and in identifying biosignatures, particularly for O_2, CO_2, H_2S, and CH_4. Notwithstanding recent advances in understanding how hypersaline microbial mats function (Bebout & Garcia-Pichel 1995; Des Marais 1995; Hoehler et al. 2001b), many uncertainties remain concerning the importance of physical controls, especially the light limitations, energy fluxes, and the chemical environment. These uncertainties have not yet been addressed fully on an ecosystem scale. In past years, numerous field and laboratory experiments have contributed to the body of knowledge on these communities (e.g., Castenholz 1994; Nübel et al. 2001; Overmann & van Gemerden 2000; Weiland et al. 2001). Such experiments are, by nature, limited in their applicability to the study of microbial interactions over a variety of time scales (from hours to years to millennia), and in their capacity to explain complex biogeochemical interactions between (aerobic) primary producers and (anaerobic) consumer and fermenter bacterial groups. Simulation modeling can be used with new experimental results derived by Investigation 4 to explain complex biochemical interactions of photosynthetic microbial ecosystems at progressively larger temporal and spatial scales. Generally, computer modeling is a highly cost-effective means to create a "virtual laboratory", in which we can expand and test understanding of Astrobiology concepts. Models allow us to explore scenarios and assess implications of experimental findings in an efficient manner that can compliment more expensive field-based measurement activities.

The overall objective of this research investigation is to refine and evaluate simulation models of energy relations, biogeochemical cycling, trace gas exchange, and biodiversity in microbial mat ecosystems with the goals of extrapolating biosignatures of early Earth ecosystems to the scale of a planetary biosphere. We will extend the predicted results from our

models over billions of years of ecological change due to environmental forcing conditions.

The significance of the proposed modeling work is to efficiently and comprehensively evaluate the limits of microbial evolution and ecosystem functions in ways that will support NASA missions aimed at explaining biosignatures on distant planets.

Specific Study Objectives:

- Predict (using simulation modeling) and validate (using experimental measurements) biochemical cycles, and biosignature emissions of O_2, CH_4, and reduced S gases from the water surface to the atmosphere above photosynthetic microbial mats.
- Investigate the spectral properties and light use efficiency of microbial mat ecosystems using satellite image analysis and in-situ spectroradiometery.
- Extend the generalized model of photosynthetic microbial mats in hypersaline subtidal environments to simulate other prominent microbial ecosystem types (with their peculiar physical and geochemical properties) that may have influenced the atmosphere of early Earth.
- Incorporate results from studies of diversity in microbial mats into the generalized model of microbial ecosystems and biospheres.
- Discern variation in biosignature emissions due to spatial and seasonal environmental fluctuations of a biosphere.

3.5.2 Research Tasks

Build On and Extend State of Knowledge

We have developed a simulation model called MBGC (Microbial BioGeoChemistry) to infer effects of major environmental controllers on microbial community structure and function (Decker & Potter 2001). Microbial growth, metabolic reaction rates, and mass flows are represented within and between vertical sediment layers of a microbial mat in the hypersaline environment (Figure 3.5-1). In addition, diel cycles are simulated to capture natural variation in environmental boundary conditions, such as light and temperature.

The fundamental structure of our MBGC simulation model follows that of a previous model (de Wit et al. 1995) of population dynamics and biogeochemistry within benthic mats containing cyanobacteria (CYN), purple sulfur bacteria (PSB) and colorless sulfur bacteria (CSB). Specific additions were incorporated recently into our MBGC model in order to (1) represent CYN photosynthesis metabolism operating as a light-driven quantum efficiency function, (2) complete the microbial sulfur cycle, and (3) enable a comparison of the model predictions to hourly field-measured biogeochemical fluxes. More specifically, we have added sulfate-reducing bacteria (SRB) to MBGC, which can consume organic carbon and produce the H_2S oxidized by PSB and CSB. In addition, molecular hydrogen (H_2), decomposition of dead organic matter (DOM), CO_2 fluxes, and realistic (time-varying) temperature and light controls on major metabolic pathways are included in MBGC as extensions of the original de Wit model structure.

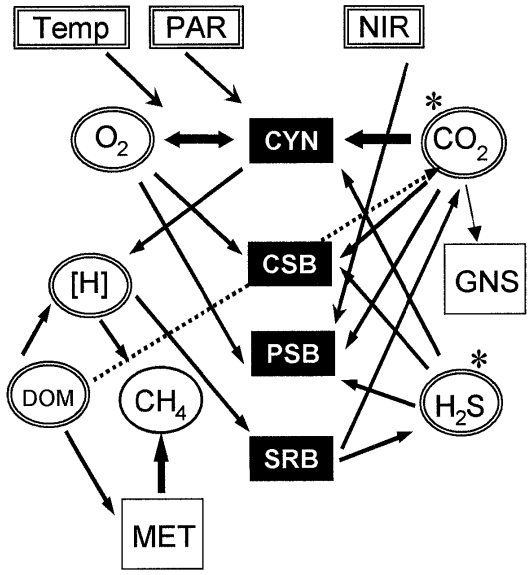


*Figure 3.5-1. Schematic representation of the MBGC model (Decker and Potter, 2002), including bacterial pools (squares), light (PAR, NIR) and temperature input as model drivers, and gas concentration pools (circles). Each vertical layer of the model is comprised of all model pools shown and adjacent layers are coupled by gas fluxes between layers. Fluxes between pools are shown as arrows. Existing components of MBGC are shown as double outline symbols, whereas new components that we are proposing to add are shown as single outline symbols. Key to new component symbols: MET are methanogenic bacteria, GNS are green non-sulfur bacteria, * are isotope tracers.*

MBGC was constructed with several noteworthy initial assumptions. Major bacterial groups compete for, or are inhibited in growth by, metabolic substrates such as O_2 and H_2S. Photosynthetically active radiation (PAR between 400-700 nm) limits the growth of the CYN population, while near infra-red (NIR) radiation limits the growth of PSB population. Mass flows control gas exchange rates between sediment layers. Surface water flow affects O_2 diffusion rates from the mat to the water through the diffusive boundary layer (DBL). Finally, concurrent aerobic chemosynthesis and anoxygenic photosynthesis by PSB is possible, but its occurrence depends on the concentration of bacteriochlorophyll-a (Bchla).

Early results from MBGC simulations show that the model reproduces major diel fluctuations in trace gases in hypersaline subtidal mat layers and into the overlying water column (Figure 3.5-2). To extend our knowledge further of the principal controls over the light/energy relations and biochemical pathways by which these mat ecosystems consume and emit trace gas biosignatures, we are testing various approaches to simulating the net hourly emissions of O_2, CO_2, and H_2S gases to the atmosphere above the mat ecosystem over the course of several days during which actual measurements of these same fluxes have been made.

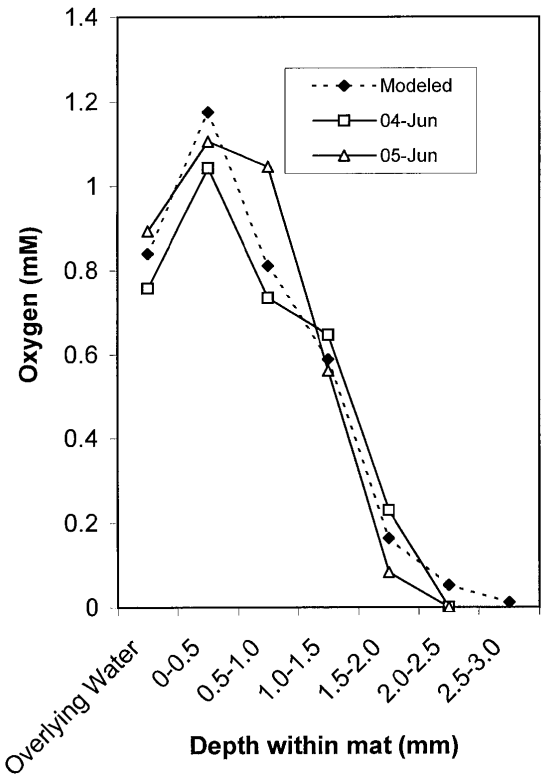


Figure 3.5-2. Oxygen concentration within the top layers of a cyanobacterial mat at mid-day for two dates in June 2001 in salt ponds near Guerrero Negro, Baja (Source: S. R. Miller), compared to results of a MBGC simulation using light and temperature values measured during the same period of the field measurements.

At Baja California salt ponds near Guerrero Negro, Mexico where such diurnal fluxes have been measured by other NAI investigations, we have recently collected near-simultaneous *in situ* spectral data and satellite images of radiation absorption and reflectance of subtidal microbial mat communities. After correction for the absorption of incident solar radiation by the overlying water column and the atmosphere, we can attempt to isolate the absorption spectrum of the mat community to infer spatial variability of PAR and NIR attenuation by the various bacteria groups (Figure 3.5-3).

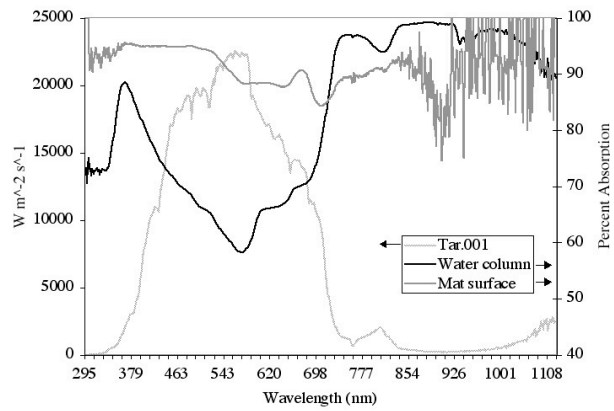


Figure 3.5-3. Spectral results from absorption and reflectance studies of subtidal microbial mat communities in September 2002. The water column was found to absorb 60-75% of incident PAR energy and more than 95% of incident NIR energy entering at the pond surface. The subtidal mat absorbs 85-95% of the residual PAR and NIR energy that is transmitted through the water column from the pond surface. TAR is the solar irradiance input at the water column surface in units of W m^{-2} s^{-1}

In order to further build on and extend state of knowledge of biosignatures from these early Earth ecosystem analogs, we propose to evaluate the following hypotheses within our modeling framework. Our primary means of testing each of these hypotheses will be to compare measured fluxes and pools to new predictions from the MBGC model that is modified in various alternative versions.

- Light use and photosynthetic rates in MBGC mat simulations are strongly influenced by prescribed properties of the overlying water layer and that of its biological constituents.
- The fate of atmospheric carbon fixed in the MBGC mat can be predicted by incorporating fluxes and stocks of fixed C (organic acids) and electrons (via organic acids and H_2) into the existing MBGC simulation model.
- Sulfate reduction in the photic (oxygenated) zone of the photosynthetic MBGC mat can be maintained by simulated pools of SRB that are capable of metabolism in oxic zones of the model ecosystem.
- Methane production rates in the photosynthetic MBGC mat depend upon the presence of simulated pools for both competitive (with SRBs) substrates (e.g., H_2, organic acids) and non-competitive substrates (e.g., osmoregulant such as methylamines) interactions that are sensitive to a variety of environmental conditions.

Technical Approach and Methodology

Ecosystem model development

We will continue to expand and refine the MBGC simulation model of microbial mat ecosystems using all available computer programming tools at NASA Ames. Our technical approach will involve synthesis of measurement data and observations from all published sources and from experiments carried out by our co-investigators on this proposal. This will result in a combined effort to develop new metabolic response algorithms and computation approaches with which to quantify trace gas biosignatures as characteristic exchanges of energy and minerals between the simulated ecosystem and the atmosphere. The modeled gas fluxes will be validated at short time scales (hours to days) and then run over a series of evolutionary scenarios that will represent millions of years of change in planetary climate and geochemical conditions. The MBGC structure will allow us to simulate changes in microbial communities in response to scenarios of changing redox conditions (ambient O_2 levels and geochemical H_2S sources) and predict the outcomes of competition among several major bacterial groups for survival and productivity.

Our overall plan of work is to evaluate MBGC modeling approaches that can accurately simulate the carbon and energy cycles in microbial mats, from the point at which CO_2 is fixed into products of photosynthesis, through decomposition and fermentation of organic acids, and finally to the biogenic emissions of reduced gas forms (biosignatures) such as CH_4 and H_2S. Our MBGC model simulations will be designed to aid our co-investigators in Investigation 4 in understanding the potential fate of carbon that is fixed during daytime photosynthesis and transformed during subsequent dark periods as non-photosynthetic bacteria utilizes this carbon for growth.

It will be necessary to first add several variables to the MBGC model to simulate the metabolism and population growth dynamics of methanogenic bacteria (MET) and green non-sulfur bacteria (GNS). We will also add pools and fluxes to assess the electron budget. We will work closely with co-investigators in Investigation 4 to develop the necessary understanding of metabolic processes of these new MET and GNS pools in the model. We anticipate, for example, the inclusion of both competitive and non-competitive substrate interactions by which growth dynamics of MET populations can be simulated and evaluated.

Secondly, we will add mathematical tracer components to the MBGC model as another computational approach to understanding the potential pathways of decomposition of biogenic organic matter and its eventual emission back to the atmosphere in reduced gas forms. Tracers such as stable carbon isotopes and ^{14}C and $^{35}SO_4^{2-}$ radioactive radioactive markers will be added to the MBGC model in an attempt to replicate the inferred (from measurements) pathways, beginning from initial fixation during CYN photosynthesis, to biogenic emissions of carbon biosignatures such as CO_2, H_2S and CH_4.

Once our MBGC simulations of subtidal hypersaline microbial mats are completed, we will generalize the model to simulate other prominent microbial communities and geochemical environments. Chief among these other microbial communities for MBGC simulations will be inter-tidal marine mats, which experience higher levels of oxygen circulation (by wave action), and green non-sulfur mats, like those that grow commonly in thermophilic environments ranging from 35°C to 72°C. These green sulfur mats may exist either anaerobically via photosynthesis or aerobically by fermentation and use sulfide or H_2 as electron donor for CO_2 fixation.

Satellite Image Analysis

To obtain simultaneous field measurements and satellite images of radiation absorption and reflectance in microbial mat communities, we will first determine optimal days and times to collect spectroradiometry data at selected locations where ongoing NAI investigations of microbial mat biogeochemistry and biosignatures are being conducted. Local meteorological conditions, seasonal sun angles and satellite overpass schedules will be evaluated. These combined data sets will provide the required information to select representative sampling periods for field sampling trips. NASA Earth Observing System (EOS) satellite images will be obtained to coincide as closely as possible to all selected close-range spectral sampling periods.

We will deploy optimal field-based methods for configuration of portable spectroradiometer and GPS equipment used for microbial mat reflectance sampling through the presence of a natural water surface or column. Calibration of close-range spectroradiometer data under variable conditions of light and wind will be evaluated. Field data sets will be returned to NASA Ames for entry into a geographic information system (GIS) and comparison with newly acquired EOS satellite imagery from the same periods of time. We will develop optimal methods for remote sensing calibration and classification of microbial mat communities. Collection of close-range spectroradiometry data, precise GPS coordinates, physical measurements of the surface water, and local meteorology on the same dates as EOS satellite image acquisition will enable us to make substantial corrections (e.g., for atmospheric interference and sun angle effects) that normally

confound calibration and classification procedures for satellite imagery. We will work to produce a new GIS of diversity in microbial mat pigments and surface physical properties, which together regulate microbial gas exchange with the atmosphere and other biogeochemical processes. This GIS will be used to drive the MBGC simulation model of microbial mat ecology and biogeochemistry at multiple spatial scales.

Biosphere model development

We will transfer subsets of trace gas biosignature algorithms and process-level metabolic information from individual ecosystem simulation runs using the MBGC model to extrapolate emission fluxes of O_2, CO_2, H_2S (and other reduced forms of S), and CH_4 to the scale of an entire planetary biosphere. Our technical approach will involve the development of relatively simple zonal biosphere models that can represent geographic (e.g., latitude zonal) and seasonal variability of these emission fluxes, beginning from about 2.5 Gyr ago to the past several million years. This is same approach that was used by Potter et al. (1996, 1998) to construct modern Earth budgets of seasonal trace gas bio-emissions.

We will evaluate our zonal biosphere models that represent geographic and seasonal variability of O_2, CO_2, H_2S, and CH_4 emission fluxes, beginning from about 2.5 Gyr ago to the past several million years. Global microbial sources of these historical gas emissions from shallow aquatic environments will be computed and compared to modern global emission sources. Digital versions of these historical gas emission predictions will be shared with other NAI teams who are interested in coupling biosphere to atmosphere evolution.

Relevance of Proposed Work

The development and refinement of our MBGC model addresses the following Astrobiology Roadmap Goals: (4) to understand how past life on Earth interacted with its changing planetary and Solar System environment, (5) to understand the evolutionary mechanisms and environmental limits of life (specifically with regards to the biochemical limits), (6) to understand the principles that will shape the future of life, both on Earth and beyond (especially Objective 6.1 - Environmental changes and the cycling of elements by the biota, communities, and ecosystems), and (7) - Determine how to recognize signatures of life on other worlds and on early Earth.

3.5.3 General Plan of Work and Key Milestones

Year 1 - Add new MBGC model components and evaluate research hypotheses using measured data from CYN microbial mats. Select new field sites for remote sensing of microbial mat ecosystems.

Year 2 - Evaluate research hypotheses using measured data from several types of microbial mats. Visit new field sites for near (and remote) sensing of microbial mat ecosystems. Submission of journal manuscripts.

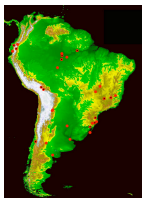
Year 3 - Develop and evaluate new research hypotheses using measured data from several types of microbial mats. Develop first set of biosphere model codes.

Year 4 - Develop and evaluate next set of biosphere model codes. Select and visit new field sites for near (and remote) sensing of microbial mat ecosystems.

Year 5 - Continue as above and finish all research activities with submission of journal manuscripts.

3.5.4 Management Structure and Statements of Contribution

Day-to-day management of the research investigation will be the responsibility of **Christopher Potter** (Lead CoI), a NASA Civil Servant at ARC. Potter is an ecosystem scientist and modeler who will be responsible for coordination and integration of the major components including modeling design, data analysis-interpretation, and dissemination of results through written reports. **Kelly Decker** (CoI) and **Steven Klooster** (CoI) will operate under an existing Memorandum of Understanding (MOU) and the Cooperative Agreement between NASA ARC and the Foundation of California State University at Monterey Bay (FCSUMB). Both Decker and Klooster will be responsible for MBGC model code programming using software available within the Ecosystem Computational Facility (ECF) at NASA ARC. Decker will also be responsible for dissemination of results through written reports. Klooster will also be chiefly responsible for analysis and interpretation of satellite image and spectral data from microbial mat sites.



3.6 Investigation 6 Hind-Casting Past Environments

3.6.1 Objectives and Significance of Research

Our own biosphere has undergone adaptations as the Earth's climate has changed, and we will use this natural "experiment" to understand how environmental drivers affect local and continental-scale ecosystems. Variation in atmospheric circulation patterns has been linked to the El Niño

Southern Oscillation (ENSO), and our previous work has shown that South American vegetation responds to those changes. Further, we have mapped vegetation responses at 8-km resolution. Using proxies in the paleontological record (such as tree rings), we have reconstructed sea surface temperatures back to the mid-13th century, and have proceeded to year 300 B.C. as more data became available. The current investigation will rely upon remote sensing data to expand our (and other) reconstruction to include ecosystem properties during this period. In addition, we propose to use pollen records to "synchronize" our reconstruction with a variety of proxy measurements and interpret behavior of ecosystems over a longer period of time and over a wider variation in environmental conditions.

The limited length of data from instrument records can hinder a detailed understanding of decadal and longer-term land-atmosphere-ocean climate variability. Tree rings, fossil pollen, banded coral, and other proxy data can help resolve the nature of such variability over millennia, much longer than is possible using data from conventional instrumentals.

Strong climate index associations with proxy data are a consequence of the close relationship between the "El Niño" Southern Oscillation (ENSO) or the North Atlantic Oscillation (NAO) and Sea Surface Temperature (SST), precipitation and storm track trajectories worldwide. These extended proxy records can also aid in model construction of historical and biosphere function, an important NASA ESE (Code Y) goal.

For this project, we will use past changes in South American vegetation to predict and demonstrate the evolution of a drying, cooling, and perhaps dying, planet. Specifically, we will ascertain if changes in Advanced Very High Resolution Radiometer Normalized Difference Vegetation Index (AVHRR-NDVI) in South America resulting from "normal", "El Niño", or "La Niña" conditions can be linked to variations in SST. We will also use imagery and data sets from instruments on newer satellites, specifically data from the Enhanced Thematic Mapper Plus (ETM+) instrument onboard Landsat 7 and data from the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra satellite. Previously, a correlation between the 32 South American sites we used and SST data from Ship Track 1 was done yielding an r^2- value of about 0.8; under this task, we will expand our research in this promising line. Changes in atmospheric circulation patterns have been linked to ENSO at a global scale, and we found that South American vegetation responds to those changes. Some such responses may have a proxy version in the paleontological record.

Using the correlation found previously, we will "hind-cast" (as opposed to forecast) changes in AVHRR-NDVI during the last 1,300 year using reconstructed SST data. This will allow us to predict what vegetation communities were present in the past seven plus centuries. Linking AVHRR-NDVI to level "zero" (approximately the present time) of pollen profiles, it is possible to "hind-cast" past changes of NDVI over a much longer time period. Since many pollen profiles reach back to 15,000 years BP, it should be possible to synchronize them with the global C curve, the Lake Vostok temperature curves, and other records. Should we determine a historical C budget, we will need to have C data from the Large Scale Biosphere-Atmosphere Experiment in Amazonia (LBA) study and elsewhere. Since time resolution will not allow direct linkages of pollen fluctuations with ENSO events, we will need to use published data and radiocarbon dating to estimate the likelihood of ENSO being the cause of some past vegetation community changes, previously undiscovered or unexplained.

3.6.1.2 Build On and Extend State of Knowledge

Numerous proxy climate reconstructions have been developed that are directly or indirectly related to our research area. Examples are the reconstruction of the winter NAO index using tree-ring widths records from eastern North America and Europe (D'Arrigo et al. 1995, 1996; Cook et al. in press). Several other investigators have linked ENSO and North Pacific climate indices with proxy records (Fritts et al. 1979; Cleaveland et al. 1992; Stahle & Cleaveland 1993; Villalba et al. 1997; D'Arrigo et al. 1999; Wiles et al. in press). Banded coral records have the potential to extend the ENSO event record back for more than 100,000 years (Tudhope et al. 2001). Our current collaboration with R. Armstrong (University of Puerto Rico-Mayagüez) is exploring this record in the Caribbean.

Our previous research used South America as an analog of the biosphere of a planet that is cooling and drying, i.e., one that is leaving the "habitable zone" around the star which it orbits (Kasting 1996; D'Antoni 2000). The main features of the current South American vegetation were established after the last Ice Age (~ 10k yrs). As we demonstrated at 8-km resolution, most of the continent vegetation indices are altered by the El Niño Southern Oscillation (ENSO) events, but the largest effects are concentrated in the Northern Amazon basin, the dry forest of Coastal Ecuador, and the Paraná-Plata watershed (D'Antoni et al. 2002). Further, we identified NDVI variation in the area most intensely studied by dendrochronologists in South America. If sustained, the large effects of ENSO must have had a significant role in shaping the modern landscape of the continent. This assumption led us to search for ENSO signals in the paleontological record (see references above). Using available tree-ring

width records for South America (NOAA, Paleoclimate), and modern data for the SST of the Atlantic and Pacific Ocean (NOAA), we generated a multivariate statistical model that showed intriguing trends. We then refined the model using an artificial intelligence model (NeuroShell by Ward Systems Group, Inc.) and produced a reconstruction of the Atlantic and Pacific SST from year 1246 AD through 1995 AD (Fig. 3.6-1) (D'Antoni & Mlinarevic, 2002).

3.6.2 Research Tasks

3.6.2.1 Data Analysis and AI Modeling

For this effort we will use the pollen calibration model [$C_f = T_m \ P_m$] for pollen studies (Bradley 1999). Here C is climate, T is a transfer function and P is pollen. The subscript *m* denotes "modern" and *f* represents "fossil". We will use simple (linear and non-linear) and multiple linear regression, and regression on principal components to establish the relationships between SST, NDVI and pollen. We will then train an artificial intelligence algorithm to refine our predictions. For tree-ring width data analysis, we will follow the above mentioned literature and the critical suggestions of Bradley (1999). Once our results are available, we will collate and compare all pertinent data sources for ENSO and NAO index reconstruction for the length of time available in the continental proxy records of South America. We will use relationships of the Ames CASA model (Potter et al. 1998; see Investigation 5, this proposal) and other ecosystem models that use NDVI inputs to make gridded predictions of past 20-yr terrestrial carbon budgets, which have been successfully linked to ENSO and NAO in the tropical zones, and globally. We will use gridded historical estimates of global ice cover overlaid to mask out terrestrial productivity in the extra-tropical zone during the last glacial age.

With this integrative approach we expect to bring the contribution of South America to the reconstruction of the Earth's terrestrial biosphere productivity for the past thousands of years, based on synthesis of modern satellite observations, ecosystem models, global climate records, and other proxy data sets from field studies.

3.6.2.2 Fieldwork

Established collaboration with local scientists in South America include field measurements of photosynthesis in the dry tropical forest of Ecuador, in a specific point of the riparian Paraná forest north of Buenos Aires and in the dry "caldén forest" near Santa Rosa. The LBA program has measured photosynthesis in several points of the Amazon basin area and those data are readily available. An area with no significant changes from ENSO to non-ENSO conditions, the Ambato valley and its surroundings in Catamarca (Argentina) will be used as a control. To reinforce the link between tree-ring width and SST, we will encourage the involved scientists (Villalba, and the group from CRICYT) to fill the gap between the end of their tree-ring research and the present day in order to enlarge the time overlap of AVHRR and tree-ring data.

3.6.3 Relevance to NASA's Astrobiology Programs

The Astrobiology Program Goal Number 6 deals with the forces and drivers that have shaped or will shape the environment of Earth. Goal 6.1 deals specifically with the structure of ecosystems due to the cycling of elements by the biota, communities and ecosystems, both present *and past*. Understanding vegetative assemblages in local ecosystems or at the continental level requires an understanding of the environmental drivers (especially rainfall patterns) that caused those assemblages to be there. This work will show how the vegetation of portions of South America has changed over thousands of years and will indicate a possible future of communities, using Earth as an analogue of a drying, cooling, and perhaps dying planet.

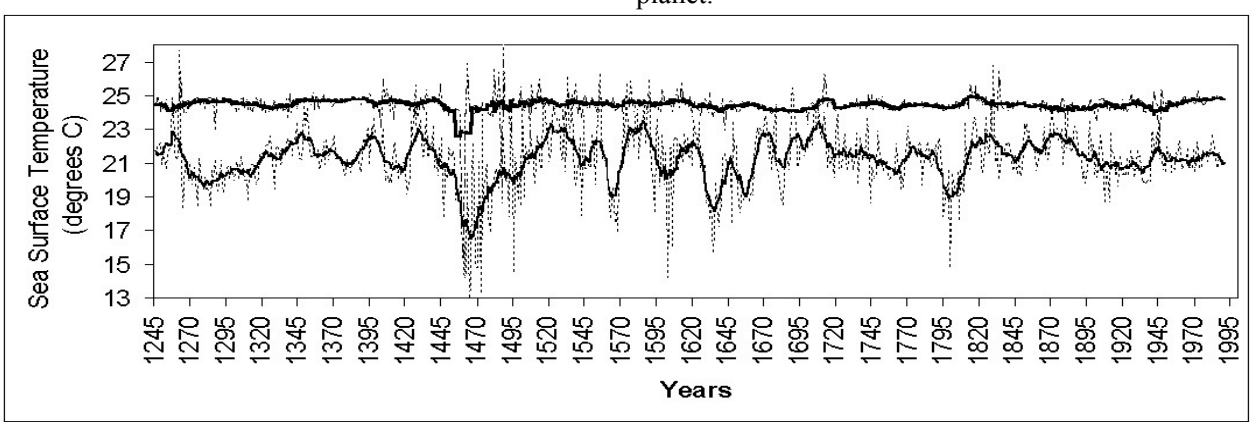


Figure 3.6-1. Reconstruction of past sea surface temperatures (SST) from 1246 AD to 1995 AD. Atlantic SST predictions are shown in the upper two curves with the thinner weight line as the raw prediction; the bold line is the five year smoothed average. Pacific SST predictions are shown in the lower two curves with the thinner weight line as the raw prediction; the bold line is the five year smoothed average.

3.6.4 General Plan of Work and Key Milestones

Due to the nature of this investigation we will need repeated measurements in the field, from year 1 through year 5.

Year 1 - Establish network of collaborators and share protocols for fieldwork. Measurements (see Fieldwork, above) starting in the Austral spring and continuing through summer. A trip to Parana-Plata basin in Argentina and Uruguay is planned. Link field measurements with remote sensing data and SST data. Milestone: identify Y1 data with an analog in our 20-year remote sensing database and with NOAA's SST record. Send or take three photosynthesis meters to S. America.

Year 2 - Repeat measurements in the field. A trip to Catamarca, Argentina is planned. Link field measurements with remote sensing and SST data. Milestone: identify Y2 data with an analog in our 20-year remote sensing database and with NOAA's SST record. Send or take two photosynthesis meters to S. America.

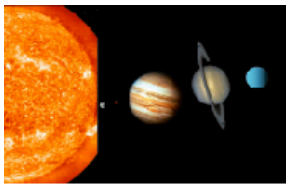
Year 3 - Run test simulations and compare with compiled climate, tree ring and other data. Collate with instrument data where available and tune AI model. Refine simulations. A trip to Calden Forest, Argentina is planned.

Year 4 - Continue refinement of models and incorporation of field and instrument data. Assembled proxy data incorporated into model refinements. A trip to a Nothofagus forest in Argentina and Chile is planned.

Year 5 - Compile all new data, expand database. Repeat as in year 4 and make final simulations. Extrapolations of climate into past done. Identify data with an analog in our 20-year remote sensing database and with NOAA's SST record. Cross-validate our record with all available and pertinent proxy records available. A trip to Parana-Plata basin in Argentina and Uruguay is planned.

3.6.5 Management Structure and Statements of Contributions

Dr. **H. D'Antoni** will supervise work done for this module and report to Dr. Des Marais. He will also work with paleoclimate and paleo-environment data. Dr. **J. Skiles** will be responsible for gathering and analyzing data from the remote sensing instruments mentioned above, overall data analysis, computer simulations and for the area of plant ecology and plant eco-physiology. Our foreign collaborators will contribute the field data they gather in each of the years of the project.



3.7 Investigation 7 Interplanetary Pioneers

Here we focus on life moving beyond its planet of origin, a question of evolutionary interest and because the human exploration of space *is* the movement of life from Earth. Today, our research effort provides a home within NAI for an integrated research program relating to this major area of Astrobiology. With this renewal, *we propose a focused research plan as a nucleus for an expanded emphasis on this area of interplanetary travel of life as the field of Astrobiology matures.*

Moving beyond the planet of origin requires a vehicle for transport, the ability to withstand transport, and the ability to colonize, thrive and ultimately evolve in the new environment. The core of this study will be to identify organisms and ecosystems that are likely to withstand the rigors of space, using as a guiding principle the hypothesis that desiccation resistance and natural exposure to high levels of radiation are good predictors of radiation resistance. Once this work – collecting, testing in the lab and in a space simulator, looking at mechanisms underlying the results – has been established, we will expand to include a flight component and to bring in workers from related fields to study other aspects of natural transport.

As a means of transport the work proposed focuses on natural transport, such as on a meteorite, where the primary factors determining success are finding a suitable vehicle for transport, and the ability of the organisms to withstand space radiation, space vacuum, desiccation, time in transit, and the physical rigors of leaving the parent body and landing on a new one. The product of the probabilities of each factor provides an estimate of the likelihood of success. As a result of this proposed effort we will increase our understanding of several of these parameters.

The proposed work is ambitious, especially for the budget permitted. The funds are primarily for work that takes place under Rothschild or Mancinelli's direction and include supplies, salary and travel, the last of which is crucial to allow the use of the unique facilities of the DLR in Germany, and facilitate collaboration with our European and Australian colleagues. The non-US citizens are not requesting monies. *It is important for the NAI to support the proposed effort because it provides a catalyst and a vehicle for interaction among disparate groups and application of on-going work to a fundamental question in astrobiology.*

This work is clearly connected philosophically with other portions of this proposal, from questions of defining the habitable zone to understanding microbial ecosystems and the biosignatures they can produce. We would anticipate through our monthly group meeting and more frequent informal contacts to form synergistic exchanges with these groups.

3.7.1 Objectives and Significance of Research

- To identify terrestrial organisms and ecosystems that will survive space radiation and vacuum. To assess the mechanisms underlying survival.
- To expand this nucleus to other aspects of transport beyond the planet of origin, focusing on the suitability of meteorites as a vehicle of transport.
- To provide a conceptual framework and collaboration for this new field.
- To use this knowledge to inform related fields such as evolutionary biology, global change, the search for life elsewhere, and planetary protection.

3.7.2 Build On and Extend State of Knowledge

Can life survive beyond its home planet? In attempts to answer this question microbes tested in the space environment as of the beginning of 2003 include *Bacillus subtilis* spores, bacteria, bacteriophage T-1, Tobacco Mosaic Virus, and osmophilic microbes (Horneck & Brack 1992, Horneck 1993; Mancinelli et al. 1998, Nicholson et al. 2000). *B. subtilis* spores will survive for years in space if in a multilayer or mixed with glucose to protect them against high solar UV-radiation flux, but are killed in minutes if exposed in a mono-layer (Horneck 1993; Horneck et al. 1994, Horneck et al. 2001). Viruses lose viability in weeks (reviewed in Horneck & Brack 1992). The halophile *Haloarcula*-G and the cyanobacterium *Synechococcus* (Nägeli) can survive for at least weeks in the space environment (Mancinelli et al. 1998) and probably much longer (Mancinelli, unpublished data). Funded in part by the current NAI grant to NASA Ames, team member Mancinelli and colleagues (Mancinelli et al. 1998) were the first to show that a vegetative cell, in this case a halophile, could survive exposure to the space environment. *We believe that many organisms suitable for space simply have been ignored or not yet isolated. This is the focus of the proposed work.*

Are there mechanisms to transport organisms off their planet of origin and deliver them safely elsewhere? We know that meteorites such as ALH84001 have journeyed from Mars to Earth, and they can contain fissures which should provide a suitable environment for microbes. Modeling studies have suggested that microbes on a planet, such as Mars, could survive the impact and forces needed to cause a meteor to be jettisoned from the planet, escape to space and survive the impact of landing on a planet such as Earth. This pioneering work by team member Horneck and collaborators (Mileikowsky et al. 2000; Horneck et al. 2001) showed a substantial rate of survival of bacterial endospores after a simulated meteorite impact at shock pressures of 32 GPa. Further, spores of *B. subtilis* mixed with minerals representing the mineralogy of generic meteorites survive in the space environment better than unprotected spores (Horneck et al. 2002).

It is easy to envision a scenario where extraterrestrial life within a meteorite could enter microcracks, in the meteorite, seal off a small volume within them, and thus be both protected from the vacuum environment and shielded from the cosmic ray and UV environment. If this is true, the transport of life beyond its planet of origin becomes far more likely.

But surviving in cracks within meteorites may not be easy. Shocks created by hypervelocity impacts induce failure and concentrate heating, particularly along strong impedance mismatches or at point contacts including pre-existing failure planes. This bad news could be good news for organisms. Survivors may find themselves in a temporarily habitable environment. *No one has attempted to place microbes within the naturally occurring cracks and crevices of a natural meteorite and determine their survival during an impact or in space as we are proposing.*

The interplanetary medium poses obstacles to the survival of Earth-based, and presumably, all carbon-based life. Space is extremely cold. It is subject to unfiltered solar radiation, solar wind, and galactic cosmic radiation. It has exceedingly low pressures, and has a much lower gravity than Earth (Rothschild 2003; Horneck 2003, Mileikowsky et al. 2000). Space is a nutritional wasteland with respect to water and organic carbon. The organisms most likely to survive these conditions are microbes, although some seeds, fungi, lichens or invertebrates might be able to make the journey. For microbes, gravity is not an issue, and cold tolerance is widespread among spores.

There are four potential "show-stoppers": a meteorite is not a suitable vehicle for transit, exposure to external radiation or the space vacuum causes death or even loss of reproductive potential, or the time in transit is prohibitively long. We will address the first three here.

During the short term, most damage to microbes exposed to the space environment is due to UV radiation, but heavy ionizing radiation has a greater probability of being lethal (rev. in Horneck et al. 1994). Reactive oxygen species are produced by ionizing radiation during flight, and are an

important component of radiation damage in space (LBNL-40278, 1997). Types of DNA damage due to UV and ionizing radiation in space are, in order of increasing danger to the cell, damage to bases, single strand breaks and double strand breaks (LBNL-40278, 1997).

Desiccation tolerance has been reported for a variety of organisms including bacteria, yeast, lichenized fungi, plants, insects and crustacea (e.g., Clegg 1986; Crowe et al. 1992; Csonka & Hanson 1991; reviewed in Potts 1994). One of the supposed mechanisms of death due to anhydrobiosis (extreme desiccation) in prokaryotes is the dehydration of DNA leading to breakage (Dose et al. 1992; 1991 Dose et al. 1995).

The ability to cope with high salt and/or desiccation appears to be a good predictor of protection from radiation damage as has been shown in microbes on Earth. This is because DNA damage accumulates during desiccation because there is no DNA repair (Setlow 1992). We know that organisms living in evaporitic salt crusts are highly resistant to desiccation, space vacuum and UV radiation through ground (Rothschild 1990; Rothschild et al. 1994) and spaceflight experiments aboard ESA's BIOPAN facility (Mancinelli et al. 1998). Duricrusts, thought to be indicative of salt crusts, were found at both Viking lander sites (Clark 1978, Clark & van Hart 1981). Deposits considered to be salt pans are seen on images of the martian surface (Forsythe & Zimbelman 1995). On any world in which liquid water becomes limited, we would expect salt formations to become an important niche for life.

Of course organisms have evolved mechanisms to avoid or repair damage. Organisms other than archaeal halophiles use organic compounds as osmotica, whereas the halophiles use K+ as their internal osmoticum. Oxidative damage, which results from space radiation as well as occurring on earth during aerobic metabolism (Brawn & Fridovich 1981), may be avoided by detoxification mechanisms such as the enzyme superoxide dismutase. Nearly all these organisms also contain catalase which catalyzes the decomposition of hydrogen peroxide to oxygen and water. Peroxidases are used by some bacteria and protists to decompose hydrogen peroxide to water by oxidizing organic compounds. Moreover, water- and lipid soluble antioxidants such as glutathione or ascorbate, and tocopherol, respectively, scavenge free radicals. Carotenoids are a class of pigments that act as quenchers of single-state oxygen (rev. in Siefermann-Harms 1987). *Understanding mechanisms for survival is important, and will be initiated during the proposed work.*

3.7.3 Technical Approach and Methodology

Our approach will be to identify novel organisms or ecosystems that have the potential to survive interplanetary transfer by bringing them into Rothschild's and Mancinelli's labs at NASA Ames and screening for UV and desiccation resistance. The most promising samples will be further screened in Germany at the DLR space simulators, and, if possible, ultimately flown on the EXPOSE facility on ISS. We will initiate studies of the mechanisms of desiccation and radiation resistance by screening DNA damage, antioxidants and detoxifying enzymes after exposure. To supplement the core project, we will experimentally test the suitability of meteorite cracks to house terrestrial organisms en route to another body.

1. Identify and collect samples that are halophilic, desiccation or radiation resistant from field sites and culture collections. *(Rothschild, Mancinelli, Johansen, Anitori, Kranner)*

Halophilic and desiccation resistant microbial samples. Some samples were collected during the first funding cycle of this project. We will collect additional halophile samples from the salt evaporation ponds of the Cargill company along the southern edge of San Francisco Bay, near NASA-ARC. The ponds range in salinity from sea water (ca. 3%) to saturation (>25%) with a pH range of 6.5-7.2.

To screen and isolate desiccation resistant organisms from the samples, the brine will be washed 3 x by centrifugation. The final pellet will be suspended with sterile 25% NaCl to bring the concentration to ~10^7 cells per µL. Fifty µL aliquots will be pipetted into 96-well microtiter plates and dried for 3 h at 30°C in a forced air incubator. After drying, the plates will be placed in a standard laboratory desiccator at 22°C. Periodically plates will be removed, each well filled with 130 µL of nutrient medium (e.g., OS liquid media, ATTC manual), incubated at 30°C, and checked for growth by monitoring increases in turbidity using a 96-well plate-reading spectrophotometer. Those exhibiting growth and desiccated for the longest period of time will be isolated on agar plates.

Desert crust. Johansen will provide microbiotic crusts from throughout the western United States and western Australia. Available collections include eukaryotic algae from such soils (Chlorophyceae, Tribophyceae, Bacillario-phyceae). The soils range from xeric, hot habitats to those in arid, temperate climates. Cyanobacteria from the crusts are identified in Johansen's lab using DNA sequences from the 16S rDNA and 16S-23S ITS region. Desiccation resistance will be tested in a similar manner to that for halophiles.

Radiation resistant samples. Two types of samples will be tested. *Deinococcus radiodurans* and those from team member Anitori who is

working on a unusual radon-rich hot spring (48-63°C) situated in a region of South Australia's Flinders Ranges which has a long history of hydrothermal activity. Radon-222 is present in gas which bubbles up from the sandy bottom of the spring. The gas has a radiation level of ~29,000 Bq/L; the radiation level in the water is 2000-5800 Bq/L. Paralana is unique in that a flourishing microbiota is present in association with the radon environment (Anitori et al. 2002; Anitori et al. 2003). 16S rRNA studies have indicated that Paralana contains, among others, cyanobacteria (e.g. *Fischerella* spp.), and halophilic Archaea (*Natronococcus xingiangensis*). Desiccation resistance will be determined as for the halophiles.

Lichens: Desert species or high altitude species are well adapted to desiccation and probably quite high UV irradiation. Two desert species, *Ramalina maciformis* (Negev) or *Teloschistes capensis* (Namib) are good candidates. Desiccation resistance will be determined as for the halophiles. Viability will be tested with the Live/Dead stain if that is shown to be appropriate, or by CO_2 exchange. If they recover photosynthetic activity, they are alive.

2. In Mancinelli's lab, expose organisms to increasing doses of UV radiation using a solar simulator. Determine survival. (*Mancinelli, Rothschild, Purcell*)

Organisms exhibiting the highest level of desiccation resistance, as determined from (1), will be prepared for UV radiation resistance as described above for desiccation resistance screening, except instead of being placed in a desiccator the dried samples will be exposed to UV radiation from a deuterium lamp (Oriel model 6316) in Mancinelli's lab. Samples will be collected periodically to determine survival using reproduction ability on the appropriate medium for the organism and Molecular Probe's Live-Dead Stain. Those organisms surviving the highest dose will be grown on the appropriate medium.

3. Expose most promising samples to space simulators at the DLR e.V., Institute of Aerospace Medicine, Radiation Biology Section, Köln, Germany *(Rettberg, Horneck, Rabbow, Mancinelli)*

Organisms exhibiting the highest survival rate for desiccation (1) and UV radiation resistance (2) in the solar simulator will be tested in the space simulators located at the DLR. For example, for halophiles the organisms will be grown to mid-log phase and washed by centrifugation as described previously. After the final wash, the pellet will be suspended in the appropriate wash salt to a dilution equaling 2.5 x 10^8 cells. Forty µL aliquots of the diluted suspension will be placed onto 7 mm diameter quartz discs and dried in forced air incubator at 30°C for three hours.

All of the samples will be exposed to space vacuum, with half of the samples exposed to solar UV-radiation and half kept in darkness shielded from solar UV-radiation. Duplicate samples will be kept in the laboratory to serve as unexposed controls. Periodically (hours, days, weeks, and months) a set will be collected from the space simulation chamber and analyzed for survivability. Live versus dead cells will be monitored using reproductive capacity in the appropriate medium as well as with a dual fluorescent stain from Molecular Probes (Live/Dead viability kit).

Determination of Survivability. Microbial survival will be determined by comparing the number of viable cells in the laboratory controls (i.e., samples not exposed to vacuum desiccation or solar UV radiation) with the number of viable cells recovered from the exposure chambers after completion of the test.

Data Analysis of Survivability. The survivability of the laboratory ground simulation chamber time course experiment samples will be calculated as a percent of the initial number of organisms placed in the flight and ground simulation control chambers. The number of survivors will be compared to the number of survivors from samples stored in the laboratory under ambient conditions. In addition, because samples will be collected periodically from the simulation chambers, their percent survival will be determined as a function of time. The data will then be compared to survival percentages of organisms on past flights and other organisms tested on this flight.

4. Look for mechanistic explanations for radiation or desiccation resistance by assessing DNA damage, antioxidants, detoxification enzymes, and pigmentation before and after exposure to solar simulator at Ames *(Rothschild, Purcell, Kranner, Lancaster).*

While a complete assessment of mechanisms of radiation or desiccation resistance is out of the scope of the present proposal, several team members have expertise in different aspects of cellular damage, and will apply their expertise to promising samples.

DNA damage (Rothschild). Double and single strand breaks will be determined qualitatively. Double strand breaks will be analyzed by agarose gel electrophoresis with a digital gel documentation system equipped with image analysis software. Breakage of DNA will be determined by loss of high molecular weight DNA. Single strand breaks will be determined similarly, but following denaturing the DNA. If more quantitative data is required, a modification of the familiar "nick-translation" method which omits the nicking step will be used. Incorporation of radioactive nucleotides into the DNA is proportional to the number of single strand breaks. To assess oxidative damage resulting in base

modification, Rothschild's lab will determine the ratio of 8-hydroxy-2'-deoxyguanosine to the unoxidized 2'-deoxyguanosine using reverse phase HPLC with a Coulchem II electrochemical detector. Prior to HPLC analysis, the DNA will be digested with P1 nuclease (1.5 h at 37°C), and incubated in alkaline phosphatase (1 h at 37°C). DNA will be isolated using commercially-available kits such as the Wizard® Genomic DNA Purification Kit (Promega). This technique has been in use in Rothschild's lab since the autumn of 2002.

Antioxidants and plastid pigments (Kranner). HPLC analysis of reduced glutathione (g glutamyl-cysteinyl-glycine; GSH) and glutathione disulfide (GSSG) will be performed according to Kranner (1998). HPLC assays of ascorbate and dehydroascorbate will follow the procedure of Tausz et al. (1996). Tocopherols (α-, β-, γ, δ-tocopherol) and plastid pigments will be analyzed according to Pfeifhofer et al. (2002).

Detoxifying enzymes (Lancaster). Catalase will be assayed using the Amplex Red Catalase Assay Kit (Molecular Probes: A-22180). The superoxide dismutase (SOD) assays are based on monitoring the degree of the autoxidation of pyrogallo (Marklund 1985). Peroxidase activity in crude cell extract will be assayed using the QuantBlu fluorogenic peroxidase substrate from Pierce Biotechnology. Glutathione reductase activity will be measured by monitoring the decrease in absorbance at 340 nm due to the conversion of NADPH to $NADP^+$. All three assays will be monitored by absorption using a multiwell plate reading spectrophotometer in Rothschild's lab.

With local funds, Rabbow will study the role of exogenous and endogenous protection mechanisms allowing survival of bacterial endospores in outer space, for example, inside of artificial meteorites. Rettberg will study the role of the repair systems in the radiation (UV and ionizing) resistance of various genetically well-defined strains of *Deinococcus radiaodurans* irradiated under the conditions simulating outer space (e.g., vacuum, extreme temperatures) using molecular biology techniques (e.g. DNA chip technology).

5. Prepare for flight experiments as part of ROSE consortium. Fly most promising samples when the external platform is built on ISS. *(Mancinelli, Horneck, Rettberg, Rabbow, and others)*

Candidate organisms will be flown in flight experiments through membership in the ROSE (Response of Organisms to the Space Environment) consortium on The European Space Agency's (ESA) external EXPOSE platform aboard the International Space Station as permitted. *Members of our team have already been selected as PI's through a peer- reviewed process to fly experiments as part of the ROSE consortium.* They are Horneck (SPORES) and Mancinelli (OSMO).

6. Use these results to determine if these organisms are likely to survive in earth orbit, and thus would make a good future candidate for long-term (multi year) space flight. Initiate assessment of meteorites as environment for microbes *(Consolmagno, Rothschild)*. Initiate studies of the survival of the most promising organisms during simulated meteoritic impact (*Schultz, Rothschild*). Model potential for such life forms elsewhere *(Raven, Wolstencroft)*.

To test the suitability of meteorite crevices for interplanetary transport, Consolmagno will select a typical non-carbonaceous meteorite, and measure its porosity using a large helium pycnometer (described in Consolmagno & Britt 1998). He will image a small sample by SEM backscatter and measure and characterize microcrack porosity. At Ames, the meteorite will be heat sterilized, and a known and easily traceable type of microbe (e.g., *Bacillus subtilis*, *Synechococcus* or yeast) will be introduced into it. The meteorite will be exposed to simulated space conditions, then cored or cut to determine the survival characteristics of the microbes as a function of depth within the rock and time of exposure. Survival will be determined by reproduction as described previously.

The meteorite will be relatively unweathered to ensure unclogged pores (Bland et al. 1998). It will be at least 3 cm in every dimension to provide a significant volume with at least 1 cm of shielding, suggesting a meteorite e~100 g in mass. Fusion crust will be avoided, as it is not typical of the state of the material in space and could affect the effective permeability of the meteorite. Meteorites will be obtained from the Antarctic Meteorite collection maintained at NASA's Johnson Space Center. Meteorites suitable for this work include EET 96 032 (435 g L4, weathering grade A/B, fracture B) and ALH 82 104 (400 g, L5, weathering grade A, fracture A/B.)

The next step is to determine if impacts kill the microbes when the pore spaces are squeezed shut by the shock wave of an impact. Collaborator Schultz will test this by taking meteorite sample impregnated with living microbes and subjecting them to controlled velocity impacts using the Vertical Gun Facility at NASA Ames. Previous experimental studies demonstrated that oblique impacts reduce the peak pressures within the impactor and induce failure prior to penetration into the target (Schultz and Gault 1990). Intact fragments (up to 10-50% the mass of the initial projectile) survive and are sprayed downrange. It is possible for fragments (and contained organisms) to survive collisions long thought to be devastating for transport. Although Schultz has separate funding through Exobiology to assess survival during hypervelocity impact, the

experiments outlined here are distinct, and collaboration with members of the NAI would allow broader applications and involvement.

3.7.4 General Plan of Work and Key Milestones

General plan of work:

- Identify and collect samples likely to withstand space flight.
- Screen for survival after exposure to a solar simulator in Mancinelli's lab, NASA/ARC.
- Expose most promising samples to space simulators at the DLR e.V., Köln, Germany.
- Look for mechanistic explanations for radiation or desiccation resistance.
- Prepare for flight experiments as part of ROSE consortium. Fly most promising samples when the external platform is built on the International Space Station.
- Use these results to determine if these organisms are likely to survive in Earth orbit, and thus would make a good future candidate for long-term space flight.
- Assess meteorites as environment for microbes. Study survival of the most promising organisms during simulated meteoritic impact.
- Model potential for such life forms elsewhere.

Key Milestones

Year 1 - Initial collection and screening of samples using solar simulator at Ames. Survival will be monitored. Test techniques on monitoring DNA damage on new organisms (e.g., DNA isolations). Select and obtain meteorites. Team meeting.

Year 2 - Continue isolation and tests of new model organisms. Initiate studies on mechanisms of survival. Begin tests of promising samples in space simulation facility, DLR. Measure meteorite fissures (Although the EXPOSE first flight is scheduled for October 2004, we anticipate that it will slip by a year).

Year 3 - Continue isolation and tests of new model organisms. Continue experiments on mechanisms of survival. Continue tests of new model organisms. Measure colonization of microbes in meteorite cracks. Begin flight component, Team meeting.

Year 4-5 - Continue research including flight component. Begin work on impact survival of test organisms that show high survival rates. Team meeting year 5.

3.7.5 Management Structure and Statements of Contribution

Rothschild (lead CoI), will be responsible for the overall management of the proposed work. **Mancinelli** (CoI), is the only US PI on the BioPan and ROSE experiments. He will be responsible for the work on prokaryotes, and testing all organisms in the Space Simulation Facility at the DLR. He, along with Horneck, will co-ordinate flight experiments. **Horneck** (CoI), will work with Mancinelli on all experiments conducted in Germany, and be the primary advisor for sample testing in flight simulators, and flight experiments. She will coordinate with ESA on the flight experiment logistics. **Consolmagno** (collaborator), will be responsible for the experiments on the survival of microbes in meteorites. **Johansen** (collaborator), will provide desert crust samples from his collection, and isolated cyanobacteria from microbiotic crusts from the western US and western Australia. **Rabbow** (collaborator), will study the role of exogenous and endogenous protection mechanisms allowing survival of bacterial endospores in outer space, and will be responsible for the work of the SSIOUX group examining this. **Raven** (collaborator), will examine generalized energetic and kinetic constraints on photosynthesis, and to relate these requirements to the range of conditions on other bodies on which life may be possible. **Rettberg** (collaborator), will study the role of the repair systems in the radiation resistance of *D. radiodurans*. **Schultz** (collaborator), will lead the study of the effect of impact on survival of microbes in meteorites. **Wolstencroft** (collaborator), will explore the generalized energetic and kinetic constraints on photosynthesis using a theoretical approach, and relate these requirements to the range of conditions on other planets on which life may be possible. **Anitori**, will be responsible for the isolation and characterization of samples from the Paralana site and Shark Bay. He will provide a link with the Australian Centre for Astrobiology. **Kranner**, will be responsible for work on lichens, antioxidants and plastid pigments.

3.8 Management Plan

Ames fulfills the following three distinct roles in NASA's astrobiology program: (1) management of the Astrobiology Institute; (2) team membership in the Institute, if this proposal is accepted; and (3) non-Institute research and technology development for astrobiology. This proposal deals directly only with (2).

David Des Marais, Principal Investigator (PI), has overall authority and responsibility for this proposed work. To provide overall scientific direction to the effort, he will chair a steering committee comprised of the Co-Investigator (CoI) leads who will supervise each of the seven investigations and the program for education and public outreach. Des Marais will supervise a staff (see Volume II, Section 11.9) that will administer this project, including helping to meet the requirements of the NAI management.

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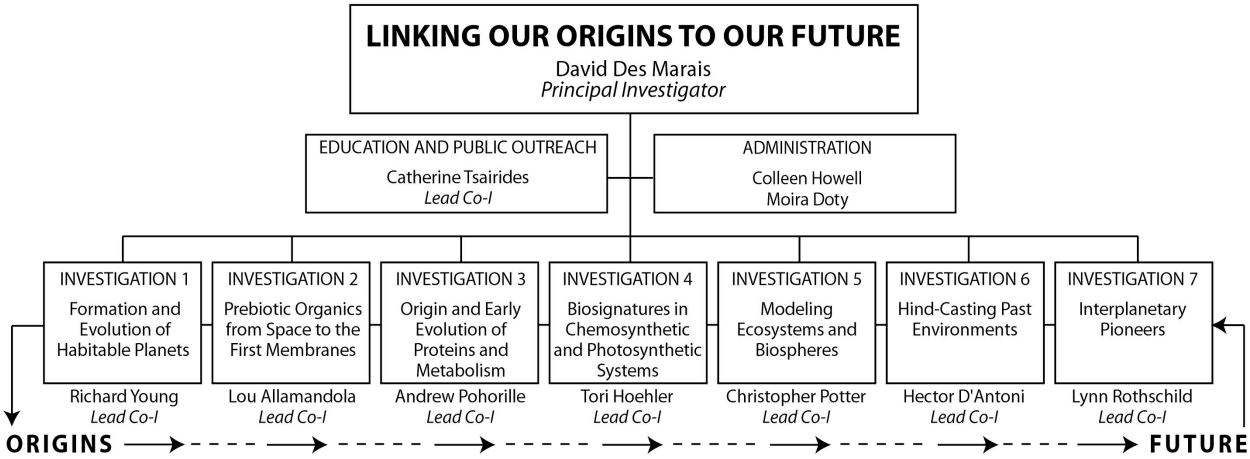
D'Antoni, Lynn Rothschild, and Catherine Tsairides, each a Co-Investigator (CoI) lead, will lead the seven investigations and the E/PO program. Each CoI lead will be responsible for detailed management of funding and personnel resources, for the laboratories and facilities of his/her investigation. Each CoI lead is also responsible for coordination and integration of the various individuals and institutions that comprise the team and for interacting with each other to identify new research ideas, new areas for collaboration, and exchange of resources, including personnel, among the implementing institutions.

This program will be integrated further through monthly meetings and less frequent workshops that will address selected elements of the program and create opportunities for new collaborations and research directions. Both activities will embrace the lessons learned during our current membership with NAI. For example, monthly presentations of biological themes will be paired with those of related astrochemical or planetary themes. Also, discussions of science tasks will be paired with those related to training, education, and public outreach. These meetings are also modeled after the highly successful Center for Star Formation (CSF), a research effort led by Ames in collaboration with UC Berkeley and UC Santa Cruz. Indeed, CSF will coordinate its own meetings with Investigation 2 of this proposal. To improve participation with our external colleagues at collaborating institutions, we will utilize NetMeeting, which has been adopted recently by NAI.

We have participated actively in NAI focus groups, and will continue to do so in the next round of membership. For example, Investigations 1 and 2 will work with the proposed Astronomy and Astronomical Biosignatures focus groups. Members of Investigation 3 are working with NAI members from the Colorado team and the University of Florida to propose a focus group on the origin and early evolution of self-reproducing systems. Investigations 4 and 5 will participate in the Mars, the Ecogenomics, and the proposed Astronomical Biosignatures focus groups. Investigations 6 and 7 will work with other NAI teams to develop groups addressing the future of life. Through these interactions with other NAI member institutions, we will continue our role in forging the interdisciplinary connections that will contribute to the overall viability of the institute.



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5.0 PLAN FOR STRENGTHENING THE ASTROBIOLOGY COMMUNITY

5.1 Education and Public Outreach

The NASA Ames Astrobiology team is committed to fostering the broad involvement of the science community in Education and Public Outreach (E/PO). Within the outreach program we adhere to the NASA Office of Space Science Education and Public Outreach Criteria and the Origins Roadmap 2003. *The major criteria that the Ames team is focused on are:*

- *The potential for the proposed education and public outreach activity to expand its scope by having an impact beyond the direct beneficiaries*
- *Reaching relatively large audiences*
- *Being suitable for replication or broad dissemination*
- *Drawing on resources beyond those directly requested*

We highlight collaborations the Ames Astrobiology team has initiated with two institutions that serve as portals for large segments of the public to explore science and the excitement of astrobiology. California Academy of Sciences (CAS), located in Golden Gate Park, San Francisco, and Yellowstone National Park (YNP) will provide the team an opportunity to work within a collaborative process. *We have been proactive in seeking high-leverage opportunities to maximize the usefulness and effectiveness of material, programs and services created by NASA missions.* The team will be creating new exhibit plans, products, and activities for CAS, YNP, and the New York Hall of Science. In addition, we will conduct specific activities associated with the Carnegie Institution of Washington, and the Ames Education Office. The exhibits, activities and educational materials developed will be distributed in a coordinated manner across these organizations for maximum impact to amplify the effect of "strengthening" the astrobiology community.

5.1.1 Programs in Astrobiology

Astrobiology at California Academy of Science

Founded in 1853, CAS is the oldest scientific institution in the American West and currently holds one of the largest natural history collections in the world. It is also one of the only such places in the world that houses a natural history museum, planetarium and aquarium together. Thus, it is one of the few institutions devoted to the dissemination of science to the public that has a range of facilities with a breadth that approaches that of the field of astrobiology. CAS is currently planning a $370 M renovation of its existing museum. This will result in more modern facilities redesigned in a manner worthy of their interactive style, so as to engage the museum's many visitors. The renovation represents a remarkable opportunity for the NASA Astrobiology Institute (NAI) Ames team to leverage those funds and convey its science to the public. The community that the NAI will reach through this collaboration is amazing by the standards of E/PO grants of the size of ours. The Ames team will serve on the design team and contribute to the exhibit development team. CAS and Ames education teams will partner researchers with educators incorporating science content with methodological background and training in conducting inquiry based programs and activities. *We will facilitate high quality product and program development by researching product usage and audience needs in conjunction with the high leverage organizations.* The Ames science team will contribute to the development of lessons and materials that will be created and presented to educators during the museum's formal education professional development program. The Astrobiology Educator Guide and the Astro-Venture program will be incorporated into the professional development workshops held at the CAS each summer. In turn, CAS will benefit from its interaction with the Ames team by gaining access to experts who can provide perhaps the only overarching theme capable of connecting CAS's disparate subjects. Moreover, in some cases the specific exhibits planned by CAS center on issues that are synonymous with NASA's main goals. This is exemplified by the exhibits "Earth and its Place in the Universe" and "Water is Life"; the latter funded in large part by a $2.5 M NSF Informal Science Education grant. In both of these exhibits, NASA Astrobiology themes—the role of water, and conditions that allow the origin, development, and persistence of life—are central.

Because the scientists at CAS are oriented towards systematic biology and evolutionary biology they will rely on this partnership to provide input on other scientific concepts and cutting-edge research for exhibit development. With NASA Ames Research Center so close geographically to CAS, we foresee a strong collaborative relationship between Ames and CAS in the development of these ideas. Specifically, we see our expertise as crucial to bringing these projects to their full potential in two ways. First, our team members will be active in ensuring that the exhibits are, and continue to be, scientifically current - a quality that has historically garnered CAS acclaim. Second, we plan to extend their features that focus on Earth environments to include examples from other worlds. Connections to science Investigations 1 and 2 can aid in the continuation of the water theme to put it into the larger context of Solar System history and future, as well as include comparisons to sub-surface oceans on icy satellites of Jupiter, and possible

liquid water on or near the surface of Mars. Another connection can be seen in Investigations 4, 5, and 7 helping to expand the presentation of extremophiles as possibly representative of what life might have looked like in the ancient past, and/or elsewhere. The Ames Astrobiology team has created this formal and informal research education partnership with CAS featuring the topic of astrobiology to bridge the three parts of the museum—natural history, planetarium, and aquarium—together. A diverse suite of programming activities at CAS, YNP, and NYHOS are in development.

- BioForum (lectures for teachers) "Astrobiology: search for life in the universe"
- 3-hour docent training tours on astrobiology for "Earth and Space"
- Planetarium show
- Planetarium lecture series
- Members lecture on NAI research
- Science Now exhibit panel on NAI research
- Science Now web page on NAI research

Timeline:

- 2003 - Scientific content discussions, professional development programs for docents area educators, and research partners
- 2004 - Scientific content discussions with exhibit designers
- 2005 - Production of prototype astrobiology exhibit elements for display in a temporary museum in downtown San Francisco
- 2006 - Evaluation and assessment of design elements
- 2007 - Modify and design astrobiology permanent exhibit elements
- 2008 - Opening of "Earth and its Place in the Universe" and "Water is Life" exhibits including astrobiology elements

Astrobiology at Yellowstone National Park

The Ames/YNP partnership will help the general public understand and appreciate the unique natural resources preserved in YNP. Information on YNP's resources forms the basis for interpretation offered to the general public in the form of exhibits, publications, audiovisual programs, the park's official web site, a variety of ranger-led programs and activities, and its formal education program. YNP provides insights into the origin and evolution of life and the potential for life to exist beyond Earth. The Ames Astrobiology team has forged a partnership with YNP to provide a tiered public engagement program through the Department of Interpretative (DOI) Research at YNP to initiate a focused outreach project that highlights both astrobiology and the work of the NAI in the Park. Specifically, the NASA/Ames Astrobiology team will work with the National Park Service's Division of Interpretation and Education, which bases its operations, programs, and functions on the Long Range Interpretive Plan. This Plan describes YNP's visitor experience goals for the Division of Interpretation based on identification of primary park resources and associated themes, and in conformity with the NPS' s mission, management policies, and other legislative guidance.

The Plan is a living document that is reviewed annually and adjusted to respond to changing park issues, research updates and reports, and visitor needs and studies. Through this partnership the NASA/Ames Astrobiology team will be a national leader in supporting resource education and interpretation. Its collaboration with the Division of Interpretation will provide the park's diverse audience with appropriately designed and developed media and other programs in which astrobiology research is presented. This presentation will be consistent with the goals in the Long Range Interpretive Plan, and it will provide insights into the origin and evolution of life and the potential for life to exist beyond Earth. The many venues we discuss in the Education Plan will be coordinated through the YNP DOI to disseminate information to tourists and web audiences. A major advantage is that YNP interpretive staff provides strong background in the science of education to complement our strengths as scientists. YNP's infrastructure for developing and distributing interpretive materials will help us reach very large audiences with maximum efficiency. For instance, the Resources and Issues Manual is the primary tool by which park managers and interpretive staff learn the information they need to affect decision-making and for interfacing with tourists. YNP has an established system for production and emplacement of signage and trail guides, which reach 1 million visitors per year. YNP will also coordinate all outreach activities associated with the new Old Faithful Visitor Education Center, including not only exhibits, but also educational programs for park visitors of all ages (Junior Ranger programs, curriculum development, etc.). In the last two years the NASA Ames Astrobiology team has initiated collaboration with the YNP Division of Interpretation to find ways to tell the astrobiology story through the many YNP venues. We have been invited to:

- Contribute chapters to the Resources and Issues manual
- Develop wayside signs and edit trail guides
- Participate in planning and development of the new Old Faithful Visitor Education Center (OFVEC)
- Explore opportunities to educate tourists and interpretive staff through informal talks
- Explore new interactive uses of existing child education tools (e.g., Junior Ranger Program)

- Explore how the YNP web site can best be used to communicate information about astrobiology.
- Participate in annual Yellowstone Association Institute formal education program

Timeline:

- 2003 - Revise and publish chapters in Resources and Issues manual; continue development of signage; initiate revision of trail guides; OFVEC proposal writing and planning; dialog about informal talks and web-sites
- 2004 - Progress from initial signs to additional signs; publish revised train guides; OFVEC planning; Junior Ranger Program revision; initiate informal talks
- 2005 - OFVEC exhibit production; informal talks; initiate web-site projects
- 2006 - OFVEC evaluation and revision; complete web-site projects
- 2007 - OFVEC opening

Formal Education Product Development and Dissemination. New York Hall of Sciences (NYHOS), in cooperation with Technical Education Research Center (TERC), and the Ames Astrobiology team will provide content development for the "Search for Extraterrestrial Life" exhibit at the NYHOS. We will participate in the design of the K-14 Discovery Center experience and hands-on activity stations which will deepen the visitors experience of the exhibit. A series of hands-on activity stations and a manual for investigations related to the activity stations that museum staff will use with the formal education student groups that frequent the museum will be developed.

Timeline:

- 2003 - Discovery Station content
- 2004 - Discovery Station Docent manual published

NASA Ames/Lunar and Planetary Institute. *The Professional development standard for teachers of science requires learning essential science content through perspectives and methods of inquiry, science learning experiences for teachers must involve teachers in actively investigating phenomena that can be studied scientifically, interpreting results, and making sense of findings consistent with currently accepted scientific understanding.* A YNP field experience in collaboration with The Lunar and Planetary Institute/Broker Facilitator role with NASA's Office of Space Science utilizing the Space Grant Consortium is a strong partnership that we have entered to provide professional development for 6-12 educators with field site studies. The field site and university connections rotate yearly.

Development and Presentation Team. NAI integrated Outreach team members Ames, MBL, and Penn State have designed and developed the first Astrobiology Short Course (five hours) accepted for presentation at the National Science Teacher's Association Conference where participants interact with scientists and educators from the NASA Astrobiology Institute as they learn about the latest content, research and curricula material *This team will explore new connections within the NAI teams and also continue past relationships.*

Children's book on the Microbial World in collaboration with The Carnegie Institution of Washington. In collaboration with CIW the Ames team will contribute to the creation of a 64 page, full color children's book on the Microbial World with over 70 original illustrations and high quality photographs. The Ames Astrobiology team will develop the teacher's guide and activities-based book with our collaborating partners, align the book to national standards, and participate in the field testing of the product. Research sites such as Yellowstone will serve as context for the story. The activities from this book can be incorporated into the Junior Ranger Program at Yellowstone National Park.

Astro-Venture is an educational, interactive, multimedia web environment focusing on Astrobiology. The Ames Astrobiology team provides content and design input into the storyboard development for the concept. The team participates on the review panel for all modules and lessons for the classroom.

5.1.2 Approach to Communicating Astrobiology

California Academy Of Sciences

CAS offers a variety of formal standards based K-14 workshops from an existing National Science Foundation grant, *meeting the criterion for leveraging non-NASA funds.* By incorporating an active and innovative formal education program with the museum we are targeting the underserved communities around the Bay area. We collaborate on presenting the Astrobiology Educator Guide and Astro-Venture program to the professional educators in the San Francisco City Schools. These programs were designed as interactive inquiry, standards-based activities that cover a range of astrobiology topics. Bio-Forum: high school, middle school, community college, and student science teachers are invited to symposia on a variety of topics of which astrobiology will be a selected topic involving the Ames team researchers as speakers. Continued development of programs for educators will evolve as the research team designs the new exhibits at the Academy.

Professional Development for Scientists. CAS and Ames education teams will work on developing science team partnerships and exhibit presentation material. Resource materials will transfer from the museum floor the formal classroom.

Exhibit Development. Our team will assist in the development the new concept and content for the new planetarium. We will be developing and testing ideas for the new exhibit spaces being created. Our team will prepare breaking mission stories, technology, and images for the news and exhibit program that can be transferred to YNP and the NYHOS.

Museum Tours. Lively and informative expeditions through the exhibition halls are led by museum docents who are education specialists. The Ames Astrobiology team will participate in the continued training of the docents at NASA Ames Research Center in collaboration with the Ames Education Office.

Yellowstone National Park Partnership

Yellowstone's diverse resources, ecological processes, and cultural history provide important opportunities for research and education. The Ames Astrobiology team will be a national leader in resource education and interpretation providing the diverse and sophisticated public with the high level of astrobiology research content incorporated into the plan. The integrated NASA Ames Team participated yearly on site at Yellowstone to develop the formal and informal education plan. The programs described below have been designed and accepted in the plan due to the planning team's collaboration with the national park system, which is opening the door to astrobiology on a significant level as we pursue our goal of inspiring the general public.

Old Faithful Visitor Education Center. The development of exhibits for a new OFVEC offers the NASA Astrobiology lead team an opportunity to work with the National Park Service's Division of Interpretation and an interdisciplinary team of geoscientists including geologists, volcanologists, geothermal specialists, microbiologists, sociologists, educators, exhibit specialists, and interpretive planners. The NASA Ames Astrobiology team will provide information on the astrobiology component of the thermophile story, based on their ongoing scientific research in YNP. Planned products that evolved from the planning committee include:

WaySide Exhibit and Trail Guides. Interpret astrobiology research in YNP through the development of outdoor wayside exhibits. These will be located park wide at sites that best illustrate the most compelling aspects of astrobiology research as it relates to the goals and themes stated in the Long-Range Interpretive Plan.

Expedition Yellowstone. The formal education team from Yellowstone will bring classroom lessons and activities to the outlying school districts that are rural and underserved many of which are Native Americans. The K-14 school districts will also have students transported back into the park for an on-site experience.

Junior Ranger Program. In collaboration with the Junior Ranger Program staff, we will develop a question and answer element or inquiry activity in the Junior Ranger newspaper designed for older children (9-14) and their parents. This activity might logically relate to development of wayside exhibits where children would have to visit a location and provide feedback as part of the overall process presented in the newspaper, leading to their achievement of Junior Ranger status.

Resources and Issues Handbook. We are contributing to the development of an expanded section of the Resources and Issues Handbook. This annually produced publication is a convenient, one volume reference manual used to train the interpreters, educators, and park staff in all offices needing quick and concise information on the full spectrum of YNP's natural and cultural resources, and controversial or critical management issues. Additionally, the Handbook is sold in bookstores at 8 visitor centers, museums, and contact stations in the park. The Ames team will provide astrobiology related content, which will then be passed through the editorial process and included in the Handbook. Sources for detailed/expanded levels of Ames team research will be listed in the Handbook.

Real Time Media. To inform the broad audiences that visit the park virtually we will work with YNP to incorporate remote broadcasts from the research sites, interactive exhibits and educational materials developed by the research team that involves the audience in the experiment.

Informal Public Talks. We will organize "fire-side" chats with the integrated Ames team and the community of astrobiology researchers to take place at YNP for the interpretive rangers and the general public.

Yellowstone Association Institute Course. A three-day field course entitled "Yellowstone's charismatic microorganisms" to interested visitors educators and the general public. The course will interpret microbial resources in YNP and will link them to life history on Earth and Astrobiology.

5.1.3 Audiences

The NASA Public Engagement program aims to include all of the diverse members of the American Public, with their varied backgrounds, wide range of experience and different ages. These large impact collaborative partnerships bring astrobiology to over four and a half million national and international general audiences reaching close to one million directly *inspiring the K-14 population of which half a million are the non traditional underrepresented audiences.* Each year 1.5 million visitors enjoy the many exhibits at CAS. The Education Division reaches over 300,000 more, many of them from urban, disadvantaged backgrounds, through tours, outreach, teacher workshops, lecture series,

museum facilitation, and special classes. Today, as one of the 10 largest natural history museums in the world, CAS brings the message of research to nearly one and a half million visitors each year. At YNP each year 3 million national and international tourists from varied geographic, economic and ethnic backgrounds visit and most of them go to see Old Faithful. The New York Hall of Science Exhibits/Discovery Learning program reaches close to 300,000 visitors in New York, 100,000 of which are underrepresented populations.

5.1.4 Project Impacts and/or Metrics

The Office of Space Science criteria, *which facilitates high quality product and program development by providing evaluation services, and by sharing their collective expertise and best practices.* For the CAS, YNP, and NYHOS large scale E/PO projects and programs three stages of evaluation are part of the assessment process: formative, summative, and field testing. The integrated NASA Ames Team participates yearly on site at Yellowstone to develop the formal and informal education plan. The Division of Interpretation and Education then applies sociological and other visitor survey data to develop multi-faceted, appropriately designed programs and media. The recommendations contained on the Long Range Interpretive Plan are based on appropriate uses of media and programs in relation to subject matter, setting, and visitor behavior patterns and expectations. Field testing will be part of that process with the exhibit and discovery station projects. Our "Summative" evaluation will provide us with the results of the effort: how effective it was, whether it met the stated intentions, whether it had other unanticipated effects. Summative evaluation is a major component of the CAS exhibit so as to publish the lessons learned as we develop future projects for the new museum. Methods of evaluation include focus groups, surveys, observations, follow-up interviews, pre- and post-testing, and many other techniques will be incorporated into our formal education program

5.1.5 Personnel and Their Expertise

Catherine J. Tsairides, Lead CoI, E/PO, will manage all aspects of the project directing the YNP, CAS and NYHOS partnerships. **Linda Young**, CoI, YNP Project and Deputy Chief of Interpretation, Planning and Media, will be responsible for selecting appropriate interpretive media and locations, and where relevant, develop media (content, design, fabrication), install and maintain media over the life-span of the device. Dr. **David Ward**, CoI, Montana State University, as a member of the YNP Old Faithful Visitor Education Center Scientific Advisory Board, will be the team's liaison for planning and developing exhibits in this important new venue. Dr. **Terrence Gosliner**, CoI, California Academy of Sciences, specifically for this project, will work with the integration of scientific content and its dissemination to audiences at the CAS. Dr. **Carol Tang**, CoI, California Academy of Sciences, will be responsible for facilitating the public outreach component at the CAS through exhibit development, lectures, workshops, outreach and professional development.

5.1.6 E/PO Costs

E/PO conference travel expenses, as well as salary for Catherine Tsairides, Lead CoI, will be covered as outlined in the outreach budget (section 11.8). California Academy of Sciences will receive $5K to provide formal astrobiology workshops and printing costs for advertising the programs. Dr. David Ward will receive $5K towards salary costs for the Yellowstone OFVEC Planning Committee, and YNP exhibit development. A Science Writer will receive $12K for content development.

5.2 Additional Elements

Here we summarize how Ames will make specific resources available to support the professional community, as well as the research and training described in this proposal. Some of these resources are offered at no, or reduced, cost to the NASA Astrobiology Institute. Also summarized is the involvement of the Ames team in professional and training activities, and in flight missions. This broad involvement indicates not only the high quality of the Ames team, but also the many important opportunities that Ames team membership in NAI will create for other NAI members and the astrobiology community.

5.2.1 Professional Community

5.2.1.1 Journal Editorships

Members of the Ames proposal team serve the professional research community in a variety of ways. The following team members will continue to serve in editorships and/or editorial boards of professional journals:

- Des Marais, D.: *Astrobiology*, Editorial Board; *Geobiology*, Editorial Board
- Lissauer, J.: *New Astronomy Reviews*, Editor
- Sandford, S.: *Meteoritics and Planetary Science*, Editor
- Schulte, M.: *Geochemical News*, Associate Editor, *Astrobiology*, Editorial Board
- Summons, R.: *Astrobiology*, Editorial Board
- Toon, O.B.: *Journal of Geophysical Research*, Associate Editor
- Treiman, A.: *Meteoritics and Planetary Science,* Associate Editor

5.2.1.2 Professional Workshops

Team members also will organize and participate in workshops that will advance the intellectual

content of the field, as well as contribute to the planning and development of missions.

For example, Dr. D. Des Marais chaired the Blue Dot Workshop (1997) and co-chaired the Pale Blue Dot Workshop II (1999). Each of these events provided the content for substantial NASA Conference Proceedings publications that were published the following year. These workshops identified major issues and approaches that address the search for evidence of life on habitable planets that orbit other stars. Thus they contributed directly to the conceptual development of a search strategy for NASA's Terrestrial Planet Finder mission, which is envisioned to be launched early in the next decade. *We propose to maintain this intellectual discourse by organizing an oral session "Searching for life beyond our solar system," to be held at the American Geophysical Union Fall meeting*, sometime during the proposed period of funding.

Dr. D. Hollenbach is the director of the Center for Star Formation Studies (CSF), a very successful NASA-funded group since 1985 of astrophysicists from the University of California at Berkeley, the University of California at Santa Cruz, and NASA Ames. CSF studies star and planet formation. *We propose to initiate a series of one-week summer workshops biennially focussed on the research of our Ames astrobiology group.* These workshops would bring together the diverse elements of the Ames group, the astrophysicists from CSF, and outside experts in the focussed research areas of the Ames group. The Ames NAI team, CSF astrophysicists, and students from San Francisco Bay area universities and beyond would benefit from this mutual interaction, which will stimulate new collaborations, more coherent interdisciplinary work, and cross fertilization of ideas.

5.2.1.3 NASA Advisory Committees

Team members participate in NASA advisory committees. For example, L. Allamandola recently served on the Origins Subcommittee of the Space Science Advisory Council (SSAC). D. Des Marais is a former member of SSAC itself. Des Marais participated actively on the Project Science Integration Group (PSIG) for the 2009 Mars Science Laboratory (MSL) Mission. Des Marais continues to serve on Mars Exploration Payload Advisory Group (MEPAG) and as an advisor for MSL.

5.2.1.4 Scientific Meeting Organization

Team members contribute to the organization of future scientific meetings. For example, L. Allamandola is a member of the Scientific Organizing Committee for the future international symposium entitled, "The Astrophysics of Dust."

D. Des Marais serves on the steering committee for the international "Earth System Processes II" (ESP2) meeting, which will address the past, present and future Earth processes from a multidisciplinary and interdisciplinary viewpoint, analogous to the field of astrobiology. Indeed at least one session at the ESP2 meeting will specifically address astrobiology.

R. Young is a member of both the Scientific Organizing Committee and the Local Organizing Committee for the 2003 Division of Planetary Sciences Meeting of the American Astronomical Society. He also served on the Scientific Organizing Committee of the Astrobiology Science Conference in 2002.

L. Rothschild is the chair of the organizing committee for the 2004 Astrobiology Science Conference (AbSciCon04), to be held at Ames Research Center. AbSciCon has become a major forum for astrobiology. More than 600 scientists, technologists, students and lay public attended AbSciCon02. D. Des Marais will serve as a member of the scientific organizing committee for AbSciCon04.

5.2.2 Training

The Ames NAI team training effort includes the following activities: (1) participation by students and postgraduate research associates in the proposed research activities, (2) an undergraduate course at Stanford University, (3), a community college course, and (5) astrobiology-related workshops that involve students. These activities will contribute large quantities of novel and current source material for the Ames team's astrobiology education and public outreach program (Section 5.1).

Development of source material both for university and for postgraduate training, as well as K-12 education, will be the responsibility of the Ames scientists and other participants in the training and education and public outreach programs. Translation of source material into curriculum, web pages, public presentations, and other education or public outreach products will be the responsibility of professional educators, curriculum developers and communication specialists working with the scientists.

5.2.2.1 Training by Direct Involvement in the Proposed Research Program

For decades, the basic research program at Ames has substantially involved graduate students, postdoctoral research associates and more-advanced professionals. One example among many is the participation of National Research Council (NRC) Fellows in astrobiology-related research at Ames. *Over the past 10 years, more than 130 NRC Fellows have completed two-year training programs here; more than 50 of these were advised by investigators who are participating in this astrobiology proposal.* A substantial fraction of these former associates are still active in

astrobiology-related activities, as evidenced by the fact that some *20 of them are either investigators or named collaborators on this proposal*. Many former postdoctoral associates are participating in other proposals, and at least two are principal investigators.

This proposal seeks to continue this successful tradition of research training. *It requests support for an average of six graduate students and eight postdoctoral associates per year* (see personnel listings in the detailed budget sheets, Volume II).

Also, David Blake is directing an engineering project for astrobiology, resulting in deliverables for potential direct use in Mars '09 MSL mission technology development with the Harvey Mudd University engineering students and faculty. A new program involving David Blake will include an on site training program in astrobiology technology development for US Air Force Academy cadets

All of the participants that are mentioned above will work alongside NRC Fellows and other students and young professionals who participate in closely related programs at Ames. They will move freely between Ames laboratories and those of its collaborators, and several will work with interdisciplinary teams on field expeditions. They will benefit from the numerous astrobiology seminars and workshops held at Ames. This next generation of astrobiologists will receive truly interdisciplinary training, for they will witness the ongoing development of astrobiology at the place of its birth.

5.2.2.2 Stanford University Astrobiology Course

We propose to continue to sponsor a course on Astrobiology and Space Exploration in the Human Biology Department at Stanford University. The course "Astrobiology and Space Exploration" (Human Biology 107) has been offered at Stanford University for more than a decade by several members of the staff at Ames Research Center. The course has changed its scope and content over the years, and has reflected the latest theory, research and planning in the various fields that now comprise astrobiology. Dr Malcolm M. Cohen, Chief of the Human Information Processing Research Branch at Ames, is currently directing this astrobiology course. Dr. L. Rothschild, a lead CoI of the Ames NAI team, will assume direction of the course in 2004. C. Tsairides wll contribute to the organization and content of this course. Further, she will identify course content that can be utilized in the proposed program for E/PO.

The format of this class consists of seminars and lectures at Stanford, with thirty students per class. The prerequisites are to have had a college level course in biology, chemistry, physics, behavioral science, or mathematics. The course, originally offered in the Department of Aeronautics and Astronautics, and entitled "Colloquium on Life in Space," was moved to the Interdisciplinary Human Biology Program in 1994. Primarily designed for advanced undergraduate students in the sciences, the course emphasizes evolutionary themes that lead from the big bang to the human exploration of space. The goal of the course is to provide a context from which to examine the three fundamental questions of Astrobiology: "What is the origin and history of life? Do life forms exist beyond the Earth? What is the potential for human expansion beyond the home planet?" Space, time, and life itself are examined from a universal perspective, and the place of humanity in the universe is seen to emerge as a unique integration of chance and necessity, of chaos and of order.

ASTROBIOLOGY AND SPACE EXPLORATION
--- Course Schedule for Spring 2003 ---

April 1	M. Cohen: Introduction - Cosmology, Astrobiology, and Space Exploration
April 3	S. Shostak: The Big Bang, Our Universe, and All That Jazz
April 8	J. Cuzzi: Stellar Evolution and Habitable Planets
April 10	L. Allamandola: From Molecules to Microorganisms
April 15	L. Rothschild: Pushing the Envelope for Life; Life at the Edge
April 17	B. Runnegar: Evolution of Life from Microbial to Multicellular
April 22	D. Morrison: When Worlds Collide - Extraterrestrial Threats to Life
April 24	L. Rothschild: Replaying the Tape (Lecture and Class Discussion)
April 29	D. Des Marais: Environments for Life in the Universe
May 1	M. Cohen and E. Holton: Newton, Gravity, and Biology
May 6	C. Chyba: Looking for Life in All the Right Places – Europa, Titan and Elsewhere
May 8	E. Holton: Gravitational Biology and Adaptation to Space
May 13	M. Kliss: Life Support Systems for Space Exploration
May 15	C. McKay: Exploration and Colonization of Mars – Why and How
May 20	M. Cohen: Behavioral Adaptation and Working in Space
May 22	M. Cohen: Group Interaction and Crew Performance
May 27	M. Cohen: Circles in Space - Skylab, Shuttle, Mir and Space Station
May 29	S. Shostak: Search for Extraterrestrial Intelligence
June 3	M. Cohen: The Place of Humanity in the Universe
June 5	T.A.: Final Review (OPTIONAL)
June 6	Staff: Final Examination (12:15 - 3:15 p.m.)

While the course already exists and is available to a few Stanford students, we propose here to widen its impact greatly. As part of this proposal, the lectures will be recorded, edited, and made available on the internet. A web site for the lectures will provide a continuing opportunity for further elaboration and discussion of important issues during the intervals between annual presentations. This electronically based education and outreach effort represents the development of a living electronic textbook on astrobiology that will include an integration of efforts from the life sciences, space sciences, information sciences, and the outside community. These services will be provided as part of Ames' Institutional commitment.

The course Website is http://www.stanford.edu/class/humbio107

The course schedule for Spring 2003 is given below. The course offering for 2004 will emphasize more strongly those aspects of astrobiology addressing the space sciences and Earth system sciences.

5.2.2.3 The Center for Star Formation workshops

The Center for Star Formation workshops that were mentioned earlier under the "Professional Community Section 5.2.2.1, would indeed include graduate students and postdoctoral researchers. Thus the workshops would provide the opportunity to attract promising young researchers to the field of astrobiology from the astrophysics and planetary science communities. It is anticipated that a number of new collaborative research projects involving graduate students and the Ames astrobiology group would emerge from these workshop interactions.

5.2.2.4 The NASA Center for Computational Astrobiology and Fundamental Biology Students Internship Program

The NASA Center for Computational Astrobiology and Fundamental Biology plans to hold its fourth Summer Students Internship Program. Eight students participated in 2000 and also in 2001; 14 students participated in 2002. For 2003, members of the Ames NAI proposal team will once again recruit a group of students with diverse backgrounds interested in applying theoretical and computational methods to biology, chemistry, astrophysics and ecology. This program is open to undergraduate and graduate students who are U.S. citizens or permanent residents. Students will be working on a variety of projects that reflect the breadth of computational astrobiology.

Students will spend ten weeks at Ames working on individual research projects. Before arriving at Ames each student will be assigned a mentor and a project that matches her/his interests and skills. Our goal is to choose projects so that at the end of the internship each participant will accomplish tangible results. In addition, the students will participate in a series of collaborative activities that will expose them to a broad range of science being done at NASA-Ames, including that by the Ames NAI team. At the end of their internship, the students will prepare a publication-style report from their work. As they have in previous courses, these reports can sometimes lead to peer-reviewed publications.

To learn more about NCCAFB see http://cca.arc.nasa.gov.

5.2.2.5 Astrobiology Academy

The NASA Astrobiology Academy is a unique summer institute of higher learning whose goal is to help guide future leaders of the U.S. Space Program. While at Ames for a six-week period, the students participate in individual research guided by world-class investigators at the center, a rigorous lecture series, and a group project that fosters leadership skills and interdisciplinary collaborations. This course provides research opportunities in state-of-the-art astrobiology laboratories coupled with broad-based views into the inner workings of the space program and research missions. The students assigned to a principal investigator will work independently on a technical project of their choice. Several members of the proposed Ames NAI team participate as principal investigators. This mentor-student relationship gives students insight into the challenges and rewards of primary scientific research. Approximately 60% of the typical workweek is dedicated towards this aspect of the Academy. Academy participants also experience other aspects of NASA during field trips to other centers, tours, lectures, etc. A formal lecture series focusing on all the various aspects of astrobiology is coordinated with some of NASA's top scientists. The students participate in a group project that typically involves them designing a scientific and technology plan for a space flight mission relevant to astrobiology. The academy has an Alumni network of over 300 Academy graduates.

5.2.2.6 Linking Life's Origins and Ecology, Ecosystem-to-Biosphere Modeling: Graduate and Upper-division Undergraduate Training and Education and Outreach Component (Lead CoI: Chris Potter, CoI: Kelly Decker)

Objectives. This portion of the proposal explicitly addresses the undergraduate and graduate-level training objective and the objectives of Section 3.4 – 3.5 of the NAI CAN proposal announcement. The simulation model we are developing is well suited for use in a classroom or a computer laboratory. To this end, our overall objective is to create an interactive web page that

can be used for undergraduate and graduate education in courses that discuss microbial ecology, biogeochemistry, ecological simulation modeling, and astrobiology. This type of web-interactive modeling tool has been successfully used to teach principles of ecology and biology within a traditional in-campus context (e.g. Maxwell and Sheley 1997; Voinov 2002) with the aid of a professor. Once the page is constructed, we will have several professors use the tool in the classroom and ask them to help us evaluate the efficacy of the model as a tool for teaching astrobiology, microbial ecology and biogeochemistry.

Model description. The current and proposed model structures are outlined in the introduction to this module. In summary, we propose to adapt our Stella™ model so as to enable biogeochemical simulations of various photosynthetic mat types such as intertidal, stromatolite-forming, and cool thermal (Yellowstone) microbial mats. The adaptations of the model will also incorporate data acquired through partnerships with microbial astrobiologists at Ames and elsewhere.

Web site construction. The web site will be designed around the model and software necessary to use the model. The model was constructed in a Stellaä environment and there is a free Stella Reader available for downloading that allows students to alter the model input, run simulations and display or print output without saving the changes. The web site will include links to the model via this free software program. This is similar to how the model has been made available to several members of the astrobiology community via the use of Science Organizer. The site will also have directions for laboratory exercises that are tailored for different classes and sections of classes (see supplementary information). There will be exercises for grades 9-14 as well as for upper-division and graduate-level students. Instructors can opt to use exercises from the web site or create their own. At each step in each exercise, students can double click on instructions for use of this tool. There will also be a section to offer advice to teachers. At the end of each exercise, we will ask students and teachers to evaluate the web site as a learning exercise (see below).

Relevant disciplines. The first goal of this project is to provide web-integrated exercises for students within astrobiology, microbiology, ecosystem ecology, and ecosystem modeling. Students within these specialized disciplines are at an upper-division level, and can alter the model components that are relevant to their field and choose relevant data to graph. At this level, some or all of the line and box structure of the model itself can be revealed.

A second goal of this project is to develop a menu-driven interface of the model for use by students in grades 9-14. Visually, the model can be simplified for different uses depending on how much the student needs to understand of its inner workings. At the 9-14 grade level, students will be able to alter different portions of the model, and choose to display graphical output via pull-down menus. Additionally, simplified sub-models will be extracted from the mail model to illustrate simple ecophysiological relationships such as oxygen production during photosynthesis of the cyanobacteria. This will be an interactive option on a mat web page that is linked to the Yellowstone page.

Exercise examples. Below are some sample questions that students should be able to answer using the model.

- How might this microbial mat affect the atmosphere?
- How does a change in day length affect the output of O_2? How does it change the distribution of H_2S within the mat?
- How does a change of temperature affect bacterial growth?
- What happens if you remove the cyanobacteria? The sulfur-reducing bacteria?
- What happens if you change reaction rates for: sulfur reduction? Oxygenic photosynthesis? Methanogenesis?

Efficacy testing and feedback. Below are some sample questions that we will ask educators after they use our model. There will be a section on how the web site was used, what was required of the students, and how the teacher assisted. There will be a section on how effective the web instructions were. There will be a section on what the students learned from the experience, and what the shortcomings were. Finally, there will be a section suggesting modifications to the web site.

- For what course did you use this model?
- What did you wish to demonstrate using this model?
- What sort of classroom instruction did you provide to the students in 1) the use of this model and, 2) to help them interpret the output?
- What did you want the students to learn from this exercise?
- What was the model effective in teaching the students?
- What was the model ineffective at teaching the students?

Literature Cited

- Maxwell, B.D. and R.L. Sheley (1997). Noxious weed population dynamics education model. Weed Technology 11:182-88.
- Voinov, A. (2002). Teaching and learning ecological modeling over the web: a collaborative approach. Conservation Ecology 6(1) 10.

5.2.2.7 University of New Mexico Field Course - Minority Involvement

CoI A. Treiman is leading the organization of a grade 6-12 teacher training workshop, for summer 2003, with significant minority service. Treiman is working closely with Dr. Horton Newsom of the University of New Mexico (and other colleagues) to arrange a week-long training experience, focusing on geology and astrobiology, that merges field study in Arizona and northern New Mexico with laboratory/classroom studies of Mars and life in extreme environments. The workshop will last a week, of which three days will be field experience at Grand Canyon, Meteor Crater, Sunset Crater cinder cone, and hot springs of the Jemez Mountains NM. Classroom/lab exercises at the University of New Mexico will cover: processes of erosion, impact, tectonics, and volcanism; remote sensing of biological materials; and the biology of life in extreme environments. The latter exercises will be taught or assisted by other CoIs of this proposal (Hoehler and/or Blake). C. Tsairides, Lead CoI E/PO, will contribute to the preparation and presentation of course materials. The web site announcement of the workshop is at http://www.lpi.usra.edu/education/desert2003.

These workshops provide unique learning experiences for teachers in two respects. First is the close coordination of field study, laboratory study, and classroom exercises. For instance, extremophiles were a focus of last year's teacher training workshop to Yellowstone. The teachers saw extremophiles in their life habitats, and then did a series of lab and classroom experiments (with those same materials) about how these biota could be detected remotely (by reflectance spectra). This work is shown in the web site for the workshop, http://lpi.usra.edu/education/yellowstone2002. The second unique aspect of these workshops is the close association between teachers and active researchers. The teachers learn directly from experts working now in those disciplines, can ask questions at will, and can observe the investigator-oriented mindsets of the researchers. Commonly, the experts disagree, and that can be a learning experience in itself.

The 2003 workshop aims for a significant enrollment of minority teachers, especially Native Americans from Arizona and New Mexico. The organizers will pay registration fees for 5 or 6 Native American teachers, who will be selected through Dr. Newsom's close connections with the 'minority' university program SIPPE. It is hoped that these teachers, who would likely be otherwise unable to attend, will share their perspective on the landscape and its life. We will provide them with a geological and astrobiological perspective, and hope that it will complement their own perspectives to yield a deeper understanding of the importance of place to human and non-human life. We also hope to provide free registration for several teachers from minority-dominated or inner-city school districts in Texas and/or Louisiana.

These teacher-training workshops are positioned to provide a limited number of teachers, 40 for the coming summer, a deep understanding of planetary geology and astrobiology. We expect that participation will strongly influence the teachers' own classes – our evaluations have shown this to be true. We will also ask that the teachers spread their knowledge through their schools and districts via word-of-mouth and tutorials. In this way, we hope to achieve significant leverage from the limited number of teachers that can be served in our workshops.

5.2.2.8 Additional Astrobiology Courses

Several members currently teach university courses related to astrobiology, as follows: Planetary atmospheres and processes (O.B. Toon, Univ. Colo.); geophysics of terrestrial planets (N. Sleep, Stanford Univ.); processes in star and planet formation (G. Laughlin, Univ. of Calif., Santa Cruz); and planetary sciences (J. Lissauer, Stanford Univ.).

5.2.3 Teaming with a Minority Institution

5.2.3.1 Jackson State University

For the last three years Dr. Andrew Pohorille has served as a member (and the chair in 2001) of the External Advisory Board for the Computational Center for Molecular Structure & Interactions NSF CREST Center at Jackson State University. This summer, NASA Center for Computational Astrobiology and Fundamental Biology at Ames will hold its Third Summer Students Internship Program (see Section 5.2.2.4). Dr. Pohorille will recruit a group of students with diverse backgrounds interested in applying theoretical and computational methods to biology, chemistry, astrophysics and ecology. They will be working on a variety of projects that reflect the breadth of computational astrobiology.

5.2.4 Staff

With the exception of students and postdoctoral research associates, the Ames researchers and most of our academic colleagues who will carry out the work proposed are civil servants or tenured faculty who will not charge their time to this proposal. The contribution of civil service salaries and travel support exceeds four million dollars over the proposed performance period.

We will continue to augment this workforce. In the past five year funding period with NAI, *Ames hired three new civil service astrobiologists, two of whom appear as CoIs on this proposal.* To augment further the existing scientific staff needed to carry out our proposed research, the Ames Astrobiology and Space Directorate will hire one new astrobiologist during the proposed grant

period, and fill additional civil service positions in subsequent years as permitted by NASA resources and personnel ceilings. Although it is not assigned a dollar value in our accounting of the Ames contribution, this ongoing, long-term development of our human resources for astrobiology represents a major Ames commitment to this program.

5.2.5 Facilities

Ames Research Center commits unique specialized facilities and equipment in order to strengthen the research effort in the astrobiology community. Major components of this committed resource are listed below.

5.2.5.1 Astrochemistry Laboratory

This laboratory complex in Building N45 will support the spectroscopic and other laboratory measurements crucial to our proposed studies. This laboratory provides the capability to: (1) prepare realistic interstellar and cometary ice analogs under a wide variety of temperature and pressure conditions, (2) subject the analogs to radiation mimicking the interstellar and solar radiation fields, (3) measure the absorption spectra of the analogs from the ultraviolet through the far-infrared at all stages of sample preparation, (4) chemically analyze the photoproducts using techniques like HPLC, MS, GC-MS, chromatography, NMR, etc., and (5) characterize physical and chemical properties of the membranes produced by hydration of the amphiphilic molecules

The equipment needed to carry out items #1, 2, and 3 are in place and routinely operating at NASA-Ames. This equipment now includes two research level Fourier transform infrared spectrometers, two completely computer-controlled UV-Visible, moderate resolution monochromator systems, one Alexandrite laser system capable of continuous tuning from 200 nm to 1 μm for in-situ luminescence studies, five high vacuum sample preparation systems which operate routinely in the 10^{-8} mbar range, and four cryostats. Four of the cryostats are closed cycle helium refrigerators that operate between 10 K and room temperature, the fifth is a helium flow system that operates between 4.2 K and room temperature. Four of the cryostat-vacuum systems are mated to the spectrometers such that complete UV to far-IR spectral coverage from 180 nm to 50 cm^{-1} is available. The fifth cryostat-vacuum system will be devoted to full time residue production if this proposal is funded.

The analytical techniques now available at Ames to use to carry out item #4 are wet chemical analysis, High Performance Liquid Chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS), deuterium labeled MS, and IR spectroscopy. The spectroscopic equipment, HPLC, as well as many of the wet chemical analytical tools necessary for this work are on hand in the Astrochemistry Laboratory at Ames while the GC/MS is located in the Life Sciences Laboratory at Ames.

5.2.5.2 The Ames Electron Microscopy Laboratory

The Electron Microscopy Laboratory at Ames Research Center provides a variety of analytical capabilities that will be made available as a remote research facility for members of the Institute (see Section 3) who participate in our proposed research. This facility includes the following instrumentation and equipment: Hitachi S-4000 Field Emission SEM with EDS / digital imaging; JEOL 2000FX Analytical Electron Microscope with EDS / digital imaging; Hitachi H-500H Cryogenic Electron Microscope, with digital imaging; liquid helium, liquid nitrogen, cryotransfer and analytical cold stages; automated Philips/Norelco X-ray Diffractometer; Renishaw Confocal Raman Spectrometer / microscope; Nicolet Nexus 670 FTIR; Oxford TOPS-3 mini-cryo (4 Kelvin) scanning tunneling microscope (STM); Perkin-Elmer Pyris 1 differential scanning calorimeter (DSC); Perkin-Elmer Lambda 900 UV-Vis-NIR spectrophotometer; Perkin-Elmer Pyris 1 thermo-gravimetric analyzer; Agilent HPLC; Digital Instruments Nanoscope IIIA multimode atomic force microscope (AFM); Molecular Imaging Picoscan AFM; Class 100 clean room with laminar flow clean hood; Nikon Microphot FXA petrographic microscope; Nikon SMZ-2 binocular microscope; Gatan dual ion-mill; Sorbol MT-2 microtomes with diamond knives; photographic dark room; VCR Group sample dimpler; VCR Group cold beam ion deposition system ("chrome coater"); Polaron Metal Sputter Coater; and an Edwards vacuum evaporator for metals and carbon.

5.2.5.3 Biogeochemical Laboratories

The biogeochemical laboratories at Ames maintain an array of instrumentation relevant to the chemical and visual characterization of microbial communities, including: gas chromatographs (Hewlett Packard, Inc.; SRI, Inc.; Trace Analytical, Inc.) equipped with FID (hydrocarbon), FPD (sulfur gases), TCD (atmospheric "fixed" gases), HgO-reduction (H_2, CO), and MSD (mass selective) detectors; an ion chromatography system (Dionex, Inc.) suitable for the determination of dissolved ion concentrations at micromolar levels; and a Finnigan-Mat Delta Plus mass spectrometer with GC-combustion interface, suitable for compound-specific analyses of $^{13}C/^{12}C$ and $^{2}H/^{1}H$ at picomolar sample sizes.

Greenhouse Facility. Building N239 at Ames houses a rooftop greenhouse facility capable of maintaining live microbial mats under conditions of irradiance, flow, and substrate supply that

approximate natural environments in the field. This facility was developed during the previous grant period of the Ames NAI team, and represents a valuable heritage for future astrobiological research in microbial ecology (see Section 3). Recent studies demonstrate that the structure of the microbial community and the rates of major biogeochemical processes are retained, relative to field samples, for > 6 months during incubation in the greenhouse. This facility includes a UV-transparent enclosure and also flow boxes (flumes) that can maintain a total of 1.3 m^2 of active microbial mats. One table that contains these flow boxes is also equipped with a computer-controlled XYZ-positioning stage capable of deploying an instrument package (chemical and optical microsensors, cameras) at a desired location on/within the mats, with 10-mm resolution. The positioning stage and instrument package can be operated remotely by collaborators via a web-based interface.

Molecular Biology Facility. We are currently developing a laboratory for genetic analysis and characterization of microbiological samples. The new molecular biology laboratory is equipped with a –80°C So-Low freezer, an MJ Research thermal cycler for PCR, electrophoresis gel rigs, a Fotodyne UV gel imaging system, incubators, and an Eppendorf refrigerated centrifuge with fixed angle rotor.

5.2.5.4 Ames Computational Facilities

Computational Facilities at Ames Research Center continue to enjoy a world-class status. These facilities have long enhanced theoretical research in planetary science and astronomy at Ames (e.g., in support of *Investigations 1 and 2* in this proposal). More recently, they have enhanced research efforts in "Bioinformatics" (related to *Investigation 3*). These capabilities will thus enhance the overall research effort, as indicated in Section 3 of this proposal, with benefits to NAI members of other institutional teams, as the network of collaborations develops. These computational efforts and capabilities are discussed further in section 5.2.7.

5.2.6 Flight Missions

Ames NAI team members are intimately involved in a host of upcoming space flight missions that are centrally important for astrobiology. Below is a short description of each mission, the particular roles played by Ames team members, and how these roles will benefit research in astrobiology.

5.2.6.1 Kepler Mission

Kepler is a recently approved Discovery mission that was conceived and led by an Ames science team with Dr. W. Borucki as Principal Investigator. This mission sets the stage for understanding the abundance and distribution of habitable planets in the Universe. "The scientific goal of the Kepler Mission is to explore the structure and diversity of planetary systems. This is achieved by surveying a large sample of stars to: 1) Determine the frequency of terrestrial and larger planets in or near the habitable zone of a wide variety of spectral types of stars, 2) Determine the distributions of sizes and semi-major axes of these planets, 3) Estimate the frequency and orbital distributions of planets in multiple-stellar systems, 4) Determine the distributions of semi-major axis, albedo, size, mass and density of short-period giant planets, 5) Identify additional members of each photometrically discovered planetary system using complementary techniques; and 6) Determine the properties of those stars that harbor planetary systems. Transits by terrestrial planets produce a fractional change in stellar brightness of 5×10^{-5} to 40×10^{-5} lasting for 2 to 16 hours. The orbit and size of the planets can be calculated from the period and depth of the transit. The Kepler instrument is a 0.95-meter aperture differential photometer with a 105 $degree^2$ field of view. It continuously and simultaneously monitors brightnesses of 100,000 A-K dwarf (main sequence) stars brighter than 14th magnitude. From measurements of the period, change in brightness and known stellar type, the planetary size, orbital size and characteristic temperature are determined. From this the question of whether or not the planet is habitable (not necessarily inhabited) can be answered" (cited from http://www.kepler.arc.nasa.gov/summary.html). Kepler is scheduled to launch during October, 2006.

Ames NAI team member Dr. Jack Lissauer is also a CoI on the Kepler Mission. He is responsible for science planning, scientific data interpretation, and assessment of implications of the data for multiple planet systems. It will soon be possible to combine observational and theoretical efforts for the first time in order to evaluate quantitatively the frequency and characteristics of extrasolar planets, and to evaluate whether or not they are habitable. A major objective of *Investigation 1* is to conduct theoretical studies of terrestrial planets regarding their formation, orbital stability, evolution, and climatology. This research will be directly relevant to interpreting data obtained by Kepler, to be launched in 2007, and more advanced future missions, such as the planned Terrestrial Planet Finder Mission.

Members of the *Investigation 1* and Kepler science teams will co-host a workshop in 2008, the fifth year of this proposed Ames NAI membership. The two teams will share findings of mutual interest that could enhance the science return of the Kepler Mission.

5.2.6.2 2003 Mars Exploration Rover Mission (MER) Twin Rovers

These spacecraft are the products of a large effort led by Dr. Steve Squyres, the Athena payload team, and JPL. "The scientific objectives of the Mars Exploration Rover mission are the following: 1) Search for and characterize a variety of rocks and soils that hold clues to past water activity. In particular, samples sought will include those that have minerals deposited by water-related processes such as precipitation, evaporation, sedimentary cementation, or hydrothermal activity. 2) Determine the distribution and composition of minerals, rocks, and soils surrounding the landing sites. 3) Determine what geologic processes have shaped the local terrain and influenced the chemistry. Such processes could include water or wind erosion, sedimentation, hydrothermal mechanisms, volcanism, and cratering. 4) Perform "ground truth" — calibration and validation — of surface observations made by Mars orbiter instruments. This will help determine the accuracy and effectiveness of various instruments that survey Martian geology from orbit. 5) Search for iron-containing minerals, identify and quantify relative amounts of specific mineral types that contain water or were formed in water, such as iron-bearing carbonates. 6) Characterize the mineralogy and textures of rocks and soils and determine the processes that created them. 7) Search for geological clues to the environmental conditions that existed when liquid water was present. Assess whether those environments were conducive to life." (cited from http://mars.jpl.nasa.gov/mer) NASA will launch twin rovers to Mars in May and June, 2003, and will land on Mars in January 2004.

D. Des Marais is a member of the MER Science Operations Working Group (SOWG). As such, he will serve operationally as one of the leads of the SOWG Long Term Planning science theme groups (LTPSTG). The LTPSTGs develop and sustain a strategic plan that looks several sols (martian "days") ahead of the current tactical operations. Also, the LTPSTGs supervise and maintain the scientific discourse during the mission, leading to an integration of all-important tactical efforts and the achievement of mission success criteria, as well as the objectives for astrobiology and other key scientific disciplines. The involvement of Des Marais with the MER mission will enable scientific perspectives and priorities developed from the NAI research program to be factored into the MER mission. In turn, the findings and perspectives gained from this mission will help to guide research by the Ames team, as well as NAI, that is related to future initiatives in NASA's Solar System Exploration program.

Several of our proposed investigations will provide basic science in direct support of MER and future Mars missions. The aqueous mineral alteration studies in *Investigation 4* will provide a context for the interpretation of MER data. Similarly, the development and quantification of the concept of energetic habitability by *Investigation 4*, in concert with studies of classical habitability by *Investigation 1* , will establish stringent boundary conditions that may greatly narrow the search parameters for subsurface life on Mars.

5.2.6.3 Mars 2005 Reconnaissance Orbiter, CRISM Spectrometer

The 2005 orbiter mission includes the CRISM remote sensing instrument, developed and managed by the Johns Hopkins University Applied Physics Laboratory. CRISM's mission is the following, "…to find the spectral fingerprints of aqueous and hydrothermal deposits and map the geology, composition and stratigraphy of surface features. The instrument will also watch the seasonal variations in Martian dust and ice aerosols, check the water content in surface materials and collect atmospheric data — leading to new areas of exploration and discovery." (cited from http://crism.jhuapl.edu).

This near-infrared spectrometer will obtain high spatial resolution coverage of the entire planet in the near infrared wavelength. These observations should reveal geological deposits that might have recorded the activity of liquid water and thus the potential for habitable conditions on Mars.

D. Des Marais is a participating scientist for astrobiology on the CRISM instrument team. In this role, he will recommend sites for detailed near-infrared observations, with the intent of identifying and characterizing mineralogical assemblages that indicate the effects of aqueous alteration of the martian crust. The sites of interest would include the martian equivalents of the ophiolite-hosted spring systems to be examined by *Investigation 4* of this proposal. The proposed studies of these spring systems would indeed enhance the prospects for detecting analogous fossil spring systems during the Odyssey Mission.

5.2.6.4 Stratospheric Observer for Infrared Astronomy (SOFIA)

SOFIA is an airborne observatory that will study the universe in the infrared spectral region. SOFIA will serve in the development of observational techniques, of new instrumentation and in the education of young scientists and teachers in the discipline of infrared astronomy. NASA and the DLR, the German Aerospace Center, are creating the airborne observatory - a Boeing 747SP aircraft to accommodate a 2.5-meter reflecting telescope. SOFIA will be the largest airborne observatory in the world, and will make observations that are

impossible for even the largest and highest of ground-based telescopes. SOFIA will be based at NASA's Ames Research Center in California, and is expected to begin flying in 2004.

The expertise of the Ames Astrochemistry Laboratory Group (*Investigation 2)* has been and will continue to be used to develop design specifications for the mid-IR instrument to be flown on SOFIA. Furthermore, our spectral database and experimental capabilities will be used to interpret the mid-IR spectra that will be measured by SOFIA. Mid-IR spectroscopy is particularly suited to probe the prebiotic organic composition of cosmic materials. Thus, this will add to our understanding of the interstellar organic inventory of the Galaxy and sharpen our ability to distinguish between true and false spectroscopic biosignatures.

5.2.6.5 Space Infrared Telescope Facility (SIRTF)

SIRTF is the next in NASA's series of Great Observatories. It will be launched in April, 2003 for a 2.5 to 5 year mission. It consists of a 0.85-meter diameter lightweight telescope and three cryogenically cooled science instruments to provide imaging and spectroscopy from 3 to 180 microns. Incorporating large-format detector arrays and innovative choices in orbit and cryogenic architecture, SIRTF offers orders-of-magnitude improvements in capability over previous infrared telescopes. SIRTF will study phenomena ranging from our Solar System to the distant reaches of the Universe. SIRTF represents an important scientific and technical cornerstone of NASA's Astronomical Search for Origins Program.

The Astrochemistry Laboratory's database (*Investigation 2*) will be used to interpret the mid-IR spectra measured for many deeply embedded objects and PAH emitting objects, both galactic and extragalactic. The extragalactic aspect of this will deepen our understanding of the organic inventory of the cosmos and the evolution of these carbonaceous materials over the past several billion years. The Galactic aspect will reveal the nature of the raw materials that are the building blocks of planetary systems in the Milky Way. The team for *Investigation 2* will be involved in both aspects. The Astrochemistry Lab group has the world's largest database of PAH IR properties taken under realistic interstellar conditions. These will be used to map the distribution of carbon in many, many galaxies for the first time. Within our Galaxy, the infrared database of celestial ice analogs that is maintained by the Astrochemistry Laboratory will be used to interpret observations of dense star and planet forming clouds.

CoI David Hollenbach is also Co-I on the SIRTF Legacy Proposal entitled "Formation and Evolution of Planetary Systems " associated with the SIRTF Mission. His responsibilities include leading the protoplanetary disk gas modeling activity and data reduction effort. SIRTF Legacy projects are large and coherent science projects, not reproducible by any reasonable number or combination of smaller General Observer investigations. They are projects of general and lasting importance to the broad astronomical community with the SIRTF observational data yielding a substantial and coherent database. The selection process for SIRTF Legacy awards was highly competitive, with only 6 investigations selected from 35 proposals.

5.2.6.6 Stardust-Comet Dust Sample Return Mission

Stardust is the first U.S. space mission dedicated solely to the exploration of a comet, and the first robotic mission designed to return extraterrestrial material from outside the orbit of the Moon. The Stardust spacecraft was launched on February 7, 1999. The primary goal of Stardust is to collect dust and carbon-based samples during its closest encounter with Comet Wild 2, a rendezvous scheduled to take place in January 2004. Additionally, the Stardust spacecraft will bring back samples of interstellar dust, including recently discovered dust streaming into our Solar System from the direction of Sagittarius. These materials are believed to consist of ancient pre-solar interstellar grains and remnants from the formation of the Solar System.

S. Sandford, a CoI on this proposal, is also a CoI on the Stardust mission. The Ames Astrochemistry Laboratory played a direct role in development of the properties of the aerogel to be used for sample collection during this mission. We will be involved in sample analysis of the first cometary samples ever studied. For example, we will search for the presence of amino acids, amphiphilic molecules and deuterium enrichment within the organics and compare these with model predictions and the findings from similar studies in meteorites and interplanetary dust particles. These observations are direct extensions of the work proposed under Investigation 2.

5.2.6.7 Astrobiology Explorer (ABE)

ABE is the first space mission solely devoted to addressing key astrobiological questions. ABE is a cooled 60 cm diameter telescope equipped with spectrographs covering the 2.5-20 μm spectral range at a spectral resolution of > 2500, far in excess of any other mid-infrared mission. This will enable ABE, in conjunction with the spectral database available in The Astrochemistry Laboratory group (*Investigation 2 team*) to determine the prebiotic chemical inventory of all major astrobiologically important classes of astronomical objects at all stages of their history.

This comprehensive study will probe chemical composition, tracking the history of carbon from its birth in circumstellar ejecta, its voyage through the diffuse interstellar medium, incorporation in dense clouds and star forming regions, and ending with the composition of objects in the Solar System. ABE will also characterize the organic chemistry and carbonaceous dust composition of many galaxy types for the first time, including Dwarfs, Ellipticals, Disk Starburst, ULIRGS, and galaxies with active nuclei (AGNs).

S. Sandford, a CoI of *Investigation 2* of this proposal, is also PI of the ABE mission. Lou Allamandola is CoI of both proposals. The Ames Astrophysics Laboratory group has played a key role in the concept development, design, and proposal for this mission that has been funded for study. It is among the first MIDEX missions to succeed getting this support on the first attempt. Members of *Investigation 2* are intimately coupled to this mission and will be involved at all stages of its execution.

5.2.6.8 Earth Observing System (EOS)

EOS is the principal science mission of NASA's Earth Science Enterprise (ESE). EOS consists of a series of satellite observing missions, a science component, and a data system that supports a coordinated series of polar-orbiting and low inclination satellites for long-term global observations of the land surface, biosphere, solid Earth, atmosphere, and oceans. *Investigations 5 and 6* propose to make extensive use EOS satellite imagery for astrobiology research. Moreover, the investigators for *Investigation 5* are funded as PIs on current EOS investigation teams for Interdisciplinary Science (IDS) and the Large-Scale Biosphere Atmosphere Experiment in Amazonia (LBA), and can thereby effectively leverage all relevant ESE program support."

5.2.6.9 Future Missions

The New Frontiers Program is a new NASA planetary exploration program consisting of medium class missions ($650M) to be initiated in 2003 as recommended by the 2002 NRC Planetary Science Decadal Report. L. Allamandola, R. E. Young, and K. Zahnle are participating on science planning teams for Comet, Jupiter, and Venus missions that may be conducted under the New Frontiers Program. Their responsibilities include defining science objectives, and determining science instrument requirements.

Comet Surface Sample Return Mission Concept is being developed in response to NASA's New Frontiers program. L. Allamandola is among a small number of individuals who are developing a mission to respond to this flight opportunity. Dr. Joe Ververka of Cornell University is coordinating this effort as its PI. This mission is to return a significant amount of comet surface material, the primordial ingredients of our solar system. If the research proposed in *Investigation 1* is funded, its findings could influence the development of the concepts and capabilities of this mission.

Terrestrial Planet Finder (TPF) *Investigation 4* will directly support the design and interpretation of TPF and related missions (e.g., establishing frequency window, spectral resolution requirements), by identifying candidate search molecules and developing mechanistic models to help understand the controls on the atmospheric abundances of such species.

5.2.7 Information Technology

5.2.7.1 The NASA Center for Computational Astrobiology and Fundamental Biology

The principal objective of the NASA Center for Computational Astrobiology and Fundamental Biology (NCCAFB) at the Ames Research Center is to advance our understanding of the origin, evolution and distribution of life in the Universe, using theoretical and computational tools. NCCAFB, adopting the multidisciplinary spirit of Astrobiology and Fundamental Biology synthesizes diverse methods and viewpoints. The Center draws on scientists with different backgrounds and interests across different organizations at Ames and in other institutions involved in NASA-related research. The Center's capabilities are greatly leveraged by Ames' status as the Lead Center in information technologies. The NCCAFB currently has access to over 4,000 processors on supercomputers at the NASA Advanced Supercomputing Division.

The main goals of NCCAFB are:

- Provide scientific and programmatic leadership in computational astrobiology and fundamental biology.
- Organize an active international community of scientists working in the fields of computational astrobiology and fundamental biology by extensive research collaborations, visiting scholar programs, workshops, seminars, etc.
- Develop and participate in joint research programs with experimental astrobiologists.
- Participate in developing NASA astrobiology-oriented missions and in analyzing data from these missions.
- Promote applications of high performance computing, communication, data analysis, and database management to astrobiology.
- Promote applications of computational astrobiology to other disciplines and to technology transfer to benefit life on earth.
- Examples of astrobiological problems that are being studied by scientists at NCCAFB are:
- Self-organization of organic matter into the simplest life forms phylogenetic relationships between organisms;

- Evolution of metabolisms of simple organisms in ambient and extreme environments;
- Changes in gene expression of terrestrial organisms in the absence of gravity;
- The future habitability of earth, as determined by interactions between biosphere and the chemistry and radiation balance of the atmosphere;
- Design, stability and evolution of systems that support life in space.

More information about NCCAFB may be found at http://cca.arc.nasa.gov

5.2.7.2 NASA Center for Astrobioinformatics

Scientists at Ames are in the final stage of creating a state-of-the-art bioinformatics center for analysis of genomic and proteomic data from modern biological experiments. The capabilities of this center will be greatly enhanced by collaborations with several leading institutions in the country, such as Stanford University, U.C. Berkeley and National Cancer Institute. The resources of the Center and the assistance of professional bioinformatics staff will be made available to all NAI members at no charge.

A variety of research programs within NAI have relied to an increasing extent on obtaining and interpreting genomic and proteomic data. It is expected that this trend will continue. To receive maximum benefit from the data, one must often analyze and characterize data with a variety of computational bioinformatics tools and, in the process, use other, relevant information stored in multiple large databases.

To meet bioinformatics needs, the NASA Center for Computational Astrobiology and Fundamental Biology, based at Ames, has established a NASA Center for Astrobioinformatics, a dedicated facility for genomic analysis and a repository for microarray data. Its main function is to create bioinformatics environment needed for solving NASA-specific biology problems. This environment will consist of tools, databases and knowledge management system suited for NASA goals in astrobiology, space genetics and fundamental biology. It will provide a unified, integrated platform for NASA scientists and their collaborators to share data, information and analyses and, by doing so, greatly improve the capabilities for interdisciplinary, collaborative research. The capabilities of the environment will be further enhanced by developing new tools, adapting to the needs of bioinformatics several sophisticated techniques for data analysis, originally developed at NASA to support other research areas, and by integrating bioinformatics capabilities with massively parallel computational environment at Ames.

Among the capabilities of the Astrobioinformatics Center are:

- A dedicated Linux cluster of 40 CPUs for genomic computations,
- A data repository for genomic and proteomic data,
- A database system, which contains a number of databases that are continuously updated (including a catalogue of microarray data relating to experiments in space and ground-based controls),
- Software for analysis of genomic, proteomic and microarray data,
- Tools for data visualization and manipulation,
- Novel, sophisticated tools for discovery of regulatory networks by combining microarray data with background biological knowledge.

All of the tools offered by the Center are conveniently available though web interface. The Center will become operational by April 2003 and by the end of the year most of its capabilities should be in place.

The capabilities of the Center will be further leveraged by formal partnerships with the Advanced Biomedical Computer Center at the National Cancer Institute and the Center for Biomedical Computation at Stanford University, which are already in place, and by active collaboration with Biostatistics Department and U.C. Berkeley. Several other institutions, including NCBI and U.C. San Francisco expressed interest in developing similar collaborations. The scope of the collaboration includes exchange of tools, joint work on the development of new computational methods and databases and sharing experience with knowledge management techniques.

5.2.8 Linkage to Other Agencies

The programs for research, training, education and public outreach outlined in this proposal involve several outside institutions, including other government agencies and private foundations. Non-university institutions and their staff who are participating in this proposal include the following: Bay Area Environmental Research Institute (CoI M. Rabbette), California Academy of Sciences (CoIs T. Gosliner and C. Tang), Deutsche Zentrum für Luft- und Raumfahrte (CoI G. Horneck and Collaborator P. Rettberg), Flugmedizin RWTH Aachen (Collaborator E. Rabbow), Lunar and Planetary Institute (CoI A. Treiman), National Park Service (CoI L. Young), National Research Council (Collaborator V. Orphan), Royal Observatory, Edinburgh, UK (Collaborator R. Wolstencroft), and SETI Institute (CoIs M. Bernstein and R. Mancinelli).

6.0 FACILITIES AND EQUIPMENT

6.1 Investigation 1 - Formation and Evolution of Habitable Planets

No major special facilities or equipment are required for the proposed research. Commonly available computers and workstations will be sufficient.

6.2 Investigation 2 – Prebiotic Organics, from Space to the First Membranes

Central to this project is the ability to: (1) prepare realistic interstellar and cometary ice analogs under a wide variety of temperature and pressure conditions, (2) subject the analogs to radiation mimicking the interstellar and solar radiation fields, (3) measure the absorption spectra of the analogs from the ultraviolet through the far-infrared at all stages of sample preparation, (4) chemically analyze the photoproducts using techniques like HPLC, MS, GC-MS, chromatography, NMR, etc., and (5) characterize physical and chemical properties of the membranes produced by hydration of the amphiphillic molecules

The equipment needed to carry out items 1, 2, and 3 are in place and routinely operating at NASA-Ames. This equipment and the techniques associated with its use are described in Allamandola et al. (1988). This equipment now includes two research level Fourier transform infrared spectrometers, two completely computer-controlled UV-Visible, moderate resolution monochromator systems, one Alexandrite laser system capable of continuous tuning from 200 nm to 1 μm for in-situ luminescence studies, five high vacuum sample preparation systems which operate routinely in the 10-8 mbar range, and four cryostats. Four of the cryostats are closed cycle helium refrigerators that operate between 10 K and room temperature, the fifth is a helium flow system that operates between 4.2 K and room temperature. Four of the cryostat-vacuum systems are mated to the spectrometers such that complete UV to far-IR spectral coverage from 180 nm to 50 cm-1 is available. The fifth cryostat-vacuum system will be devoted to full time residue production if this proposal is funded.

The analytical techniques we now have available at Ames to use to carry out item 4 are wet chemical analysis, High Performance Liquid Chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS), deuterium labeled MS, and IR spectroscopy. The spectroscopic equipment, HPLC, as well as many of the wet chemical analytical tools necessary for this work are on hand in the Astrochemistry Laboratory at Ames while the GC/MS is in the Life Sciences Laboratory at Ames. Our collaboration with Professor Deamer at the University of California at Santa Cruz adds the availability of thin layer chromatography, fluorescence microscopy, and membrane analysis techniques. Our collaboration with Professor R. Zare at Stanford University gives us access to laser desorption mass spectroscopic sample analysis. Thus all is in hand to carry out the work proposed here.

6.3 Investigation 3 - The Origin and Early Evolution of Proteins and Metabolism

Computer facilities in the Pohorille laboratory at NASA-Ames Research Center include a Linux cluster of 20 modern workstations, each containing 2 CPUs, two 4-processor Silicon Graphics Origin 1000 workstation, three single-processor Silicon Graphics graphics workstation, several personal computers, X-window terminals and laser printers. The P.I. also has free access to the graphics facilities in the Computer Graphics Laboratory at Ames.

Advanced simulations will be performed using resources at the Numerical Aerospace Simulation (NAS) Division at NASA-Ames Research Center. NAS is equipped with several SGI massively parallel computers, including one 1024 CPU, one 512 CPU and two 256 CPU machines. The P.I. has free access to these resources.

Department of Molecular Biology Resources at Massachusetts General Hospital:

Laboratory: The Department of Molecular Biology at MGH occupies the upper half (floors 7-12) of the 150,000 square foot Wellman Research Building. Main laboratory facilities are housed on floors 8 through 11, with greenhouses, plant labs, and growth chamber facilities on floors 7 and 12. Typically, each main laboratory floor houses three research groups, placing 30-40 researchers on a floor. Each floor has a full complement of laboratory support spaces—two instrument rooms, warm/cold rooms, a balance and chemical storage room, a large tissue culture facility, electrophoresis and gel rooms, a dark booth and a specialty dark room, and generous fume hood capacity, including an isotope dilution hood. The Szostak laboratory comprises the right half of the 9th floor.

In addition to group- and floor-specific support spaces, each main floor also houses one or more department-wide support facilities. The 8th floor contains an animal storage and prep facility. The main administrative offices, supply area, materials management and purchasing operations, and mail services are found on the 9th floor. Glasswashing, media preparation, and sterilization facilities occupy the north (departmental) section of the 10th floor. These services process all laboratory glassware, plasticware, and pipettes for the Department, as well as supplying a broad range of sterile plates, growth media and buffers. Eleventh floor departmental spaces include the

conference/meeting room. The meeting room is separated from an adjacent, oversized tearoom by a sliding soundproof wall which, when opened, provides 80+ seating for departmental seminars and other social and scientific functions. A tea/conference room, equipped with full kitchen facilities, is also found on each of the four main floors.

Computer: The Department's UNIX and Windows NT servers and workstations form the heart of a sophisticated network of Macintosh and PC computers and printers. The network provides the vehicle for a highly developed package of genetic sequence manipulation and analysis programs. Macintosh & PC computers are available throughout the laboratories, offices, and in tea and conference rooms as well. At least 2 PostScript laser printers & a color printer are available for common use on each floor with additional laser printers available in secretarial offices. Flatbed & 35mm color digitizing scanners, photographic quality color printer, automatic slide maker, and dial-in remote access servers are also available. Full Ethernet communications networks are provided for within the Department and to the greater MGH computer network. All computers within the department are fully connected to the worldwide Internet via a high-speed connection. Interactive molecular design software is also available utilizing a high performance Silicon Graphics computer. All departmental computers are provided with a wide variety of state-of-the-art scientific and office productivity software. The Department maintains a number of internationally used World Wide Web servers including the Arabidopsis and Caenorhadbitis genomes.

Office: A two-person, floor secretarial office and the offices of the three faculty members are found on each of the four main floors.

Other: MGH Core Facility: the oligonucleotide synthesis and oligonucleotide sequencing cores, are housed within and run by the Department. The Core's 3 ABI 377 DNA Sequencers can run a minimum of 1000 samples/week and the Core is in the process of upgrading to a capillary electrophoresis machine which will allow a minimum of 384 samples/8 hr. day The Core's The cores 6 Millipore Expedite workstations allow synthesis of 88 concurrent oligos for a minimal output of 1760/wk. Additional services to all departmental researchers needing peptide synthesis, protein sequencing, and/or amino acid analysis are available within the MGH community.

Major Shared Equipment: The following list is representative of other major scientific equipment distributed throughout the Department: 20 ultracentrifuges, 12 high speed centrifuges, 4 low speed centrifuges, 4 liquid scintillation counters, 2 gamma counters, 4 lyophilizers, computer-driven spectrophotometers (including melting curve capability), dozens of tissue culture and other incubators, several preparative and analytical HPLC's, an FPLC, an analytical microdensitometer, a fluorescence spectrophotometer, ultramicrotome for EM sectioning, Imaging System, Total Array System, Picking and Gridding Q Bot, and a multitude of stereo, dissecting, inverted, and microinjection microscopes. Departmental darkroom facilities are equipped with 2 X-O-Mat processors, an instant slide maker, and an automatic print processor.

Szostak Laboratory: The Szostak laboratory consists of approximately 2000 sf of bench space (20 lab benches), along with additional special purpose rooms and shared instrument space detailed above. The lab contains four chemical hoods suitable for organic syntheses, and equipped with vacuum pumps and standard equipment for organic synthesis. NMR, FTIR, and mass spectrometer facilities are available through Harvard. The lab is well equipped with state of the art instrumentation including two HPLC systems, an automated mini-prep 24 processor, Octane/SSE Dual Computer Molecular Graphics WorkStations, UV-Vis and Fluorescence Spectrometers, Rapid Quench Flow device for fast kinetics, a Biacore X surface plasmon resonance instrument for measuring binding interactions, an instant imager for imaging radioactive gels, and 2 DNA thermal cycler 480 PCR machines. Equipment particularly relevant to this grant proposal includes a Pharmacia FPLC system for protein purification and a Jasco Instruments J-715 CD Spectrometer.

6.4 Investigation 4 - Biosignatures in Chemosynthetic and Photosynthetic Ecosystems

The Ames Electron Microscopy Laboratory houses a broad array of equipment for the micro-scale visual and mineralogical characterization of rock samples. This facility includes the following instrumentation and equipment: Hitachi S-4000 Field Emission SEM with EDS / digital imaging; JEOL 2000FX Analytical Electron Microscope with EDS / digital imaging; Hitachi H-500H Cryogenic Electron Microscope, with digital imaging; liquid helium, liquid nitrogen, cryotransfer and analytical cold stages; automated Philips/Norelco X-ray Diffractometer; Renishaw Confocal Raman Spectrometer / microscope; Nicolet Nexus 670 FTIR; Oxford TOPS-3 mini-cryo (4 Kelvin) scanning tunneling microscope (STM); Perkin-Elmer Pyris 1 differential scanning calorimeter (DSC); Perkin-Elmer Lambda 900 UV-Vis-NIR spectrophotometer; Perkin-Elmer Pyris 1 thermo-gravimetric analyzer; Agilent HPLC; Digital Instruments Nanoscope IIIA multimode atomic force microscope (AFM); Molecular Imaging Picoscan AFM; Class 100 clean room with laminar flow clean hood; Nikon

Microphot FXA petrographic microscope; Nikon SMZ-2 binocular microscope; Gatan dual ion-mill; Sorbol MT-2 microtomes with diamond knives; photographic dark room; VCR Group sample dimpler; VCR Group cold beam ion deposition system ("chrome coater"); Polaron Metal Sputter Coater; and an Edwards vacuum evaporator for metals and carbon.

Biogeochemistry and Microbial Ecology Laboratories at Ames maintain an array of instrumentation relevant to the chemical and visual characterization of microbial communities, including: gas chromatographs (Hewlett Packard, Inc.; SRI, Inc.; Trace Analytical, Inc.) equipped with FID (hydrocarbon), FPD (sulfur gases), TCD (atmospheric "fixed" gases), HgO-reduction (H_2, CO), and MSD (mass selective) detectors; an ion chromatography system (Dionex, Inc.) suitable for the determination of dissolved ion concentrations at micromolar levels; and a Finigan-Mat Delta Plus mass spectrometer with GC-combustion interface, suitable for compound-specific analyses of $^{13}C/^{12}C$ and $^{2}H/^{1}H$ at picomolar sample sizes.

Greenhouse Facility. Building N239 at Ames houses a rooftop greenhouse facility capable of maintaining live microbial mats under conditions of irradiance, flow, and substrate supply that approximate natural environments in the field. Recent studies demonstrate that the structure of the microbial community and the rates of major biogeochemical processes are retained, relative to field samples, for > 6 months during incubation in the greenhouse. This facility includes a UV-transparent enclosure and also flow boxes (flumes) that can maintain a total of 1.3 m^2 of active microbial mats. One table that contains these flow boxes is also equipped with a computer-controlled XYZ-positioning stage capable of deploying an instrument package (chemical and optical microsensors, cameras) at a desired location on/within the mats, with 50-mm vertical and 1-mm horizontal resolution. The positioning stage and instrument package can be operated remotely by collaborators via a web-based interface.

Molecular Biology Facility. We are currently developing a laboratory for genetic analysis and characterization of microbiological samples. The new molecular biology laboratory is equipped with a –80°C So-Low freezer, an MJ Research thermal cycler for PCR, electrophoresis gel rigs, a Fotodyne UV gel imaging system, incubators, and an Eppendorf refrigerated centrifuge with fixed angle rotor.

6.5 Investigation 5 - Modeling Ecosystems and Biospheres

The Ecosystem Computational Facility (ECF) at NASA Ames is well equipped and staffed to support our modeling activities. ECF will supply most of the necessary computational support to the research, including data backup and storage, GIS software support, digital scanner, color printing and film recorder services. Existing equipment in direct support of this research includes several computers hosting systems modeling, GIS, spreadsheet, word-processing, statistics and image processing software (for an updated on-line description, see http://geo.arc.nasa.gov/info/ECF_Description.html). Specific to our research, four workstations, including a 4-CPU Sun Microsystems UltraSPARC server, are available for modeling and image processing tasks, while a fifth system is shared between image processing and GIS tasks. All systems have 24-bit color displays. The primary image processing package is ERDAS IMAGINE, although several other packages (listed below) are also available. Raster imagery from both aircraft and satellite sensors is used extensively in the analysis process, and ranges from standard Landsat and SPOT data to experimental hyperspectral sensors such as AVIRIS. GIS software available includes ARC/INFO (including GRID, TIN, and NETWORK), and GRASS, plus ENVI and Matlab for image analysis. In addition to our own ECF, we periodically have access (albeit not unlimited) to NASA Ames Center-wide facilities for high performance computing. Fees are likely to apply in the future for computing time on these facilities.

6.6 Investigation 6 - Hind-Casting Past Environments

We have access to the Ecosystem Computational Facility (ECF) described in section 6.5. We also have access to laboratories on the ARC center grounds. There, we have glassware, plastic ware and hardware; a microscope workstation, with the following microscopes: 1) Zeiss Photomat light microscope with automatic photomicrography equipment, phase contrast and planapochromatic optic, fluorescence equipment. 2) Zeiss Utraphot II light microscope with zoom attachment and large format photomicrography attachment. 3) American Optics Spencer light microscope with phase contrast optic. 4) Wild Stereo microscope with epi and transillumination and zoom optic. 5) High Resolution Imaging Microscope (HIRIM) with computer for cell-level spectrometry; various balances with precision to 0.01g; a Zeiss adapter 0.5 x for c-mount camera, 1-CCD Pulnix high resolution color video camera with light and speed regulation enabling the video signal to be directed to a monitor, a video printer, a video recorder or to a computer desktop and lap-top computers (Apple and PC), printers, scanners, and access to large format (91 cm) printers; a glasshouse on the building terrace; an Optronics OL-752 portable spectroradiometer, double monochromator with OL 752-O-PMT head; ADC LDC4 gas exchange

(photosynthesis) measurement system and a Minolta SPAD-502 chlorophyll meter with calibration disk.. In addition to the ECF computers and software, we have various Apple and PC personal computers, statistical and plotting software, word processing software, photo-manipulation software.

Laboratory - NASA Ames Research Center:

- Fumes-hoods
- Open growth chamber with light and UV radiation sources, timer for day/night simulation and monitor lights
- Ohaus balances (piezoelectric) (Precision = 0.1g)
- Metler piezoelectric balance (Precision = 0.01g)
- Metler analytic balance
- Electric oven
- Electric furnace
- Centrifuges
- Vortex mixers, hotplates and small equipment
- Glassware, plasticware and hardware
- Freezer
- Refrigerator
- Glasshouse on the building terrace with airflow, temperature, humidity and light controls, telephone line and power connections
- Optronics OL-752 portable spectroradiometer, double monochromator with OL 752-O-PMT head. Spectral range 200-800nm, with OL 752-150 plug-in dual calibration and gain check source module, OL 752-10E spectral irradiance [250-800nm], OL 65A programmable current source, OL 752-50 battery pack, 2-OL 752-51 spare batteries, and OL 752-70 carrying cases
- Minolta SPAD-502 chlorophyll meter with calibration disk
- Microscope workstation, with the following microscopes: 1) Zeiss Photomat light microscope with automatic photomicrography equipment, phase contrast and planapochromatic optic, fluorescence equipment. 2) Zeiss Utraphot II light microscope with zoom attachment and large format photomicrography attachment. 3) American Optics Spencer light microscope with phase contrast optic. 4) Wild Stereo microscope with epi and transillumination and zoom optic. 5) High Resolution Imaging Microscope (HIRIM) with computer for cell-level spectrometry.
- 1-Zeiss adapter 0.5 x for c-mount camera, 1-CCD Pulnix high resolution color video camera with light and speed regulation. Video signal can be directed to the monitor, the video printer, a video recorder or to the computer
- 1-C-mount lens for macro video
- 1-Sony high resolution color monitor
- 1-Sony UP-2200 Color Video Printer
- Statistical and plotting software, word processing software, photo-manipulation software
- Desktop and lap-top computers (Apple and PC), printers, scanners, and access to large format (91 cm) printers
- ADC LDC4 gas exchange (photosynthesis) measurement system

Office: Complete office facilities on Ames' premises.

6.7 Investigation 7 - Interplanetary Pioneers

Rothschild and Mancinelli each have two labs at NASA Ames Research Center. Jointly the labs have all of the basic equipment for studies of microbial physiology and ecology including a Zeiss Axioscope microscope with phase optics, fluorescence, DIC optics and both film and digital cameras for microscopical analysis. Additionally, they have a model Z1 Coulter Counter, a UV/Visible plate reading spectrophotometer and fluorometer (Molecular Devices), glassware, balance, pH meter, microprobes for pH, O_2 and CO_2 measurement, a Li-Cor model LI-185B quantum/ radiometer/ photometer with an spherical sensor, a broad band UVA/UVB meter (Solar Light Company), an underwater temperature sensor, several microfuge centrifuges, a radioisotope hood, anaerobic hood, gas manifolds, incubators, freezers, chromatography refrigerators, and assorted supplies. In addition, their labs have assorted equipment for biochemical and molecular analyses (e.g., speed vac centrifuge, thermal cycler, horizontal and vertical gel electrophoresis equipment, UV and white light boxes and cameras). Centrifuges, autoclaves, combustion oven and drying ovens are all available on the same floor. Rothschild has a EGC environmental chamber to simulate diurnal changes in light and temperature and maintain stock cultures, and Mancinelli has four New Brunswick incubator/shakers set at different temperature and light levels. For computer facilities, Rothschild and Mancinelli have six powerMacs including G4s, printers and scanners, and one PC. Facilities for experiments performed by the collaborators such as isolation of desert varnish and antioxidant analyses are available already in the relevant laboratories.

Specialized equipment includes three unique facilities. A solar simulator is located in Mancinelli's lab at NASA Ames. A space simulation facility at The Institute of Aerospace Medicine at the DLR has nine test beds for extreme environment simulation which can be modularly equipped with ionizing and UV radiation sources, atmosphere control, pumping systems, and temperature control. Depending on the space parameters to be simulated and the duration of the experiment, the most suitable facility will be selected and refurbished. Through a grant to the DLR from ESA the facilities are being

refurbished specifically for this purpose. For example, space simulation facility #2 served as ground control facility for the 9 month long EURECA mission (Horneck et al., 1995). It provides a vacuum up to 10^{-4} Pa, 8 deuterium lamps inserted in the lid to simulate solar UV radiation including the vacuum UV range (>110 nm), a cold plate to duplicate the temperature profile in space, and a sample tray with an optical filtering system to accommodate different biological test systems. Finally, organisms that are selected for flight will be flown in the EXPOSE facility on the outside of the International Space Station, an ESA-built facility designed to test the ability of organisms to survive the space environment. The flight experiments will be flown as part of the already scheduled experiments by two team members: SPORES (PI: G. Horneck) and OSMO (P.I. R.L. Mancinelli), if appropriate. EXPOSE is one of the ESA-built facilities designed to test the ability of organisms to survive the space environment, scheduled for 2004. The laboratory studies will also give rise to new experiment proposals for EXPOSE-follow-on missions.

6.8 Ames Computational Facilities at Ames Research Center continue to enjoy a world-class status. These facilities have long enhanced theoretical research in planetary science and astronomy at Ames (e.g., in support of *Investigations 1 and 2* in this proposal). More recently, they have enhanced research efforts in "Bioinformatics" (related to *Investigation 3*). These capabilities will thus enhance the overall research effort, as indicated in Section 3 of this proposal, with benefits to NAI members of other institutional teams, as the network of collaborations develops.

APPENDIX A - ACRONYMS

16S-rDNA	Small Sub-Unit Ribosomal DNA
16S-rRNA	Small sub-unit Ribosomal Nucleic Acid
ABE	Astrobiology Explorer
ABP	ATP Binding Protein
AFM	Atomic Force Microscopy
AGNs	Active Galactic Nuclei
AMP	Adenosine Monophosphate
AMR	Adaptive Mesh Refinement
ARC	Ames Research Center
ATP	Adenosine Triphosphate
AVHRR	Advanced Very High Resolution Radiometer
Bchla	Bacteriochlorophyll-a
BEQ	Biological Energy Quantum
BioPan	The Biological Spaceflight facility used by ESA for free flyers
C - Terminal	Carbonyl end of a protein or peptide
CAN	Cooperative Agreement Notice
CAS	California Academy of Sciences
CASA	Carnegie Ames Stanford Approach - Biosphere Model
CBMC	Center for Biomedical Computation (Stanford)
CCSM	Community Climate System Model
cDNA	Complementary or Cloned DNA
CREST	Computational Center for Molecular Studies at Jackson State University
CRICyT	Centro Regional de Investigaciones Cientificas y Tecnologias, Argentina
CSB	Colorless Sulfur Bacteria
CSF	Center for Star Formation
CSUMB	California State University at Monterey Bay
CYN	Cyanobacteria
D/H Ratio	Deuterium/Hydrogen Ratio
DBL	Diffusive Boundary Layer
DLR	Deutsche Zentrum für Luft-und Raumfahrt (German Aerospace Center)
DNA	Dioxy Nucleic Acid
DOI	Division of Interpretation
DSC	Differential Scanning Calorimeter
ECBILT	Royal Netherlands Meteorological Institute, Center for Climate Research General Circulation Model
ECF	Ecosystem Computational Facility
EDS	Energy Dispersive X-Ray Spectroscopy
EMPA	Electron Microprobe Analyser
EOS	Earth Observing System
ESA	European Space Agency
ESE	NASA's Earth Science Enterprise
ETM+	Enhanced Thematic Mapper Plus
EURECA	Facility used to house microbe experiments flown on NASA's Long Duration Space Flight Facility.
EXPOSE	Facility used by ESA to expose samples to the space environment on an outside platform of the ISS

FCSUMB	Foundation of California State University at Monterey Bay
FESEM	Field Emission Scanning Electron Microscopy
FID	Flame Ionization Detection
FISH	Fluorescent in situ hybridization
FPD	Flame Photometric Detector
GCM	Gas-Chromatography-Mass Spectrometery (an analytical technique)
GCM	General Circulation Model (Mars)
GIS	Geographic Information System
GNS	Green Non-Sulfur Bacteria
GPS	Global Positioning System
GSH	Glutamyl-Cysteinyl-Glycine
GSSG	Glutathione Disulfide
GTP	Guanosine Triphosphate
HBCU	Historically Black Colleges and Universities
HPLC	High Performance Liquid Chromatography
IDP's	Interplanetary Dust Particles (asteroidal and cometary)
IMF	Initial Stellar Mass Function
IR	Infrared
IRTF	Infrared Telescope Facility (a ground based telescope)
ISM	Interstellar Medium
ISO	European Infrared Space Observatory
ISS	International Space Station
JSC	Johnson Space Center
JWST	Jack Webb Space Telescope
k's	Refractive Indices
LBA	Large Scale Biosphere - Atmosphere Experiment
LPI	Lunar Planetary Institute
MBGC	Microbial Biogeochemistry
MBL	Marine Biological Laboratory
MBP	Maltose Binding Protein
MC	Monte Carlo Method
MER	Mars Exploration Rover
MER-SOWG	Mars Exploration Rover-Science Operation Working Group
MET	Methanogenic Bacteria
MeV	Millions of Electron Volts
MGCM	Mars General Circulation Model
MIDEX	Mid-sized Explorer Mission
MODIS	Moderate Resolution Imaging Spectroradiometer
MOU	Memorandum of Understanding
MS	Mass Spectrometry
MSD	Mass Selective Detectors
M-type	One category of stars, classified according to its range of masses. M-type stars are smaller than our own Sun, a G-star
NAD^+	Nicotine Amide Phosphate
NADPH	Hydrogen Nicotine Amide Phosphate
NAI	NASA Astrobiology Institute
Nakhlites	Class of Meteorite probably of Martian origin
NASA R&A	NASA Research & Analysis Programs
N-body	Three or more gravitational interacting bodies

NCAR	National Center for Atmospheric Research
NCCAFB	NASA Center for Computational and Fundamental Biology
NDVI	Normalized Difference Vegetation Index
NFP	New Frontiers Program
NIMS	Near-Infrared Mapping Spectrometer on the Galileo Spacecraft.
NIR	Near Infra-red radiation
NIRSPEC	Near-Infrared Echelle Spectrograph at Keck
NMR	Nuclear Magnetic Resonance
NOAA	National Oceanic and Atmospheric Administration
NPS	National Park Service
NRC	National Research Council
n's	Refractive indices
NSF	National Science Foundation
N-Terminal	Amino Group end of a peptide chain
OFVEC	Old Faithful Visitor Education Center
PAHs	Polycyclic Aromatic Hydrocarbons
PAR	Photosynthetically Active Radiation
PDR	Photodissociation Region
Promega	Genomic DNA Purification Kit
PSB	Purple Sulfur Bacteria
PSIG	Project Science Integration Group
QSAR	Qualitative Structure - Activity Relationship
RNA	Ribonucleic Acid
ROSE	Response of Organisms to the Space Environment
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscope
SIMS	Secondary Ion Mass Spectrometry
SIRTF	Space Infrared Telescope Facility
SOD	Superoxide Dismutase
SOFIA	Stratospheric Observatory for Infrared Astronomy
SpeX	Medium-Resolution Spectrograph on the IRTF.
SPORES	Experiment using Bacillus Subtilis spores to be flown on the expose facility aboard the Space Station
SRB	Sulfate Reducing Bacteria
SSAC	Space Science Advisory Council
SSIOUX	Space Simulation for Investigating Organics, Evol ution and E xobiology. A series of experiments using the space simulation facilities at the German Aerospace Facility in Cologne, Germany, sponsored by the ESA
SST	Sea Surface Temperature
STELLA	Computer Program used for Modeling
STM	Scanning Tunneling Microscopy
SwRI	South West Research Institute
TCD	Thermal Conductivity detector
TEM	Transmission Electronic Microscopy
TERC	Technical Education Research Center
TPF	Terrestrial Planet Finder
ULIRGS	Ultra Luminour Infrared Galaxies
UMa System	Ursae Majoris Extrasolar System

UMIST	University of Manchester Institute of Science and Technology
UV	Ultra Violet
UV-Vis	Ultra Violet Visible Wavelength Range
VIMS	Visible and Infrared Mapping Spectrometer
μXRD/XRF	Micro X-Ray Deffraction/X-Ray Fluorescence

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Astrobiological Pathways: From the Interstellar Medium, through Planetary Systems, to the Emergence and Detection of Life

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3. Executive Summary

Unifying Intellectual Focus. The NASA Astrobiology Institute team led by the Carnegie Institution will be dedicated, over the coming five years, to the study of the gradual evolution of organic compounds from prebiotic molecular synthesis and organization to cellular evolution and diversification – processes central to the missions of the NAI. Our program attempts to integrate the sweeping narrative of life's history through a combination of bottom-up and top-down studies. On the one hand, we study processes related to chemical and physical evolution in plausible prebiotic environments – the interstellar medium, circumstellar disks, extrasolar planetary systems, and the primitive Earth. Complementary to these bottom-up investigations of life's origin, we will continue our field and experimental top-down efforts to document the nature of microbial life at extreme conditions, as well as the characterization of organic matter in ancient fossils. Both types of efforts inform our development of biotechnological approaches to life detection on other worlds. In the process, we will continue to serve as a resource center for all members of the NASA Astrobiology Institute.

Motivation and Rationale. The most compelling opportunities in astrobiology stem from new interdisciplinary approaches to fundamental questions about life's origin and its distribution in the universe. We continue to be motivated by the importance of these questions and by our unusual interdisciplinary team approach to scientific research. The Carnegie Institution supports astrobiologists with backgrounds in astronomy, biology, geology, and chemistry on a single Washington, D.C., campus. We are motivated to make additional progress toward addressing these fundamental questions, while continuing to serve as a dynamic, interactive resource center for other NAI teams. The rationale for our proposal is guided by the visionary mission of NASA's Astrobiology Program, as defined by the Astrobiology Roadmap. In particular, we address the nature and distribution of planets (Goal 1), past and present habitable environments (Goal 2), the chemical origin of life (Goal 3), past life on Earth (Goal 4), the environmental limits on life (Goal 5), and the identification and detection of biosignatures (Goal 7). Moreover, through our daily interactions we will continue to strive to integrate these separate goals into a fuller understanding of the continuous processes that characterize life's origin, evolution, and distribution in the universe.

Proposed Research Plan. Our proposed research activities for the coming five years focus on life's chemical and physical evolution, from the interstellar medium, through planetary systems, to the emergence and detection of life. We propose seven integrated areas of research:

1. We will continue to apply theory and observations to investigate chemical evolution in the interstellar medium, in circumstellar disks, during planetary formation, and on Solar System bodies.

2. We will conduct analytical research on extraterrestrial samples, including meteorites and interplanetary dust particles, with an emphasis on organic molecules and evidence for water.

3. We will study prebiotic chemical and isotopic evolution on Earth, with a new emphasis on the sulfur cycle and the role of sulfur in prebiotic organic synthesis.

4. We will investigate possible mechanisms of prebiotic molecular selection and macromolecular organization, including the self-organization of amphiphiles and the selective adsorption of organic molecules onto mineral surfaces.

5. We will continue to study life in extreme environments, with field studies of hydrothermal microbial communities and laboratory studies of stress adaptation of microbes in high-pressure and high-temperature environments.

6. We will examine ancient fossils and microbes fossilized in the laboratory with a variety of analytical techniques to assess preservation mechanisms of molecular biosignatures. We will study modern geothermal systems to investigate preservation of biosignatures during silicification in these environments.

7. We will apply our enhanced understanding of life's chemical and physical evolution to develop new techniques in astrobiotechnology – procedures that will be applied to the design and testing of instruments for life detection, initially in terrestrial settings and eventually on spacecraft to be sent to other Solar System bodies.

Fuller understanding of life's origin, evolution, and distribution requires major advances on all these topics, as well as the extensive challenge of integrating these topics. During the next five years of NAI support we anticipate significant progress in each of these seven areas, as well as considerable advances derived from integrating these theoretical, experimental, and field studies.

Training, Education, and Public Outreach. As members of the NASA Astrobiology Institute we will continue our firm commitment to a dynamic, sustained program of education, public outreach, and training at the K-12, undergraduate, graduate, and postgraduate levels. This effort is facilitated by our widely acknowledged programs, including the First Light Science School, the Carnegie Academy for Science Education (CASE), the Carnegie Institution Summer Intern Program, and the Capital Science Lecture Series. Our continuing NAI outreach programs will include the following:

- We will continue to introduce astrobiology themes to CASE, which will provide in-service training and summer workshops for more than 1,000 teachers in the District of Columbia Public School system.

- We will introduce astrobiology themes to our widely distributed educational product series, as well as our astrobiology web site.

- The Carnegie Institution will continue to sponsor general audience lectures by prominent astrobiologists through the successful Capital Science public lecture series at Carnegie's headquarters in northwest Washington, D.C.

- We will initiate collaborative activities with the Minority Institution Astrobiology Collaborative, including research, internships, teacher training, and publications.

- We will develop an exhibition on astrobiology in the lobby of the Smithsonian Institution's National Museum of Natural History, one of the most frequently visited museums in the world.

- We will support approximately 8 NAI summer interns per year as part of the Carnegie Institution's program for undergraduate and high school students. This astrobiology program has already produced one Intel finalist and one Westinghouse finalist.

- We will support approximately 12-15 NAI Pre- and Postdoctoral Fellows per year.

- We will participate through teaching and research opportunities with the new Astrobiology Ph.D. program at George Mason University. The first Ph.D. candidate will be commencing research at Carnegie in the summer of 2003.

Management Plan. All investigators and collaborators have clearly defined roles and responsibilities. Team activities will be managed by an Executive Committee, consisting of representatives from the partner institutions and chaired by the Principal Investigator. Our plan for multi-institutional cooperation and coordination includes regular all-consortium symposia, frequent intersite lectures, regular visits of senior staff to partner institutions, and more extended exchanges of students, predoctoral scholars, and other staff. Through the use of the internet, the World Wide Web, and the next generation internet, we will continue to make extensive use of electronic communication. Beyond daily communications among individuals, we will continue to provide frequent Web-based news updates to all team members. We remain committed to the development of Web-based instructional and outreach materials, and we will continue our development of electronic communications to enable access to data archives and summaries of research activities.

Proposed Institutional Commitment. The Carnegie Institution will continue to make a substantial commitment of resources to astrobiology. Dr. Wesley Huntress, who initiated the Astrobiology Institute while at NASA's Office of Space Science, was selected as Director of the Geophysical Laboratory in 1998. Subsequently, the institution has added six astrobiologists to the senior scientific staff (Paul Butler, Larry Nittler, James Scott, Sara Seager, Andrew Steele, and Alycia Weinberger). The institution will provide full salary support for all 16 members of the senior research staff who are investigators on this project, 50% of the stipends of all NAI-sponsored Postdoctoral Research Associates, support for half of the 8 college and high-school astrobiology summer interns, and full support of all NAI-related technicians and Information Technology staff. An array of laboratory instrumentation with an aggregate value in excess of $10 million will continue to be made freely available to research in support of NASA Astrobiology Institute objectives. The Carnegie Institution will continue as well its major financial commitment to programs in science education and outreach. Significant contributions of salary support and laboratory instrumentation and facilities will also be made by each of the partner institutions in our consortium.

Innovation and Distinguishing Features. Our NAI team is distinguished by several features:

- Scientific breadth: We integrate experimental, theoretical, observational, and field studies by experts in astronomy, biology, geochemistry, organic chemistry, mineralogy, planetary science, microbial ecology, and biotechnology in one laboratory.

- Scientific focus: We direct this broad expertise toward fundamental astrobiological questions regarding the origin and distribution of life in the universe.

- Shared resources: We provide important resources for the larger astrobiology community. During the first five years of the NAI we hosted more than 30 researchers from other NAI teams, NAI foreign associates, and other institutions with interests in astrobiology.

- E/PO: Our consortium is dedicated to training, education, and outreach programs that reach numerous students, teachers, and members of the general public.

Summary. The mission of the Carnegie Institution's vibrant astrobiology program matches that of the NASA Astrobiology Institute itself – to explore the origins and distribution of life in the universe. Throughout the past five years the Carnegie team has played a significant and expanding role in shaping the context and content of NAI. Our dynamic and extensive research program, our acclaimed education and public outreach efforts, and our unswerving commitment to the future of astrobiology provide the primary motivations for this proposal. We look forward with enthusiasm to continued participation in this unique program.

5. Research and Management Plan

5.1 Introduction

Astrobiology, the search for the origin and distribution of life in the universe, demands a bold, interdisciplinary research strategy. Life's sweeping story began in the icy near-vacuum of the interstellar medium and the dynamic environments of circumstellar disks, where organic molecules first were formed and concentrated. Life emerged by the diverse chemical and physical processes of nascent planets and moons, and it continues to evolve in concert with its home world, on Earth, and perhaps elsewhere in the Cosmos. To explore such a history requires the integration of forefront research in astronomy, chemistry, planetary sciences, and biology. Here, we propose such a far-reaching effort.

Life's origin remains a mystery, but all life as we know it requires an environment with three key ingredients: energy, water, and access to the critical elements from which biochemistry is assembled. Our proposal outlines a narrative that traces the gradual evolution from prebiotic molecular synthesis and organization to cellular evolution and diversification.

We propose to begin our study of life's origin with investigations of the chemical evolution of the interstellar medium and circumstellar disks – environments in which simple organic molecules first appeared and concentrated. We also propose to consider the fate of this organic matter during planetary formation through observations of extrasolar planets and models of planetary system formation. These studies will be informed by our ongoing analytical research on organic molecules and evidence for water in extraterrestrial samples, including meteorites and interplanetary dust particles.

Life's narrative continues via prebiotic molecular evolution on Earth, a subject that has inspired a half-century of chemical research. We propose to expand our investigations into the role that transition-metal minerals may have played in promoting key prebiotic organic reactions. Such chemistry is closely connected to the chemical and isotopic evolution of Earth's sulfur cycle, which provides a key to understanding global geochemical and biochemical changes during Earth's first 3 billion years. Additionally, we will evaluate processes by which specific prebiotic organic species (such as chiral molecules) were selected, concentrated, and organized into macromolecular systems, both through molecular self-organization and selective adsorption on mineral surfaces.

Complementary to these bottom-up investigations of life's origin, we will continue our top-down efforts to document the nature of microbial life at extreme temperatures and pressures, including field studies of hydrothermal environments at deep-sea hydrothermal vents and in the newly opened hydrothermal district of Kamchatka. Fieldwork will be complemented by laboratory studies of high-pressure microbial survival and adaptation. We will also focus on pathways of microbial metabolism – processes that influence the evolution of planetary environments.

Our proposed studies of prebiotic organic chemistry and microbial biochemistry inform the search for life on other worlds. We therefore propose to continue attempts to identify reliable biosignatures by measuring elemental, isotopic, and molecular characteristics of ancient fossils and to compare these results with measurements of abiotic suites of organic species derived from carbonaceous meteorites and our organic synthesis experiments. These studies will guide our development of analytical techniques for remote detection of biosignatures on space flight missions.

This ambitious program of research is enabled, in part, by the unusual degree of scientific integration of the Carnegie Institution team. Since 1998 the Carnegie Institution has added six

new staff scientists in key astrobiology fields, including planet formation, detection of extrasolar planets, cosmochemistry, microbial ecology, extremophiles, and life detection. We enjoy an unusual degree of interdisciplinary interaction and collaboration because these scientists have offices and laboratories on Carnegie's close-knit Broad Branch Road campus in Washington, D.C. In addition to the core team of 18 Carnegie astronomers, chemists, planetary scientists, and biologists, we have invited nine scientists from seven partner institutions to fill key gaps in the narrative of life's origin and evolution.

Funding of this proposal will result in at least three important benefits to the NASA Astrobiology Institute. First, this proposal describes an integrated program of research that will not be attempted without NAI support. Most Carnegie NAI Investigators are engaged in several other research activities as well as astrobiology. We estimate that as many as half of the nearly 200 NAI-sponsored peer-reviewed articles (**Section 14**) produced by Carnegie team members between 1998 and 2003 (including all of the work by NAI-supported undergraduate, predoctoral, and postdoctoral fellows) would not have been completed without NAI funding.

Second, NAI fosters our interdisciplinary team approach to astrobiology research. The daily on-campus interactions among astronomers, chemists, planetary scientists, and biologists are an important outcome of NAI's sponsorship, and they have influenced strongly each individual's approach to the field. Thanks to NAI support, many collaborative cross-disciplinary projects have resulted. The net scientific contribution is greater than the sum of the parts.

Thirdly, thanks to NAI, Carnegie scientists have increasingly taken leadership roles in the astrobiology community. In addition to Carnegie's exemplary Education and Public Outreach efforts, team scientists have delivered more than 100 public lectures on astrobiology topics and have participated widely in meetings on astrobiology themes. For example, the Carnegie Institution hosted the April 2001 General Meeting of NAI in Washington, D.C., where they contributed 20 oral and poster presentations. At the February 2003 NAI General Meeting in Tempe, Arizona, Carnegie scientists and their team colleagues participated in 33 oral and poster presentations – more than 15% of the presentations at that meeting.

Astrobiologists pose profound questions about life's origin and cosmic distribution. Significant progress toward the field's inspiring goals will require broad vision, effective interdisciplinary collaboration, and the hard work of many dedicated scientists. With NAI support we will continue to strive toward those goals.

The format of the research section of this proposal breaks the discussion into seven scientific focus areas, each self-contained with the essential background material and specific proposed research. Because there is considerable synergy across the various scientific focus areas, we highlight where research in each section connects with other proposed research. Our Management Plan (**Section 5.9**) describes in detail how we will integrate these scientific initiatives into an effective element of NAI.

5.2 From Molecular Clouds to Habitable Planetary Systems

How far does prebiotic carbon chemistry advance in developing complex organic compounds in astronomical environments? Terrestrial life is carbon-based, and extraterrestrial life may be as well. Organic compounds are composed predominantly of carbon and hydrogen, but also oxygen, nitrogen, and sulfur. These five elements (CHONS) provide the most basic chemical prerequisites for life. Forged as elements in stellar interiors, carbon, oxygen, nitrogen, and sulfur undergo considerable chemical evolution in both diffuse and dense interstellar clouds (e.g., Prasad & Huntress 1980). Over 100 different molecules, produced by a variety of gas-phase and gas-grain interactions (e.g., Langer et al. 2000), have been detected in interstellar and circumstellar gas and dust clouds. Deciphering how the CHONS elements evolved from their

astronomical origins to their participation in living organisms on habitable planets constitutes one of the most fundamental questions of astrobiology.

5.2.1 Protoplanetary and debris disks (Astrobiology Roadmap Objective 3.1)

Background. The link between the material of the interstellar medium and the ultimate composition of planets lies in the manner material is processed in circumstellar disks. The very different bulk compositions of the terrestrial, gas giant, and ice giant planets, as well as the asteroids and Kuiper Belt objects, must arise from some combination of segregation and survival of material in the early solar nebula and planet formation processes. We need to understand the evolution of the chemical content of planet-forming disks as a function of both location and time.

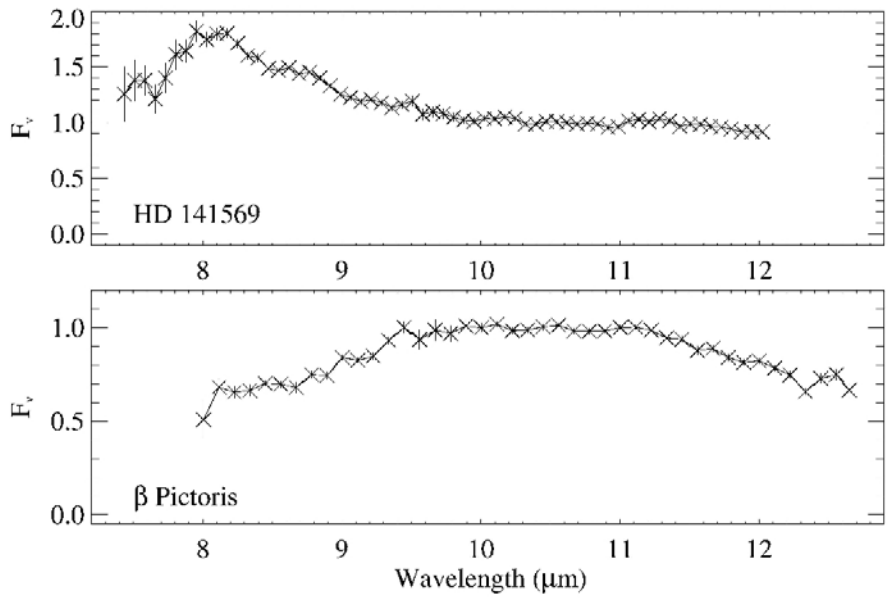

Figure 5.2.1. Spectra taken in the mid-infrared from the W. M. Keck Observatory, normalized arbitrarily at 11 μm. Top panel shows the 8-13 μm spectrum of HD 141569A, a 5-Myr old star approximately 20 times as luminous as the Sun. A prominent peak at 7.8 μm as well as a shoulder at 8.6 μm are evidence for PAH emission. However, the usually strong 11.2 μm PAH peak is not present. The line ratios suggest that the PAHs in this disk are both large and highly ionized. The bottom panel shows a spectrum of Beta Pictoris, a 12-Myr old star approximately 7 times as luminous as the Sun. Here silicate grains produce the broad peak centered at ~10 μm and there is no evidence for PAHs (Weinberger et al. 2003).

The infrared (IR) is an excellent wavelength range for studying the compositions of intermediate-age circumstellar disks (those in the process of losing their gas). Grains at temperatures of several hundred Kelvin, typical for dust at distances of 1-50 AU from the central star, emit strongly in the IR. Thus, IR emission emanates from dust grains in the region where planets formed in our own planetary system. Circumstellar disks are also known to be composed of materials, such as silicate grains, water and methane ices, and polycyclic aromatic hydrocarbons (PAHs), which have spectral features in the 1.5-25 μm (near-IR to mid-IR) region. Water ice is the most commonly detected material on surfaces in the outer Solar System and is found everywhere from comets, to the surfaces of satellites, to the small particles in the rings of the giant planets (Clark 1980; Irvine et al. 2000; Brown et al. 2000). Methane ice is less prevalent but is still the dominant feature in the spectrum of Pluto (Cruikshank et al. 1976). Methane may be quite common on the surfaces of Kuiper Belt objects. In disks, whether methane or water ice is more abundant may depend on progenitor cloud chemistry, the size of

the bodies (Pluto's surface is covered mostly with methane ice and smaller Charon's with water ice), or distance from the central star. Ices were proposed to explain the high visual and near-infrared albedos of large (>1 μm) disk grains, which are typical for the ice-silicate mixtures in our own Solar System, and to explain an increase in the surface brightness of the disk around HD 141569A at the ice-condensation radius (Weinberger et al. 1999). Determining the location of ices in circumstellar disks requires high-dynamic-range spectrometry in the near-IR.

Perhaps the most striking feature of spectra from circumstellar disks that emit strongly in the mid-IR (8-24 μm) is how varied they are. About two-thirds show evidence of PAHs (Figure 5.2.1), independent of the host star's spectral type, a few show very strong silicate features, and some show almost no silicate features. It is unclear why PAHs should dominate the spectra of some disks, while silicates should be most prevalent in others. The presence of PAHs probably indicates active chemistry occurring in the very inner parts of the disk. It may be that silicate grains are present in all intermediate-age disks but are large and cold, and therefore less efficient emitters in the mid-IR.

Research to Date. Detailed chemical studies of protoplanetary disks are hampered by the distance to even the nearest stellar nurseries, the T Tauri associations (~150 pc). At these distances, the 1-AU length scales that are necessary for studying inner disk processes are effectively unresolvable, even with the largest existing telescopes. Co-I Weinberger has published spatially resolved, mid-infrared spectra on the one disk for which the largest telescopes in the world have adequate spatial resolution (Weinberger et al. 2003); see Figure 5.2.1. She has been working on disk detection and spatially unresolved spectra in other nearby young stars (e.g., Weinberger et al. 2002). She has further proposed to use the Hubble Space Telescope (HST) for near-infrared imaging as a first attempt to detect water and methane ices in circumstellar disks.

Proposed Research. Co-I Weinberger will integrate her high-resolution mid-IR studies of disks into Carnegie's multidisciplinary effort directed at constraining the origins and evolution of chondritic organic carbon (see **Sections 5.3.1 and 5.3.2**). Specifically, Weinberger will seek to determine the spatial distribution of PAH emission (a proxy for presolar organic carbon) within circumstellar disks. She will also constrain the size and ionization distribution of PAH molecules using the ratios of 7.7, 11.2, and 12.7 μm PAH features (Allamandola et al. 1999). If the fraction of ionized PAHs can be calculated, based on the observed stellar ultraviolet (UV) flux, then it will be possible to determine the radial distribution of PAHs in the disk, even though discrete PAH spectra remain spatially unresolved. Weinberger will develop new techniques for spatially resolved, near-IR spectroscopy of nearby circumstellar disks. She has applied for HST observation time for preliminary work on this project. Her ultimate goal is to develop an IR spectrograph for Carnegie's Magellan telescopes to work behind an adaptive optics (AO) system. This should allow for observations with a resolution of a few AU in the nearest young disks. Weinberger will work on techniques for obtaining the high-contrast spectra with Magellan AO that are necessary for studying faint disks around bright stars. The funds for building the new IR spectrograph will come from other sources. An NAI Postdoctoral Fellow with instrumental expertise would be an integral part of this development effort.

5.2.2 Formation of habitable planetary systems (Astrobiology Roadmap Objective 1.1)

Background. The conventional view of Solar System formation is that the presolar cloud collapsed in a region of low-mass star formation, similar to Taurus-Auriga (Shu et al. 1993). In such a quiescent setting, the background UV flux is likely to be low and limited largely to the flux from the proto-Sun, once it forms, greatly restricting the UV flux available for prebiotic chemistry.

Research to Date. A new scenario for Solar System formation has been proposed by Co-Is Boss and Wetherill and NAI Postdoctoral Fellow Haghighipour (Boss et al. 2002), based on forming the Solar System in a region of high-mass star formation, similar to the Orion Nebula cluster, and on the rapid formation of giant gaseous protoplanets by the disk instability mechanism (e.g., Boss 1997). This scenario relies upon a high flux of UV radiation from nearby massive stars to remove the gaseous portion of the solar nebula from Saturn's orbit and beyond and then to strip the gaseous envelopes from the outermost giant gaseous protoplanets, leaving behind largely ice and rock cores similar to what may have evolved into the ice giant planets, Uranus and Neptune. Terrestrial planet formation is likely to proceed largely unimpeded and may even be somewhat hastened by the gravitational perturbations from a rapidly formed Jupiter (Kortenkamp et al. 2001). Because the inner Solar System is deep within the Sun's gravitational potential well, a halo of hydrogen gas will be retained inside 10 AU, which will protect the planetesimals and gases of the inner solar nebula. Planetesimals and cometesimals outside this distance will be subjected to a withering UV flux once the disk gas in their vicinity has been photoevaporated.

If this new scenario can be shown to be applicable to the origin of the Solar System, then the implications for the frequency of habitable planets are clearly significant. Most stars are believed to form in regions of high-mass star formation, with perhaps only a minor fraction forming in regions similar to Taurus-Auriga. If the Solar System formed in a region similar to Orion, then the prospects for finding and characterizing other Earth-like planets increase several-fold. Future astrobiology space missions such as Kepler will determine the frequency of Earth-like planets and help to determine if this optimistic conjecture is correct.

Proposed Research. Co-I Boss receives support from NASA's Planetary Geology and Geophysics program to investigate the implications of this new scenario for planetary system formation. Boss and an NAI Postdoctoral Fellow will explore the implications of this new scenario for Solar System formation as a part of the Carnegie NAI effort. UV photons from nearby massive stars result in photolysis of the ices on the surfaces of cometesimals, producing PAHs and amino acids (Bernstein et al. 2002), i.e., a thick layer of organic compounds that will form an effective sun block. Beneath this surface layer, the pristine nature of these bodies will be retained. This scenario suggests that prebiotic UV-driven chemistry would have been vigorous in the outer regions of the solar nebula, even as the planetary accumulation process was underway. Because UV radiation can destroy as well as create organics, the outer solar nebula offers a perhaps unique opportunity to create complex organic compounds and to shelter them from subsequent UV-driven losses, though storage in cometesimals. Boss and an NAI Postdoctoral Fellow will model these processes in the context of Boss's three dimensional, radiative, gravitational hydrodynamics models of the solar nebula.

Co-I Wetherill will continue to collaborate with Satoshi Inaba and Masahiro Ikoma of the Tokyo Institute of Technology in developing theoretical models for the formation of the gas giant planets Jupiter and Saturn by the alternative core accretion mechanism (Inaba et al. 2003b). This work has evolved to include calculating the importance of the early phase of formation of the atmosphere of these planets (Inaba et al. 2003a). Although volumetrically small in comparison to the eventual massive gaseous atmospheres of the gas giant planets, the early atmosphere has a profound influence on the growth rate of the solid cores and, furthermore, enhances the probability of collisions between the planetary embryos. These early atmospheres may exert a significant influence on the growth rate of the subsequent final giant planets and thus set the stage for the emergence of habitable terrestrial planets (Wetherill 1996).

5.2.3 Searching for extrasolar habitable planetary systems (Astrobiology Roadmap Objective 1.2)

Background. The ultimate goal of NASA's Navigator Program is to detect and characterize habitable terrestrial planets (Beichman et al. 1999). Achieving this extraordinarily ambitious goal requires the detection of nearby stars that are capable of sheltering Earth-like planets, i.e., stars with long-period Jupiter-mass planets – Jupiter intercepts Oort Cloud comets that would otherwise catastrophically impact the Earth (Wetherill 1994a).

Research to Date. Co-I Butler's Extrasolar Planet Search is designed to survey the nearest 1,300 Sun-like stars (F8-M5) with the precision Doppler technique at the Lick 3-m, Keck 10-m, and Anglo-Australian 3.9-m telescopes. Over the past 7 years, these surveys have led to the discovery of two-thirds of the known extrasolar planets, including the only known transit planet, all 5 published multiple planet systems, and all 5 published sub-Saturn-mass planets (e.g., Butler et al. 2003).

Most of the known planets orbit beyond 0.2 AU in eccentric ($e > 0.1$) orbits, while a surprising class of Jupiter-mass planets is found in circular orbits with periods of 3 to 5 days. Within the last year a handful of planets have been found orbiting beyond 1 AU in relatively circular orbits, suggesting that circular orbits may be more common among systems in which giant planets orbit in distant Jupiter-like orbits. Unfortunately, none of these planetary systems are hospitable to habitable Earths, because their present giant planet orbits would prevent an Earth from orbiting stably in the habitable zone (i.e., where liquid water can exist on the planet), because their orbits would have prevented an Earth from forming in the first place (Wetherill 1996), or because their inward migration to their present short-period orbits would have disrupted the formation of Earths in the habitable zone. However, less than 10% of nearby stars seem to be inhospitable to Earths – the remaining 90% could shelter exact Solar System analogues and we would not yet know it.

Proposed Research. The long-term goal of Co-I Butler's work is to survey all 2,000 nearby Sun-like stars out to 50 parsecs. Butler will begin adding most of the remaining stars to the survey in 2003 with the addition of the Carnegie 6.5-m Magellan II telescope. Butler's survey currently achieves a long-term precision of 3 m/s radial velocity, sufficient to make 4-σ detections of Solar System analogues (Jupiter-mass companions at 5 AU), and he is working to improve the precision to 2 m/s. By 2010, these surveys will provide a first planetary census of nearby stars, allow estimates of the frequency of Solar System analogues, and provide the target list for NASA's Terrestrial Planet Finder (TPF). Co-I Butler is also a member of the astrometric planet search team of NASA's Space Interferometry Mission (SIM), scheduled for launch in 2009. SIM is intended to be capable of detecting Earth-mass planets in Earth-like orbits around the closest solar-type stars.

In addition, Co-Is Boss and Weinberger will begin in 2003 a new ground-based astrometric planet search, using Carnegie's du Pont 2.5-m telescope in Chile. When equipped with a specially-designed astrometric camera, the du Pont is expected to be capable of astrometric accuracies on the order of 0.25 milliarcsecond, sufficient to allow the detection with a signal-to-noise ratio of 4 of a long-period Jupiter-like planet orbiting a solar-mass star 5 pc away. Such long-period Jupiters are thought to be signposts for the existence of Solar System analogues. Co-Is Boss and Weinberger will undertake this new effort as a part of the Carnegie NAI team.

Co-I Boss is a member of the Science Team for the Kepler Mission, a space telescope that will be capable of detecting the presence of hundreds of Earth-like planets in orbit around stars in the disk of our galaxy at the same galactic radius as the Sun. Kepler will detect these planets by the transit method, looking for dimming of starlight on the order of a part in 10,000. Kepler is scheduled for launch in 2007. Co-I Seager is using Carnegie's Las Campanas Observatory to

search for extrasolar planets by the transit method. Ground-based transit surveys are capable of detecting the presence of Jupiter-sized planets in short-period orbits around solar-type stars (similar to the "hot Jupiter" orbiting the star HD209458) and even smaller-radius planets orbiting lower mass stars.

5.2.4 Characterizing extrasolar habitable planetary systems (Astrobiology Roadmap Objective 1.2)

Background. The relative abundances of the carbon compounds CO and CH_4 are potentially very useful temperature indicators for hot Jupiters (Seager et al. 2000). The hot Jupiters have effective temperatures of around 1000 K, resulting from their extremely close proximity to the parent stars — seven times closer to their stars than Mercury is to our Sun. Equilibrium calculations for model planetary atmospheres indicate that at these high temperatures and for atmospheres of roughly solar composition, CO should be much more abundant than CH_4, whereas at lower temperatures it should be the other way around. The temperature structure of hot Jupiter atmospheres is key to characterizing them — possible hot Jupiter models span a temperature range that includes dominance of either CH_4 or CO. To date extrasolar planet atmosphere models have assumed that thermodynamic equilibrium is achieved (Burrows et al. 2001). However, in brown dwarfs, objects with similar effective temperatures, CO and CH_4 are not in equilibrium, perhaps because of convective dredge-up from deep within their atmospheres. Photochemistry may also play a role in the atmospheres of hot Jupiters.

The fact that Jupiter is rich in elements heavier than H and He ("metal-rich") due to "pollution" by planetesimals shortly after its formation raises interesting questions for extrasolar giant planets. Are the extrasolar giant planets also metal-rich? Is their metallicity related to factors in their environment (such as number of giant planets in the system or metallicity of the parent star) that are also likely affected by planet formation? Because H_2O is expected to exist in vapor form at the temperature range of most of the known extrasolar giant planets, and because of its high abundance and strong absorption features, H_2O absorption bands should be among the easiest spectral features to detect in extrasolar giant planets.

Carbon dioxide is a good indicator for the presence of an atmosphere around terrestrial-sized planets because it is the only major atmospheric feature common to Earth, Mars, and Venus. Carbon dioxide is an atmospheric feature considered as essential for detection by TPF (e.g., Seager 2003). One good spectral region for such measurements is in the mid-IR, where the contrast between the central star and the planet is large, but for CO_2 the strongest thermal IR emission line is likely to be saturated. Thus, it could be used to determine if an atmosphere is present but little else. An alternative is to look for weaker CO_2 lines, potentially detectable with an alternative method: in transmission during a planet transit across the face of the star. As shown in Figure 5.2.2, Venus has many lines throughout the near-IR. Venus will transit the Sun in June 2004 for the first time in 120 years.

Research to Date. Co-I Seager is attempting to characterize the physical properties of extrasolar planets (spectra, density and temperature profiles). To do this Seager is developing radiative transfer models of extrasolar planet atmospheres (e.g., Seager et al. 2000). The results of such modeling will be used to interpret spectral data currently being acquired with ground-based telescopes and to be acquired in the next 5 years by space-based experiments. The aim is to determine the planets' atmospheric compositions and to also to predict their spectra for the design of future experiments (both from the ground and from future space telescopes).

Proposed Research. Co-I Seager proposes to develop kinetic models for the atmospheric chemistry that are necessary to explore quantitatively the potentials of CH_4 and CO as temperature indicators. The development of such models is well-suited for an NAI Postdoctoral Fellow. Seager also proposes to investigate the potential of H_2O and carbon-compound

atmospheric features as diagnostics of planet metallicity, considering the likely observational parameters of proposed ground- and space-based search programs.

Finally, Seager proposes to model Venus' atmosphere in transmission (Figure 5.2.2) in order to use observations from the Venus transit as a test case for future extrasolar planet transit measurements (Seager & Sasselov 2000). The transit of Venus presents a unique opportunity for an NAI Postdoctoral Fellow to be trained in extrasolar planet atmosphere observations and theory, by participating in observations of the transit and in the data reduction and analysis.

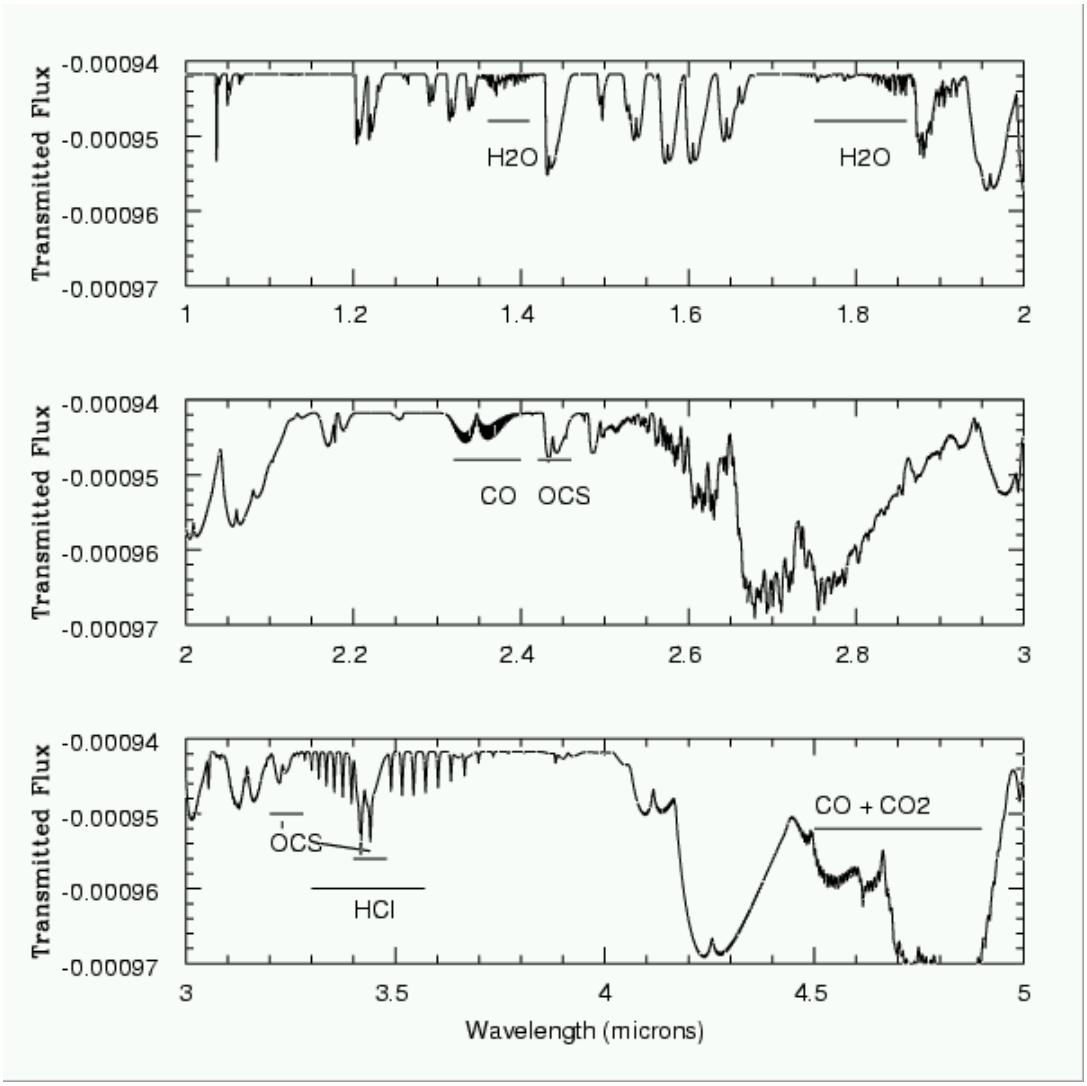

Figure 5.2.2. A model of the near-IR transmission spectrum of Venus at a spectral resolution of 10,000. The transmitted flux is the normalized in-transit minus out-of-transit flux, thus canceling out the stellar lines. Major absorption features are identified unless they are CO_2 features.

5.2.5 Habitable environments in the Solar System (Astrobiology Roadmap Objective 2.1)

Background. Much attention has been given to those few Solar System objects where the minimal conditions for life might all be present, including liquid water, essential chemical

materials, and sources of energy. Early consideration of "habitable zones" in a planetary system where liquid water is stable at the surface of a planet under some atmospheric conditions has been modified by the realization that life might be possible in subterranean environments or within deep oceans overlain by thick shells of more or less permanent ice cover on outer planet satellites.

Research to Date. PI Solomon is supported by the NASA Planetary Geology and Geophysics Program to investigate aspects of the geological and geophysical evolution of solid planets and satellites. His recent work has focused on the thermal and magmatic evolution of such bodies, as well as the interaction between magmatism and atmospheric chemistry and climate. He is also a member of the Mars Orbiter Laser Altimeter team on the Mars Global Surveyor mission, and he has been using the highly precise new altimetric measurements to address problems ranging from interior processes to water-surface interactions on Mars. Among the results has been the demonstration (Phillips et al. 2001) that much of the volcanic construction and magmatic intrusion that created the Tharsis Rise on Mars occurred before the end of the Noachian epoch. This conclusion rests on the grounds that the global response to Tharsis loading influenced the downhill directions recorded by late Noachian valley networks. Another outcome has been the suggestion that the pattern of paleomagnetic anomalies may have been strongly influenced by hydrothermal alteration of magnetic carriers, following processes that occur in very young oceanic crust on Earth (Solomon et al. 2003).

Proposed Work. As part of his contribution to NAI research, Solomon will lead a task aimed at evaluating the locus, vigor, and history of hydrothermal activity that might have been possible or even favored within targeted settings in the modern Solar System and earlier in its history. That evaluation will be informed by considerations of heat sources versus depth and time as constrained by parameterized thermal histories and their dependence on internal volatile budgets (e.g., Hauck & Phillips 2002) as well as mechanical lithosphere estimates and other proxies for lithospheric heat flow (e.g., McGovern et al. 2002). Other considerations will include the sources and budgets of subsurface volatile inventories, the nature of rock compositions and fluid pathways (e.g., fault distributions, magnetic anomaly patterns on Mars), and constraints on compounds of carbon, nitrogen, phosphorus, sulfur, and other materials important for prebiotic organic chemistry. The findings from these evaluations will form a basis for the design of targeted experimental studies by other consortium members aimed at assessing the conditions for organic synthesis in possible hydrothermal systems on Mars, Europa, and other Solar System objects.

5.3 Extraterrestrial Materials: Origin and Evolution of Organic Matter and Water in the Solar System

What was the nature of the carbonaceous matter that was delivered to the early Earth and other planetary bodies? Meteorites and interplanetary dust particles (IDPs) provide information that is relevant to every stage of the processes that ultimately led to life in our Solar System. They also provide direct links among all aspects of this proposal.

Chondritic meteorites are primarily aggregates of material that formed in the solar nebula prior to or during the early stages of planet formation. Their asteroidal parent bodies are the last vestiges of the swarm of planetesimals from which the terrestrial planets ultimately formed. IDPs are thought to come from both asteroids and comets (Bradley et al. 1988). Many appear to have experienced less modification on their parent bodies than meteorites. They are in this sense more primitive than meteorites, but their size (10s of μm) makes their analysis more difficult.

Chondritic meteorites and IDPs also contain circumstellar and interstellar grains and interstellar organic matter that are a direct record of the prehistory of our Solar System (Messenger & Walker 1997; Bradley et al. 1999; Messenger et al. 2002). There is evidence that

at least some fraction of the organic matter is a ubiquitous constituent of the interstellar medium (ISM) in our Galaxy and other galaxies (Pendleton et al. 1994; Pendleton & Chiar 1997). The bulk composition of the meteoritic material is also similar to the so-called CHON particles from comet Halley (Kissel & Krueger 1987). Meteorites and IDPs would have supplied a complex suite of organic matter to the terrestrial planets, including amino acids and nucleic acids (Cronin et al. 1988). The discovery that some amino acids in meteorites exhibit a slight "left-handedness" excess is particularly intriguing (Engel & Nagy 1982; Cronin & Pizzarello 1997; Pizzarello & Cronin 2000). If the enantiomeric excesses are in fact *bona fide* (i.e., not due to some contamination effect), then the chemistry that produced the organics in meteorites are our only example of a truly abiotic, chirally selective reaction pathway. Because of the enantiomeric excesses, some have even speculated that extraterrestrial organic matter played a direct role in the development of life on Earth (Pizzarello & Cronin 2000).

The presence of complex organic matter in chondritic meteorites and IDPs may also have played a second significant astrobiological role by modifying the Earth's early atmospheric composition. When micro-meteorites and IDPs are rapidly heated during atmospheric entry, organic matter is released pyrolytically (Kress et al. 2003). On the early Earth, the flux of exogenous material would have been high and the oxygen content of the atmosphere would have been low. As a result, this pyrolitic organic matter may have had a significant effect on its atmosphere by providing both greenhouse gases and UV-protective molecules such as PAHs (Kress et al. 2003).

Many chondritic meteorites experienced a period of hydrothermal activity that created a number of transition-metal sulfides and hydrated silicates. Some have suggested that similar minerals were essential for catalysis of prebiotic "metabolic" cycles on Earth (Wächtershäuser 1988; Russell & Hall 1997; Russell et al. 1998; Cody et al. 2001). One component of the organic matter in meteorites even forms vesicles that have been likened to primitive cell walls (Deamer & Pashley 1989; see **Section 5.5.1**). Given the abundance of complex organic matter and the presence of important catalysts, why did not life develop in meteorites? Time and pressure may have been two factors – the period of hydrothermal activity was probably quite short, and the small sizes of their parent bodies would have meant low pressures. In this regard, meteorites provide a natural model of a system clearly capable of promoting extensive organic chemistry, but probably incapable of bridging from pure chemistry to life.

The terrestrial planets were built up by accretion of many smaller bodies (Wetherill 1994b; Chambers & Wetherill 2001). Most of these objects would have undergone widespread partial melting and differentiation before being accreted. The fraction of the original carbon that would have survived this process and in what form it would have been preserved is not well understood. However, these questions are key to understanding the carbon budgets and degassing histories of the terrestrial planets.

Of all the planets other than Earth, Mars is perhaps the most likely to have developed life. It shows abundant evidence for the past activity of liquid water at its surface. There is tantalizing evidence from satellite imagery that near-surface liquid water persists at least intermittently to this day (Malin & Edgett 2000; Costard et al. 2002). Meteorites provide us with the only samples of Mars that we can study in the laboratory. All the Martian meteorites are igneous rocks, and many show evidence of having experienced aqueous alteration on Mars after crystallization (McSween & Treiman 1998). The crystallization ages of these meteorites provide upper limits for the ages of this alteration and indicate that aqueous activity on Mars has continued up until almost the present day – the youngest Martian meteorites are ~180 Ma old (McSween & Treiman 1998). Thus, meteorites also provide evidence of the nature of the sub-surface hydrosphere on Mars.

5.3.1 Organic carbon in meteorites and IDPs (Astrobiology Roadmap Objective 3.1)

Background: Organic carbon in meteorites. Through funding from NASA's Origins of Solar Systems Program, Co-Is Cody and Alexander have focused on determining the structure of meteoritic macromolecular organic matter, the dominant form of organic carbon, by solid-state nuclear magnetic resonance (NMR) spectroscopy (Cody et al. 2002, 2003a). These studies show that the meteoritic organic matter has an extremely complex distribution of C-bearing organic functional groups and exhibits enormous differences in the relative abundances of functional groups within and between chondritic classes. However, we do observe certain chemical similarities in all meteorites, suggesting a common precursor that has been variably modified by parent-body processes.

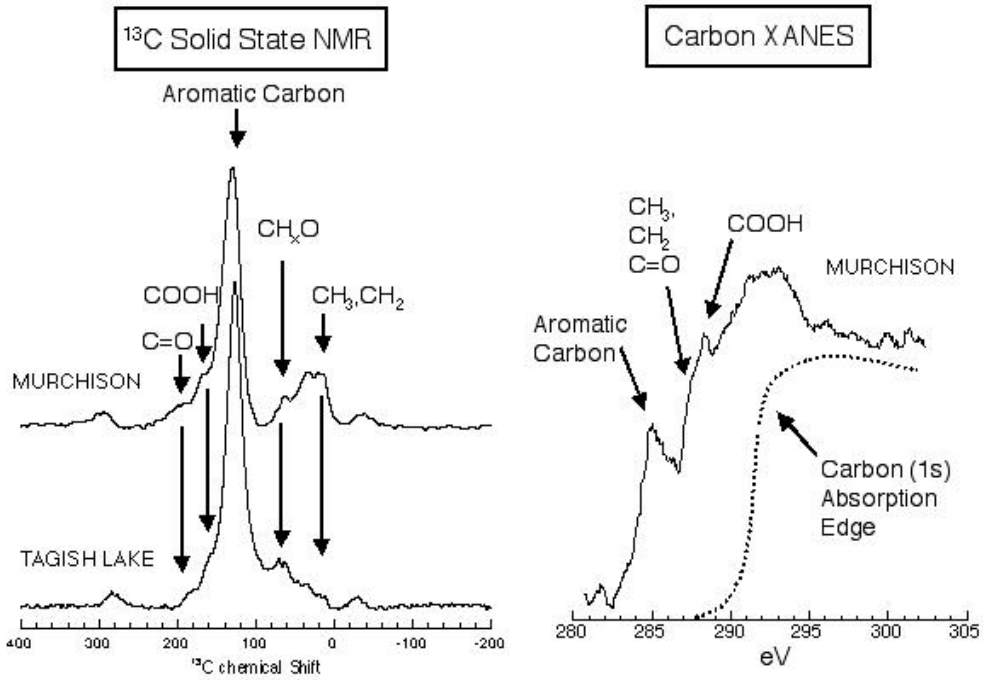

Figure 5.3.1. A comparison of the chemical information recorded by solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy vs. carbon (1s) X-ray Absorption Near Edge Spectroscopy (XANES). NMR spectra of the organic matter in the Murchison and Tagish Lake meteorites (left) reveal enormous chemical complexity in both samples while also exhibiting tremendous differences in the relative distributions of organic functional groups. C-XANES of Murchison records the same organic functionality in the pre-edge region of the spectrum. Note that C-XANES suffers from considerably greater band overlap compared with NMR. C-XANES, however, can be obtained on sub-femtogram quantities, whereas NMR requires sample weights in excess of ~ 20 mg (ideally 100 mg).

What is still needed, and cannot be done by NMR, are equally stringent constraints on N and S speciation. Pyrolysis gas chromatography/mass spectrometry (GC-MS) analyses do reveal both N- and S-containing organic moieties, but this technique may not be detecting all of the organic matter and the pyrolysis may modify the heteroatomic molecules from their pristine configurations.

The NMR and pyrolysis techniques are also relatively insensitive. We would like to obtain similarly detailed information from rare meteorites, IDPs, and cometary samples returned by NASA's Stardust mission. The H and N isotopic compositions of organic matter in IDPs exhibit a much wider range of compositions than in meteorites (Messenger 2000), suggesting that they have been less modified by parent-body processes. The organic matter in the Stardust samples is also likely to be very primitive, although possibly modified during collection. To analyze these materials, we need to develop new microanalytical techniques.

Proposed Research. Our NAI-specific work will focus on astrobiological and mission-related issues associated with the chemistry of chondritic organic matter. In particular, we will obtain high-quality information on the organic N, O, and S contained in meteoritic organic matter. High resolution X-ray Absorption Near Edge Spectroscopy (XANES) is probably the best means of obtaining this information, particularly for small samples (Figure 5.3.1). We specifically seek support for research at the Scanning Transmission X-ray Microscope (STXM) and microspectrometer at the Advanced Light Source (a 4.0-GeV synchrotron) at Lawrence Berkeley National Laboratory. Co-I Cody is a member of the principal research team for this instrument, and Collaborator Ade is the principal beamline scientist. We propose to use the STXM to acquire XANES spectra of meteoritic organic matter, exploiting the characteristic fine structure on the C(1s), N(1s), O(1s), and S(2p) absorption edges. Light-element XANES has been previously successfully applied to probe the electronic structure of organic carbon in terrestrial kerogenous materials (Cody et al. 1995a,b, 1996, 2000; Boyce et al. 2002) and has been shown to provide fairly well-resolved functional group information. C- and O-XANES has been recently applied to the study of IDPs and some meteorites (Flynn et al. 2001). The application of N, O, and S-XANES to our collection of meteorites will place constraints on perhaps the most interesting (in an astrobiological sense) of their exogenous organic compounds. We will use the XANES data, calibrated against various N, O, and S organic standards, to develop a consistent fitting scheme by which any exogenous organic solid can be analyzed and chemically characterized, even given femtogram quantities.

The application of C-XANES provides an equally useful function. We now have a thorough understanding of the carbon chemistry in meteoritic macromolecular material via solid-state NMR (Cody et al. 2002, 2003a). We will use these data to fit C-XANES spectra and obtain reasonable estimates of the critical optical parameters, i.e., the oscillator strengths associated with electronic transitions corresponding to various organic functional groups. It is important to be aware that organic functional groups detected with XANES (like IR spectroscopy and unlike NMR) may have vastly different molar absorptivities, thus some functional groups (e.g., COOH, and RCO) exhibit very strong absorption relative to other functional groups, e.g., CH_2 and CH_2O. Furthermore, C-XANES typically suffers from substantial band overlap (Figure 5.3.1), leading to complications in band assignments. Using our extensive NMR data to constrain the C-XANES data on meteorites will, therefore, provide an extremely valuable database that can be used to quantify the C chemistry within extremely small samples (e.g., IDPs), where the application of NMR is impossible. This database will be made available to the entire scientific community and thus will facilitate the quantitative analysis of any extraterrestrial organics.

Background: Organic Matter in IDPs. Interplanetary dust particles (IDPs), collected by aircraft in Earth's stratosphere, are believed to be among the most primitive extraterrestrial materials currently available for laboratory study (Bradley et al. 1987; Messenger 2000). These small (typically < 20 μm) particles are generally aggregates of much smaller grains of silicates, Fe-Ni metal, sulfides and carbonaceous material. They have bulk chemical compositions similar to carbonaceous chondrites but are more highly enriched in C. Laser-desorption mass spectrometry (Clemett et al. 1993), Fourier Transform Infrared (FTIR) spectroscopy (Flynn et al. 2002), and C- and O-XANES (Flynn et al. 2001) have all shown that much of the carbonaceous material is organic, rather than elemental C, bolstering the suggestion of Anders (1989) that IDPs

could have been an important source of organic matter to the early Earth. Moreover, large and variable enrichments of D and ^{15}N (relative to H and ^{14}N and terrestrial ratios) in most cases appear to be associated with organic matter (Messenger 2000; Aléon et al. 2001), indicating preservation of organic material inherited from the presolar molecular cloud.

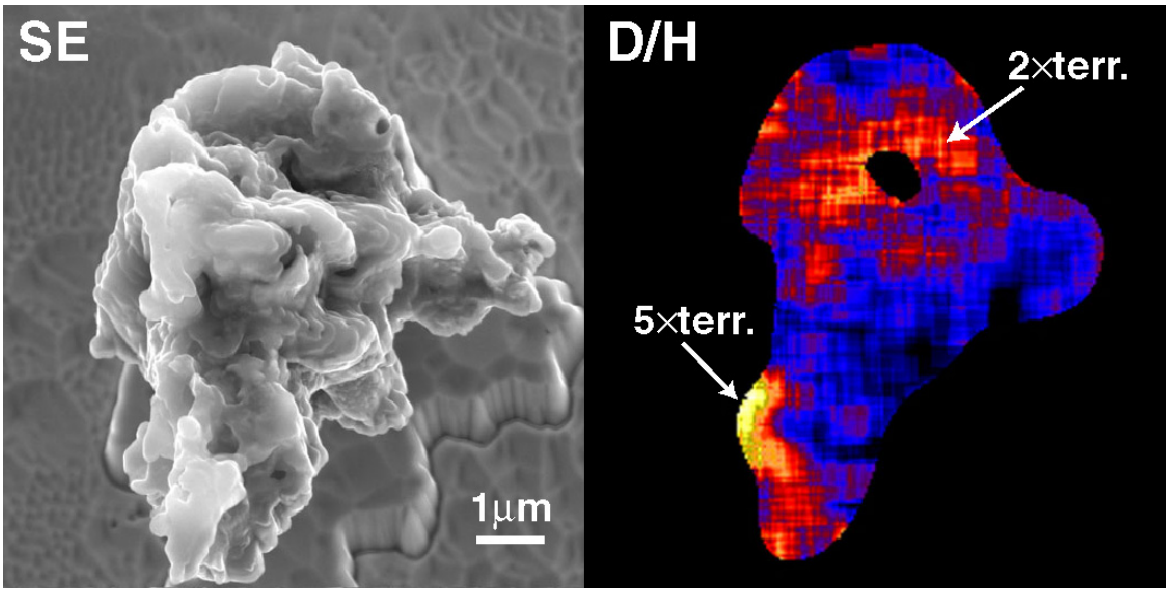

Figure 5.3.2. Images of a cluster interplanetary dust particle (IDP) fragment collected in the Earth's stratosphere. Left panel: secondary electron image. The IDP consists of carbonaceous material intermixed with fine-grained silicate and sulfide minerals. Right panel: Hydrogen isotope image of IDP generated by an ion microprobe. The D/H ratio is highly variable on a μm scale. D/H reaches 5 times the terrestrial value in the most carbonaceous region of the particle, indicating partial preservation of interstellar organic material.

Proposed Research. Co-Is Nittler, Alexander, Cody, Steele, and Stroud request support to characterize IDPs isotopically, chemically, and mineralogically on a micron and sub-micron spatial scale. The goals of the proposed research are to (1) characterize more completely the presolar molecular cloud matter preserved in the particles, (2) trace the alteration processes that have affected the organic matter, and (3) compare the IDP organic matter with that preserved in primitive meteorites. A multi-technique approach allows the most information to be obtained about the organic material in the IDPs (e.g., Keller et al. 2002). Isotopic imaging with the CIW ims-6f ion probe will be used to investigate quantitatively the spatial distribution of D/H, C/H, and $^{15}N/^{14}N$ ratios in IDPs with a ~1 μm spatial resolution (Nittler & Messenger 1998; Mukhopadhyay et al. 2002) (Figure 5.3.2). Several techniques will subsequently be used to characterize chemically and mineralogically the isotopically analyzed IDP material, including field-emission scanning electron microscopy, fluorescence microscopy (Co-I Steele), transmission electron microscopy (Co-I Stroud), and scanning transmission X-ray spectro-microscopy (STXM, Co-I Cody, see **Section 5.3.1**). The isotopic work is currently funded by a NASA grant to Co-I Nittler.

Transmission electron microscope (TEM) and STXM analyses require ultrathin (~100-nm) samples. These samples will be prepared either by ultramicrotoming of IDPs embedded in S (Keller et al. 2002) or by focused ion beam (FIB) techniques (e.g., Stroud et al. 2002). By tagging specific functional groups (e.g., PAHs amines) in these sections with fluorescent molecular probes, fluorescence microscopy can be used to characterize the spatial distribution of those groups (Clemett et al. 2002).

STXM can be used to obtain both elemental abundances and electronic structure information (e.g., bonding) with very high sensitivity (femtomole detection) and high spatial resolution. Co-Is Cody and Nittler will perform C-, N-, O- and S-XANES (see above) imaging of the IDPs. Previous XANES measurement of IDPs have indicated the presence of C=O double bonds and a much higher O/C ratio for IDP organics than seen in meteoritic organics (Flynn et al. 2001). Correlated isotopic and XANES measurements on the same material will allow us to search for correlations between type and abundance of chemical bonds and isotopic compositions. Complementary to the STXM analyses, Co-I Stroud will use TEM techniques to characterize the IDPs. High-resolution imaging, EDS analysis, and electron energy loss spectroscopy (EELS) imaging will be used to characterize silicate mineralogy, the chemical bonding environment, and speciation of organic C and N (Keller et al. 1997) and correlate these with isotopes. The IDP EELS spectra will also be compared with spectra from the well-characterized meteorite samples (see above).

5.3.2 The fate of carbon during planetary differentiation (Astrobiology Roadmap Objective 3.1)

Background. Carbon from the interstellar medium and the solar nebula is incorporated into chondritic meteorites, occurring in a variety of forms (amorphous organic carbon to ordered graphite) and with a wide range of isotopic signatures. While this material forms the reservoir of carbon assembled into planets and available for abiological and biological evolution, the planets themselves have undergone differentiation. Relatively little is known about the effect of planetary differentiation on carbon and its isotopic signatures. While we might expect planetary-scale differentiation at high temperatures to homogenize the isotopic signatures, we know relatively little about the time scales on which this happens or the ultimate fate of the incorporated carbon.

Proposed Research. Co-I McCoy, along with Postdoctoral Fellow C. M. Corrigan (Smithsonian), collaborator G. K. Benedix (Wash. Univ.), and Co-I Nittler, will investigate the mobilization and isotopic homogenization of chondritic carbon during partial melting and differentiation through both experimental melting of chondritic meteorites and analyses of carbon isotopic signatures in both these experiments and natural, partially-differentiated meteorites. In collaboration with Co-I Vicenzi, carbon in the experimental charges will be mapped at high spatial resolution (~0.25 μm) prior to isotopic analysis, using time-of-flight secondary ion mass spectrometry (ToF-SIMS). This characterization will reveal trace and free carbon, as well as potentially illustrating transport paths along grain interfaces that may otherwise go undetected using conventional X-ray microanalysis.

McCoy, Benedix, and Corrigan are currently undertaking a series of experiments to examine partial melting and melt migration in chondritic meteorites heated between 1000°C (just above the Fe,Ni-FeS cotectic) and 1300°C. These experiments should allow us to trace the movement of carbon during melting. Our expectation is that carbon is an early-melting phase that will concentrate in the Fe,Ni-FeS cotectic melt. During planetary differentiation, this outcome would result in most of the carbon being segregated into the core and removed from the zone of biologic activity. This conundrum between the requirement for carbon to initiate life and its expected behavior during melting is the driving force for these experiments.

The second phase of the work will involve analyses of carbon isotopic signatures. During the past 10 years, there has been a growing recognition that within the world's meteorite collections examples exist of the earliest stages of differentiation, arrested before reaching completion. Preliminary work by Co-Is McCoy and Nittler on one of these meteorites – lodranite GRA 95209 – suggests that carbon isotopic heterogeneity may be preserved during partial melting. Despite having reached temperatures that generated significant silicate partial melting (perhaps 1250°C),

graphite rosettes contained within metal exhibit carbon isotopic signatures ranging from $\delta^{13}C$=–55 to +75‰. Furthermore, these isotopically diverse graphite rosettes often occur within a few tens of microns of one another. This pattern strongly suggests that the initial, chondritic isotopic heterogeneity was preserved, despite significant partial melting. Analyses of controlled melting experiments, as well as additional analyses of graphite-bearing stony-iron meteorites (e.g., particularly silicate-bearing IAB irons) should allow us to constrain further the extent of heating and melting required to produce isotopic homogeneity.

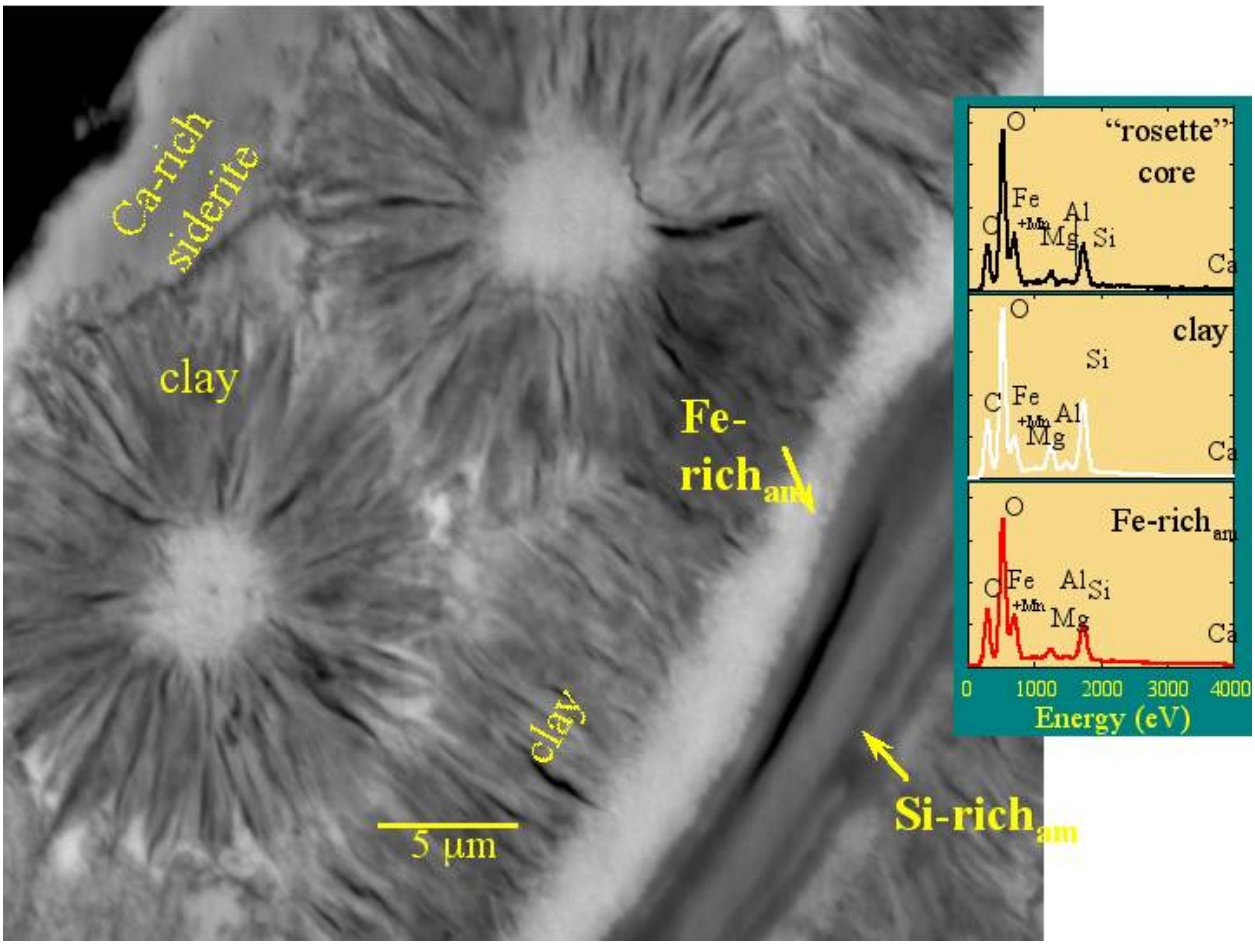

Figure 5.3.3. A backscattered electron image taken with a scanning electron microscope (SEM) of a fine-grained region of hydrothermal alteration of the Martian meteorite Lafayette. The insets are energy dispersive X-ray spectra of various regions. This particular region is dominated by clay minerals but includes Ca-rich siderite ($FeCO_3$) and a vein of iddingsite.

5.3.3 The Martian hydrosphere: Clues from meteorites (Astrobiology Roadmap Objective 2.1)

Background. A strong body of evidence exists for the claim that liquid water once was present on, or beneath, the surface of Mars. Large-scale (atmospheric observations/modeling) and regional-scale (surface morphologies) studies offer the opportunity to understand Mars as an evolving system. For instance, its now dry surface and the large D enrichment of its atmosphere suggest that most of Mars' atmospheric water has been lost to space. There are models for how this might have occurred. However, the most tangible evidence for the evolution of Mars comes

from Martian meteorites. The Martian meteorites are all igneous rocks with crystallization age that range from 4.5 Ga to almost the present day. They include primary water-bearing minerals, shock-produced glasses, and secondary alteration minerals (Figure 5.3.3). The alteration minerals are presumably the products of processes occurring in Mars' subsurface hydrosphere. Thus, the meteorites potentially provide us with a record of the evolution of Mars throughout its history, particularly the history of its hydrospheric and atmospheric water (e.g., Leshin 2000). This information will help in assessing whether life could have evolved on Mars and where it might survive today.

Research to Date. We have determined the H isotopic compositions and water abundances of primary minerals and shock-produced glasses in 10 Martian meteorites (Boctor et al. 2003). The minerals and glass are generally enriched in D, probably because they have interacted with Martian hydrospheric and atmospheric water during the shock events that produced the glasses. A more direct record of the Martian hydrosphere is found in the alteration products in meteorites. The alteration products in some meteorites are only 100s of Ma old (e.g., Swindle et al. 2000), indicating that the Martian hydrosphere remains active, at least intermittently, to this day. The isotopic compositions of H (Karlsson et al. 1992; Eiler et al. 2002) and O (Farquhar & Thiemens 2000) in the alteration minerals, estimated by conventional extraction methods from bulk samples of the meteorites, strongly suggest a linkage between the atmosphere and hydrosphere of Mars. We intend to take advantage of microanalytical techniques to provide a better understanding of the physical and chemical conditions near the interface between subsurface fluids and the Martian atmosphere.

Proposed Research. Co-I Vicenzi will conduct a detailed examination of C-bearing and associated secondary minerals in Martian meteorites using high-spatial-resolution ToF-SIMS. The temperatures for such precipitation from fluids in the Martian crust are poorly constrained but have been estimated to be < 100°C to –30°C (Romanek et al. 1998; Treiman 1993), a range well within the window for life. At such low temperatures, reaction rates are sluggish, and many of the alteration phases have grain sizes on the micron to submicron length scales, and some silicates lack periodicity all together. The Ga ion source provides the spatial resolution (~0.1-0.3 μm) for studying the compositions of complex secondary assemblages through two-dimensional (2D) and shallow three-dimensional (3D) ion imaging.

The goal of these studies will be to (1) use the parallel detection capabilities of ToF-SIMS to determine the disposition and covariation of H, C, N, O, OH, P, S, and Cl in the alteration products, (2) use the high-resolution ion images to guide magnetic sector SIMS isotope analyses, e.g., D/H, $\delta^{13}C$, and $\delta^{18}O$ (conducted in conjunction with Co-Is Nittler and Alexander), (3) perform *in situ* focused ion beam (FIB) milling in the ToF-SIMS to obtain a quasi 3D view of structures and aqueous deposition boundaries noted in the high resolution ion images. Follow-up electron and low-energy X-ray imaging will be conducted with the analytical tools available on the Carnegie field-emission gun scanning electron microscope (FEG SEM) with Co-I Nittler.

5.4 Prebiotic Chemical and Isotopic Evolution on Earth

Did the emergence and sustenance of Earth's first life depend on unique attributes of Earth's earliest sulfur cycle? This question lies at the core of NASA-sponsored astrobiological research. A major component of the solution to this question hinges on a robust determination of the most probable initial planetary conditions, i.e. the chemical compositions of the early atmosphere, oceans, crust, and mantle, and fundamentally deducing the nature of the first geochemical cycles that couple these reservoirs. Knowledge of these cycles may provide a critical set of boundary conditions for establishing a probability for the emergence of life.

From our knowledge of Earth history it appears that life arose early and may likely have been an inevitable consequence of the initial geochemical evolution the planet. However, as a

consequence of Earth's active tectonism, little record remains of the first 500-700 millions years of Earth history. What can be deduced is that Earth's earliest history began with the cataclysm of accretion (and possibly a massive Moon-forming impact event). The kinetic energy associated with Earth's formation (and the substantial impacts that followed shortly thereafter) was sufficient to vaporize much of the Earth and resulted in a magma ocean on the surface blanketed by a hot, dense atmosphere.

The sustained high temperatures associated with such a process were more than sufficient to convert any accreted organic molecules (i.e., those included in the chondritic sources) to their most thermodynamically stable light constituents, e.g., CO_2, CO, N_2, H_2, H_2O. Thus, at the very beginning it appears reasonable to assume that there existed no complex organic molecules within and on the surface of Earth. It may not have taken considerable time after these energetic events, however, for the first stable crust, atmosphere, and oceans to have formed (Sleep et al. 2000). Furthermore, there exists evidence that the first continents arose very early after crustal formation (Wilde et al. 2001; Mojzsis et al. 2001). The establishment of crust, ocean, and atmosphere inevitably initiated Earth's first geochemical cycles (e.g., carbon, sulfur, water, weathering) coupling the chemistry of these primary environments and reservoirs. The early dynamic chemical equilibrium across the primary reservoirs was, no doubt, subject to significant evolution by the addition of volatiles from the rapidly evolving mantle, atmospheric losses to space resulting from earliest photochemistry, as well as a continual rain of exogenous carbon from the slowly tailing remnants of the late heavy bombardment phase of the Solar System's history.

Within this chaotic and currently ill-defined world, life evidently arose by as yet unknown pre-biotic processes in an unknown environment. There remains considerable uncertainty regarding exactly how any organic chemistry intrinsic to a primitive terrestrial planet bridged into the initiation of an RNA world (Gilbert, 1985). It appears likely, however, that the probability of such chemistry occurring must have been inextricably bound to the nature of the Earth's earliest geochemical cycles. In the absence of favorable carbon, sulfur, and hydrologic cycles, the crucial first prebiotic stages may never have occurred, negating the possibility of the emergence of life. Given that the Earth is our only example of a planet where life began, understanding the early geochemistry of this planet is critical to understanding the probability for the emergence of life. What we learn about the Earth may provide critical guidance on the types of astrobiological instrumentation packages sent to other terrestrial planets (see **Section 5.8** for more discussion). The search for ancient life on other planets may well begin by establishing a robust determination of the earliest history of other potentially interesting terrestrial bodies, such as Mars (as discussed in **Section 5.2.5**), and comparing these with what was apparently sufficient for the emergence of life on the Earth.

With this in mind we propose a multidisciplinary approach toward understanding the early Archean sulfur cycle. Specifically, we aim to determine the biological and non-biological signatures within the sulfur isotopic record and use these data to place constraints on the early sulfur cycle which, due to a highly reducing atmosphere, was quite different from the latter half of Earth's history. We will further seek to determine whether there were particularly favorable attributes of the early Archean sulfur cycle that enhanced the quality of abiotic organic chemistry catalyzed by transition-metal sulfides that may have played a role in the emergence of life.

5.4.1 Unraveling Earth's early sulfur cycle (Astrobiology Roadmap Objective 4.1)

Background. Of the stable isotopic proxies for reconstructing ancient environments and detecting early life, sulfur isotope systematics stand above all others because they record not only metabolic processes but also the role of ultraviolet (UV) photolysis in atmospheric chemistry. Isotope analyses of sulfur-bearing minerals in ancient sedimentary rocks reveal that early microbial life had a strong preference for the lighter isotopes. In general, chemical

reactions often exhibit mass-dependent fractionation wherein the slight differences in mass (e.g., 32, 33, and 34 in the case of sulfur) manifest small but significant differences in reaction rates. These differences lead either to enrichments or depletions in the heavier isotopes in the products of a given reaction. The measurement of large mass-dependent fractionations of sulfur isotopes (associated with sulfate reduction and sulfide disproportionation) in low-temperature geologic environments (under conditions where kinetics inhibit inorganic processes) is convincing evidence of microbial activity.

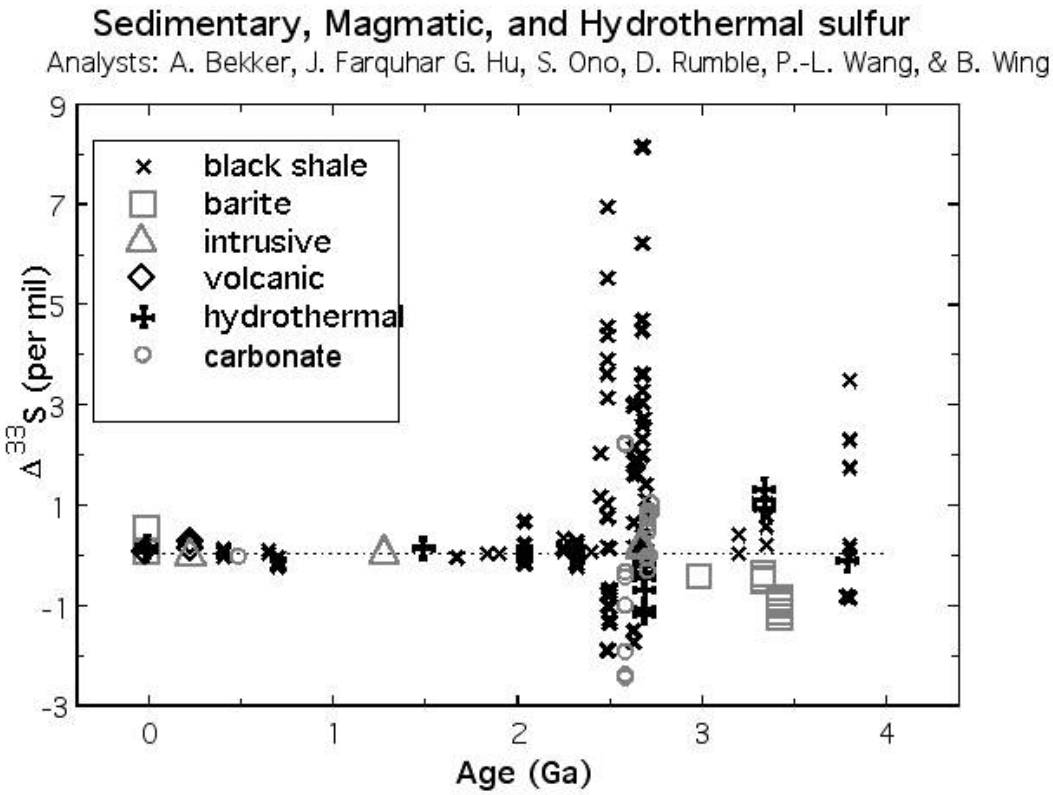

Figure 5.4.1. Values of •^{33}S (‰) for sedimentary, hydrothermal, and magmatic pyrite plotted versus age, where •^{33}S = δ^{33}S-0.515*δ^{34}S (‰). A •^{33}S (‰) near zero reveals mass-dependent fractionation, whereas deviation from •^{33}S (‰) ~ 0.0, in either a positive or negative sense, reveals mass-independent fractionation. Note the sharp break in distribution at an age of 2.3 Ga, the time proposed for the oxygenation of Earth's atmosphere. Before that time, values of •^{33}S range from -2.5 to 8 ‰; after 2.3 Ga, -0.5 < •^{33}S < 0.5.

Sulfur isotopes also exhibit the remarkable property of mass-independent fractionation (MIF). In the Earth's upper atmosphere today, certain gas-phase reactions promoted by solar UV light lead to fractionations of the various sulfur isotopes at variance with the typical mass-dependent fractionation line. There is not currently a very satisfying explanation for MIF phenomena; however, when such fractionation is observed it is invariably connected with photolytic gas-phase reactions (e.g., Farquhar et al. 2000).

These distinctive MIF fractionations are only rarely preserved at the Earth's surface, and then under unusual conditions. Polar ice cores, for example, contain sulfate aerosols derived from historic giant volcanic eruptions whose eruptive clouds reached into the stratosphere, penetrating

the ozone shield (Savarino et al. 2002). These sulphates exhibit small but detectable mass-independent fractionations. Aerosol sulphates derived from smaller eruptions incapable of rising above the troposphere exhibit only mass-dependent fractionation. The modern stratigraphic record of sulphate derived from volcanogenic sources thus reveals predominantly zero MIF effects punctuated by spikes corresponding to particularly large volcanic events.

The widespread prevalence of MIF in sulfur isotopes in Archean sediments is in strong contrast to their limited distribution today (Farquhar et al. 2000; Ono et al. 2002) (Figure 5.4.1). Analysis of drill cores of black shale from Australia and South Africa over an age range from 2.5 to 2.7 Ga shows a continuous geologic record of atmospheric production of MIF sulfur further modified by microbial activity at the seawater-sediment interface during deposition and diagenesis (Ono et al. 2002). The disparity in stratigraphic distribution between present and Archean sediments demonstrates a modern, episodic versus an ancient, continuous production and preservation of MIF sulfur. These relationships reveal a profound difference between the atmosphere as we breathe it and the atmosphere that existed during the Archean.

Numerical simulations of modern atmospheric chemistry have been used to provide a comparison of hypothetical anoxic atmospheres, postulated to have existed in Archean times. It appears that the sulfur isotope MIF anomalies are unlikely to survive in the presence of oxygen. In an oxygenated atmosphere, both the products and reactants of photochemical reactions are eventually fully oxidized to sulfuric acid, resulting in mixing and elimination of the MIF signature induced during photolysis (Pavlov & Kasting 2002). Modern preservation in sulfate aerosols from giant volcanic eruptions demonstrates the necessity of immediately storing MIF sulfur in solid particles that are less vulnerable to oxidation and isotope exchange than gas molecules.

The preservation of MIF sulfur isotopic signatures in an anoxic Archean atmosphere was not as difficult. First, in the absence of atmospheric oxygen no ozone shield would have existed to prevent photolytic reactions occurring throughout the atmosphere. Consequently, a considerably larger fraction of the sulfur records the MIF signature (Farquhar et al. 2000). Second, the products of photochemical reactions were much less likely to be attacked by oxygen, thus preserving the sulfur species that carries the MIF signature. Model calculations (Pavlov & Kasting 2002) indicate that MIF fractionations would have been preserved in aerosol particles of elemental sulfur as well as in sulfate aerosols delivered to the hydrosphere. In the absence of oxidative weathering of continents, atmospheric sulfate aerosols would contribute a sulfur isotope MIF signature to seawater. The particles of elemental sulfur would likely accumulate in sediments and be utilized by microbes, perhaps leading to the precipitation of pyrite.

Although microbial activity will impart a distinct mass-dependent isotope fractionation signature through SO_4^{2-} reduction, the precursor MIF signatures will persist. Microbes catalytically promote the precipitation of insoluble sulfide minerals, thus storing the MIF signature in sulfide minerals such as pyrite. Different species of microorganisms utilize different forms of sulfur that carry different MIF signatures. For example, sulfate-reducing bacteria would lead to precipitation of pyrite with a seawater sulfate MIF. Microorganisms that reduce atmospheric elemental sulfur particles would lead to precipitation of pyrite with the MIF of the sulfur aerosols.

With extensive reworking, the metabolic activities of consortia of microorganisms may also obscure or even eliminate MIF sulfur isotopes. For example, poly-specific microbial communities typically recycle each others' waste products. If in a given environment a sulfur-oxidizing species used the sulfide produced by sulfur-reducers, the resulting sulfate would mix with seawater sulfate and the MIF signature would eventually be diluted to undetectible levels. Depending on the situation it is clear that microbes have the ability either to preserve or obscure the stratigraphic record of MIF. Thus, the magnitude of the MIF sulfur signature in ancient

sediments reflects a fortuitous interplay between local environmental conditions, the ecology of microorganisms, and atmospheric deposition of aerosols.

Proposed Research. Co-I Rumble will lead four target investigations:

1. We plan to investigate in detail the stratigraphic record of the MIF signature in sulfur. We will seek to establish the pattern (or lack thereof) of geographic distribution in rocks of the same geologic age and measure highly resolved stratigraphic sections to investigate time-variation at specific geographic sites. We will focus attention on three critical time intervals, including:

2.5 to 2.0 Ga. We will analyze rocks that formed during this time interval to constrain, stratigraphically, when pO_2 level rose above 10^{-5} of present atmospheric levels, i.e., the point at which there was sufficient ozone to minimize the production of a strong MIF signature.

2.5 to 2.8 Ga. We will attempt to use both MIF and mass-dependent fractionation signatures to reconstruct the sulfur cycle in this transition period when O_2 photosynthesis first initiated (2.7 Ga or earlier) but while the atmosphere remained anoxic.

2.8 Ga to 3.5 (3.8?) Ga. We will seek isotopic evidence of the earliest sulfur-metabolizing organisms. This is an enormous challenge given the antiquity of this time interval, but progress may help provide the most robust signature of Earth's first life.

We will conduct fieldwork to collect suitable samples from each of the above time intervals from the relatively well-preserved Archean terrain exposed in Australia. Currently Co-I Rumble and collaborator Ono are actively participating in an Australian and international collaboration (including Johnson Space Center) to study this ancient terrain.

2. We will determine the extent to which the MIF signature is homogenous within a given stratigraphic unit. Toward this end, we will analyze individual pyrite crystals on a millimeter to micron scale with the new Thermo-Finnigan MAT 253 mass spectrometer in Co-I Rumble's laboratory at CIW. Our goal is to determine the degree to which sedimentary pyrite is homogeneous or heterogeneous isotopically. If given stratigraphic horizons are inhomogeneous, we may address whether this heterogeneity is due to microbial activity or to post-depositional metasomatism. Spatially resolved isotopic analyses will be crucial in answering these questions.

3. We will elucidate the mass-dependent microbial sulfur isotope signature through the analysis of controlled microbial cultures with Co-Is Emerson, Scott, and Steele. By measuring sulfur isotope fractionations by microorganisms, we will seek to apply laboratory fractionations to the interpretation of fractionations measured in Archean rocks. This research complements the goals outlined in **Section 5.7** of this proposal.

4. Technically we plan to develop new continuous-flow techniques for isotopic analysis of nanomole quantities of SF_6. Such new techniques will greatly aid the research described above. To accomplish our goals we will develop an ultralow-blank analytical protocol for the analysis of trace amounts of disseminated forms of sulfur in Archean sediments (and meteorites), including organic-bound sulfur, trace sulfate, and disseminated pyrite.

5.4.2 The critical role of sulfur in prebiotic (protometabolic) organic chemistry (Astrobiology Roadmap Objective 3.1)

Background. Over the past several decades our understanding of the diversity of habitats that support microbial communities has grown enormously. The recognition that life can exist even in some of the most inhospitable environments, e.g., the Antarctic dry valleys, has raised optimism regarding the slight possibility of extant life on other planets, most significantly Mars.

It is now apparent that virtually any environment that can be shown to provide biologically utilizable energy may sustain life. However, the range of environments suitable for the emergence of Earth's first life must have been much more restricted. Life could only have emerged in an environment capable of promoting the critical first organosynthetic reactions.

As it currently stands there is no satisfactory theory for the origin of life. A hypothetical, largely schematic, path from the initial abiotic world to Earth's first biology has been described as an ascendance through several key stages (e.g., de Duve 1991). In a condensed format, such a path starts with proto-metabolism, the stage where the natural environment promotes purely abiotic organosynthesis (i.e., abiotic carbon and nitrogen fixation). The consensus view holds that this protometabolic chemistry must have led into some manifestation of the "RNA" world (Gilbert 1985), the nascent stages of which involved spontaneous RNA replication. The ability to exploit crude molecular evolution was achieved at the point of primitive encapsulation (the first membrane, see **Section 5.5.1**). The development of RNA-dependent peptide synthesis, followed by the final development of translation, yields a first or "pre"-biotic system poised to intiate biological metabolism and herald in Earth's first life (de Duve 1991).

Research to Date. Although there has been substantial progress over the past three decades in research covering many of these path segments, for example in the area of RNA catalysis, there remains considerable uncertainty regarding how organic chemistry intrinsic to a primitive terrestrial planet bridged to the initiation of the RNA world. As part of our NAI research initiative, we have focused on the first stages (or the geochemical roots) of the origin of life, protometabolism. Our investigation has been and continues to be the experimental exploration of the importance of mineral catalysis (in particular transition-metal sulfides) in promoting the protometabolic stages of the origin of life.

One of the key aspects to deducing the prebiotic chemical origins of life on Earth, as well as constraining the potential for life on any other terrestrial planet (e.g., Mars), involves establishing where in either the atmosphere, oceans, or crust, natural geochemical cycling provided the most useful chemistry for pre-biotic biosynthesis. Our experiments have demonstrated the importance of sulfur (both inorganic, e.g., transition-metal sulifde, and organic, e.g., thiol) as an essential component for useful protometabolic chemistry. Certain organo-sulfur species, thioesters in particular, have long been proposed as particularly useful prebiotic intermediates, based on their large free energies of formation and their role in promoting organic chemistry that can provide the function and utility necessary to initiate anabolic biosynthesis (e.g., de Duve 1991; Wächtershaüser 1988). Their high energy content means that once formed thioesters can be used in organic reactions to drive otherwise endergonic reactions, e.g., the peptidization of amino acids. Acetyl-CoA is the ubiquitous thioester employed in many of the metabolic strategies of extant microorganisms. Both Acetyl-CoA and pyruvic acid are synthesized *de novo* by anaerobic chemoautotrophic prokaryotes (e.g., methanogens and acetogens) through the action of a complex set of enyzmes that contain within their active centers Fe and Ni (and possibly Cu, e.g., Doukov et al. 2002) sulfur clusters (Lindahl et al. 1990).

To date, the only demonstrated abiotic means for the synthesis of thioesters (relevant to the early Earth) has utilized transition-metal sulfides (e.g., Huber & Wächtershaüser 1997; Cody et al. 2003b). It may very well be that the essential prebiotic chemistry that led to the emergence of the phenomenon of life was inextricably bound to the presence of transition-metal sulfides. In addition to catalysts, however, the protometabolic environment also required a reliable source of energy and essential chemical substrates. This means that if life derived from endogenous chemistry intrinsic to the early Earth, the environment must have provided both readily available energy and light-element substrates (e.g., CO_2, NH_3, and H_2S), as well as natural catalysts to couple the energy and the chemical substrates to form biochemically useful molecules. Certainly, the most obvious energy sources are solar UV and visible radiation and

thermochemical energy derived from redox disequilibria. Photo-driven prebiotic organic chemistry would necessarily have been restricted to the atmosphere, shallow ocean, and continental surfaces. Thermochemical energy could have been available wherever mixing of reduced and oxidized species result in thermodynamic disequilibria (e.g., Shock et al. 1996).

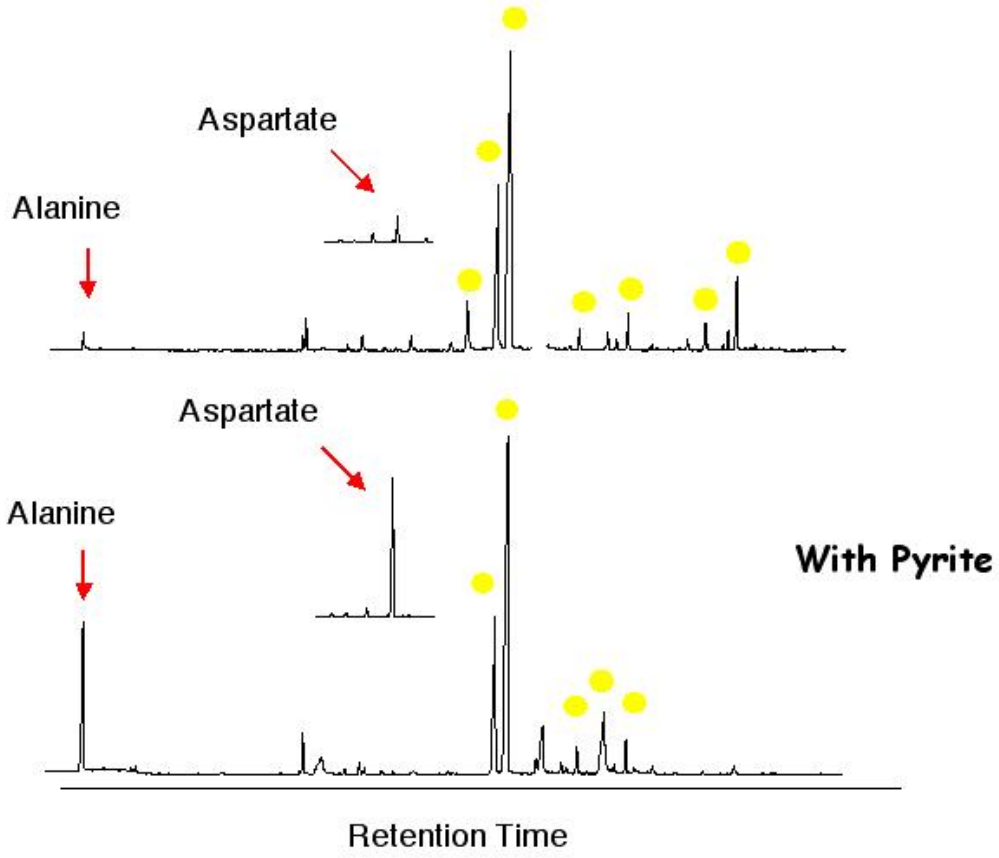

Figure 5.4.2. Chromatograms revealing the products of reactions of aqueous citric acid (100 mM) in the presence of 100 mM of ammonium formate run at 200 °C for 3 hours with and without pyrite. Of primary interest to protometabolic chemistry are the products of aqua-thermal decomposition of citric acid ending with the formation pyruvic acid (Cody et al. 2001). This reaction pathway presumably passes through the production of oxalacetic acid, although none of this important metabolic intermediate has been detected. In the presence of ammonium formate, a substantial quantity of pyruvate is converted to alanine through a reductive amination reaction. Small, but detectable, quantities of aspartic acid formed from the reductive amination of oxalacetic acid are also detected (shown here as an inset). The direct synthesis of aspartic acid (aspartate at this pH) is significant, as it mimics several key steps that occur in certain metabolic strategies. Aspartate also provides a critical threshold for the potential synthesis of pyrimidines, possibly connecting this hydrothermal chemistry with the roots of some type of RNA world. The presence of pyrite enhances the reductive amination, presumably by providing a catalytic site for the reduction of the imine intermediate.

Our previous work in this area has demonstrated the utility of a broad range of metal sulfide catalysts for the promotion of chemistry that bears (at least superficial) similarity with extant anabolic strategies of certain chemoautrophic anaerobic organisms (e.g., methanogens and acetogens). We have shown by experimental assay that many different transition-metal sulfides

provide the catalytic qualities to promote protometabolically useful carbon fixation chemistry that largely mimics the Acetyl-CoA pathway utilized by both methanogens and acetogen as their primary carbon fixation and root anabolic pathway (Cody et al. 2003b). We have shown that transition-metal sulfides catalyze the reduction of nitrate and nitrite (potentially derived photochemically) (Brandes et al. 2000). We have identified, and provided supporting experimental results for, a plausible protometabolic carbon fixation pathway that uses transition-metal sulfides as heterocatalysts (Cody et al. 2001).

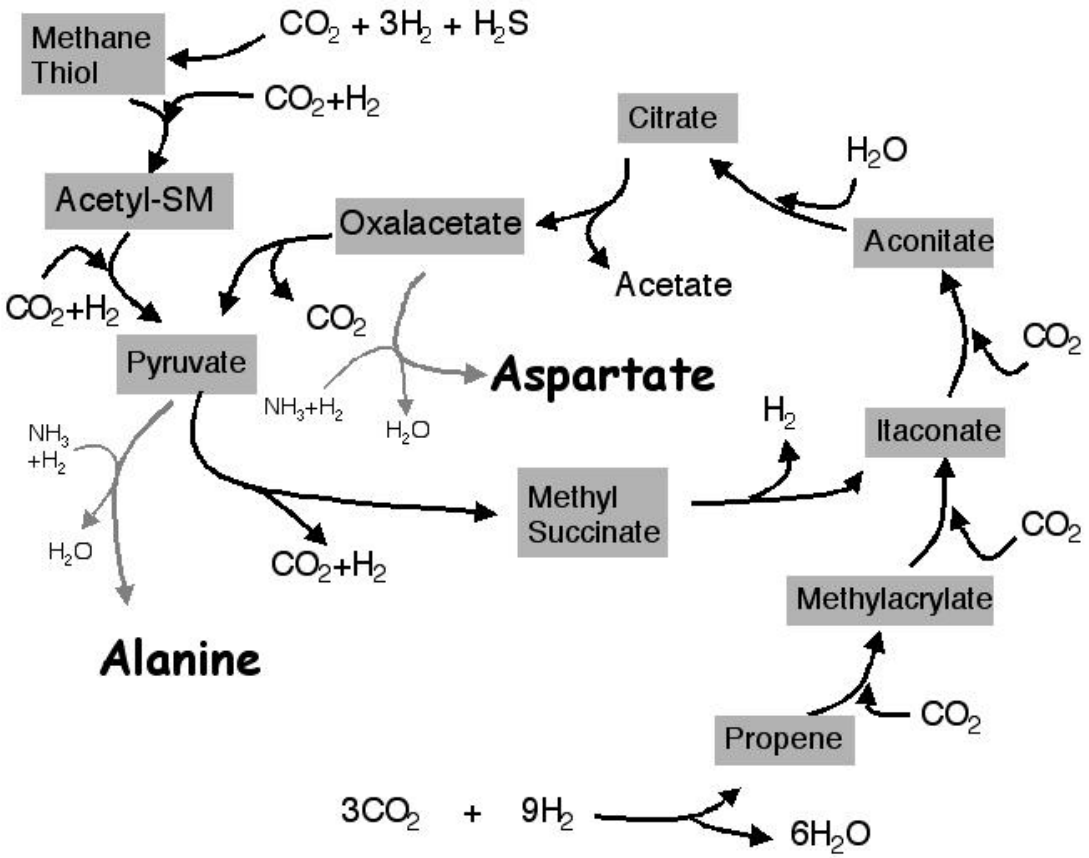

Figure 5.4.3. A composite of various *abiotic* ("protometabolic") carbon- (and nitrogen-) fixation reactions promoted or catalyzed in the presence of transition-metal sulfides and in aqueous media. This reaction network shares superficial similarities with intermediate anabolic strategies of certain anaerobic autotrophs, e.g., methanogens and acetogens. A reaction network such as this may have provided the primitive world with the essential biochemicals necessary to "jump start" an emergent chemical system, e.g., RNA world. The apparent complexity shown in this diagram does not, by any means, reflect a limit for abiotic chemistry; rather it records what has been observed to date in a very limited reaction space explored by relatively few researchers in experimental protometabolic chemistry (e.g., Heinen & Lauwers 1996; Huber & Wächtershäuser 1997, 1998; Cody et al. 2000, 2001, 2003a,b).

Recently, we have shown that we can couple nitrogen fixation for the promotion of amino-acid synthesis by utilizing segments of the above-mentioned protometabolic cycle, again in the presence of transition-metal sulfides. Specifically, we have shown that we can synthesize aspartate and alanine directly from citric acid under reducing conditions, with the reaction enhanced by presence of transition-metal sulfides (Cody et al. 2003c) (Figure 5.4.2). The hydrothermal synthesis of alanine has been demonstrated previously (e.g., Hafenbradl et al.

1995; Weber 1998, 2001; Brandes et al. 2000). The synthesis of aspartate is extremely important, as it may provide the critical bridge for the synthesis of certain nucleic acid bases, in particular the pyrimidine, orotic acid, the precursor to uracil, cytosine, and thymine (e.g., Abeles et al. 1992). Finally, we have shown that certain transition-metal sulfides will participate in reactions beyond just that of catalysts, yielding soluble organometallic iron-sulfur clusters that are capable of promoting the synthesis of alpha-keto acids from alkane thiol precursors (Cody et al. 2000). Figure 5.4.3 summarizes the results of our experiments, including those of others where transition-metal sulfides were used to promote reaction (e.g., Heinen & Lauers 1996; Huber & Wächtershäser 1997), placing the results in the context of a potentially useful protometabolic scheme for carbon and nitrogen fixation.

It must be stressed that the our experiments are not designed to explore directly the origins of life; rather they were designed and optimized to explore the ability of metal sulfides to promote "proto-metabolic" chemistry with possible relevance to the origins of life. Therefore, our previous results and the experiments proposed below should not be misinterpreted as supporting the possibility that life could have arisen at temperatures as high as 250° C.

Many important biomolecules are thermally unstable in aqueous media; for instance, functionalized sugars (both aminated and phosphorylated), peptides, polyphosphates, or thioesters will not survive long in water under hot hydrothermal conditions (e.g., Larralde et al. 1995; Shapiro 1995). Nitriles, such as cyanoacetylene and cyanoacetaldeyde — proposed as possible prebiotic precursors for pyrimidines (Robertson & Miller 1995) — will be rapidly hydrolyzed to carboxylic acids in hot water (Siskin et al. 1990). Therefore, if the synthesis of nucleotides and their subsequent oligomerization to promote an "RNA" world is requisite to the emergence of life, then such chemistry would be extremely improbable (and likely impossible) in high-temperature water (e.g., Miller & Lazcano 1995).

Nevertheless, in a pre-enzymatic "protometabolic" world, mineral catalysts were undoubtedly required to initiate a broad range of organosynthetic reactions for producing the first biomolecules. For at least some of this chemistry, it is difficult to conceive of better, naturally occurring, prebiotic catalysts than metal sulfides (e.g., the theories of Wächterhäuser 1988; Russell & Hall 1997; Russell et al. 1998). The challenge remains to demonstrate which catalysts did what and where the prebiotic chemistry ultimately converged into the phenomenon of life itself. The results of our previous experiments suggest that cool environments in close proximity to metal sulfide-precipitating hydrothermal hot-springs may have been advantageous to the emergence of life on the early Earth, as well as other terrestrial planets, such as early Mars or early Venus.

Proposed Research. To further our contribution to the prebiotic components of astrobiological research, Co-Is Brandes, Cody, Fogel, Hazen, Yoder and Collaborators Boctor and Sharma propose to continue and expand on our experimental studies of organosynthetic reactions that may have actually occurred on the Hadean and early Archaen Earth. Our focus in the CIW NAI team will be to provide a robust experimental assessment of the feasibility of such prebiotic chemistry on any terrestrial planet with an active sulfur cycle and volcanism to provide both reduced fluids and catalytically active metal-sulfide substrates. We propose the following:

Over the next cycle of research, Co-Is Cody, Brandes, Hazen, and Collaborators Boctor and Sharma propose to assess experimentally (quantitatively) the kinetics of key carbon-, nitrogen-, and sulfur-fixing reactions by focusing on establishing the dependence of temperature, substrate activities, fluid pH, and choice of catalytic substrate on the reaction rates. We have already shown what is possible under ideal, assay conditions; we now want to establish the key kinetic parameters that would allow for a robust global and historic assessment of abiotic synthesis in the early Archean. These experiments will utilize synthetic sulfides (prepared by Co-I Boctor) spanning geologically relevant compositional series (e.g., Ni^{2+} and Co^{2+} substitution in FeS and

FeS_2). Following these experiments, we will select from our extensive library of well-characterized natural specimens for a comparative analysis. Our assessment of the organosynthetic reaction rates and reaction pathways also has direct relevance for the proposed research outlined in **Section 5.6.1** on hydrothermal vent environments.

Co-Is Cody and Fogel will explore whether kinetic isotopic fractionation associated with the key organosynthetic reactions can serve as a definitive signature for protometabolic organosynthesis utilizing compound-specific gas chromatography isotope-ratio mass spectrometry (GC-IRMS) focusing initially on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Such isotope fractionation has been shown to be the case for simple hydrocarbons, e.g., methane through butane (Sherwood Lollar et al. 2002). We will expand these analyses to organic chemistry that yields more complex (hence prebiotically more interesting) organic molecules, e.g., amino acids and various metabolic intermediates. In this regard, this research dovetails with the work proposed in **Section 5.7.1** on biosignatures. Kinetic isotope fractionation will be used to help constrain reaction mechanism, as well as allow us to determine whether one can distinguish between biological and abiological synthesis of biologically relevant molecules (e.g., amino and fatty acids). As part of the overall goal of employing sulfur isotope signatures to help constrain the global sulfur cycles, we will work with Co-Is Rumble and Farquhar and Collaborator Ono to develop methods for the analysis of sulfur isotopes in organosulfur-containing species.

Co-I Cody and Collaborator Boctor propose to explore catalytic organosynthetic reactions utilizing natural metal sulfide assemblages. Throughout most of our previous studies we have utilized laboratory-synthesized pure metal sulfide phases as a means of adding a degree of chemical control to what are otherwise complex reaction systems. Natural sulfide deposits typically contain multiple phases, e.g., coexisting sphalerite (ZnS), chalcopyrite ($CuFeS_2$), pyrrhotite ($Fe_{1-x}S$), and pyrite (FeS_2). Individual (natural) metal-sulfide phases are virtually never compositionally pure. Considerable cation substitution (iron for zinc, cobalt or nickel for iron) can occur in many of the predominant phases in natural sulfide deposits. Such substitutions will likely effect significant changes in the catalytic properties of a given metal sulfide phase. We anticipate that the naturally occurring transition-metal sulfide samples may reveal interesting synergetic effects and perhaps considerably enhance catalytic promotion of useful protometabolic reactions.

We will focus initially on two types of metal-sulfide-rich samples. The first are recent samples obtained from mineralized vent chimney-wall material provided by Co-I Baross. These samples tend to grade in their mineral chemistry and assemblages across the vent chimney walls, recording a range of precipitation temperatures (Tivey 1995). Co-I Baross has proposed (see **Section 5.6.1**) that certain extremophilic microorganisms that currently live within the porous vent chimney walls may exploit the natural catalytic qualities of transition-metal sulfides in their environment for the production of simple organic substrates to support heterotrophy. We will assay the catalytic or otherwise promoting qualities of these natural sulfides under conditions that mimic those in proximity to an active vent chimney to help test this hypothesis.

We will also investigate organosynthesis promoted by metal sulfides present in natural ore samples obtained from ancient massive sulfide deposits (e.g., the ~ 3.2-Ga Sulfur Springs VMS deposits of Western Australia; these sulfides are currently being studied by Co-I Rumble and Collaborator Ono in collaboration with Australian and international scientists; see **Section 5.4.1**). In both cases, we will use similar assaying strategies that were previously developed by us (Cody et al. 2003b) for the analysis of pure transition-metal sulfides. We will utilize isotopically enriched (^{13}C) reagents so that we will be able to separate our reaction products from any naturally present organic compounds (T. McCollum, pers. comm., 2003).

Finally, Co-Is Brandes, Cody, Hazen, and Yoder propose to conduct a series of experiments to establish whether there exist abiotic isotopic signatures for small molecule production and

destruction in hydrothermal (and perhaps other) systems. Previous work has shown that in addition to potentially useful protometabolic chemistry, transition-metal sulfides also serve as powerful promoters of NO_x reduction and to a lesser extent N_2 reduction (Brandes et al. 1998, 2000, 2001; Schoonen & Xu 2001). Specifically, we propose to establish the isotopic fractionation factors for the production of methane and C_2-C_5 hydrocarbon gases from the reduction of CO_2 to compare with the natural occurrences of such gases where abiotic synthesis has been indicated (e.g., Sherwood Lollar et al. 2002) (see also **Section 5.7.2**). We will also establish isotope fractionation signatures among nitrogen species (NO, N_2O, N_2) produced during the transition-metal-sulfide-promoted reduction of NO_2 under strictly abiotic conditions. Our proposed experiments will seek to detect specific isotopic fractionation patterns based on the nature of the catalyst and reaction conditions. The proposed work aims to provide isotopic constraints to the interpretation of stable isotopic signatures of carbon and nitrogen that may be analyzed on future Mars lander missions that sample the regolith or polar ice. The reactions will be performed by Co-Is Cody, Hazen, and Yoder at CIW and the products analyzed at the Stable Isotope Laboratory at the University of Texas at Austin by Co-I Brandes.

5.5 Prebiotic Molecular Selection and Organization

By what mechanisms were biomolecules selected, concentrated and organized from the diverse prebiotic organic inventory? Many processes contributed to the synthesis and accumulation of organic molecules on the prebiotic Earth (e.g., Chyba & Sagan 1992). Synthesis in interstellar dense molecular clouds (Bernstein et al. 1995, 2002), in the primitive atmosphere (Miller 1953; Miller & Urey 1959), in hydrothermal systems (see **Section 5.4.2**) (Huber & Wächtershäuser 1997; Cody et al. 2000, 2001, 2003b), during impacts (Blank et al. 2001), in aerosols (Dobson et al. 2000; Tuck 2002), and perhaps even in igneous rocks (Freund et al. 2001) provided the primordial Earth with a diverse suite of organic molecules. Our proposed studies on prebiotic chemical and isotopic evolution on Earth (see **Section 5.4.2**) will amplify our understanding of these ubiquitous processes.

Notwithstanding the relatively facile prebiotic synthetic chemistry and accumulation of exogenous organic molecules near Earth's surface, there remains the significant challenge of understanding the origin of life. Life's molecular building blocks, unlike the diverse prebiotic inventory, are highly selected and are organized into functional macromolecules. Biological amino acids, sugars, lipids, and the metabolic intermediates epitomize this point. Terrestrial life relies predominantly on a suite of 20 different α-H levose (L) amino acids that are condensed to form proteins. By contrast, prebiotic processes produce a more diverse suite of racemic amino acids, which do not tend to form peptide bonds in most prebiotic environments. For example, the Murchison meteorite yields more than 70 different amino acids monomers, including α-methyl amino acids and β-amino acids not used in extent organisms (Kvenvolden et al. 1970). Extant life also uses enantiomerically pure pentose sugars, D-ribose and D-deoxyribose. While several studies demonstrate facile prebiotic synthesis of sugars via the formose reaction (Shapiro 1988; Weber 2001), such processes invariably lead to a complex suite of energetically similar molecules, including the eight chiral isomers of D-ribose, plus a variety of straight, branched, and cyclic tetroses, pentoses, and hexoses. In these and many other examples, extent life employs specialized synthesis pathways to achieve a high degree of molecular selectivity and macromolecular organization. The natural origin of homochirality in both monomers and biopolymers remains a mystery.

In this section we examine processes that might have promoted the selection and organization of prebiotically derived organic molecules into functional structures beneficial to emergent life. We propose to study two possible mechanisms: (1) the self-organization of amphiphilic molecules to form membrane-like bilayers in saline and non-saline aqueous solutions, and (2) the chiral selective adsorption and polymerization of amino acids and sugars on mineral surfaces.

5.5.1 Self-organization of amphiphiles (Astrobiology Roadmap Objective 3.2)

Background. Molecular self-assembly provides a potential solution to the problem of molecular selection and organization. In fact, self-assembly still plays a central role in life processes such as duplex DNA assembly from single complementary strands, protein folding, and membrane formation. Living cells are separated from their environment by membrane boundary structures that incorporate a lipid bilayer composed of amphiphilic molecules such as phospholipids and cholesterol. It is a simple exercise to show that vesicles composed of amphiphilic compounds (liposomes) spontaneously appear by self-assembly when amphiphilic molecules are introduced into aqueous phases (Bangham et al. 1965). It has been argued that such vesicles may provide reasonable models for the first membranous structures on the early Earth (Deamer 1997; Segré et al. 2001; Luisi 1996; Deamer et al. 2003).

Phospholipids will readily self-assemble into membranous vesicles. Membranous structures can also be prepared from single-chain amphiphiles such as fatty acids, fatty alcohols, and monoglycerides. Such vesicles may provide models for the formation of the earliest cellular compartments. The prebiotic early Earth would have provided multiple sources for such molecules (see **Sections 5.3.1, 5.3.2**, and **5.4.2**). For instance, carbonaceous meteorites contain a rich mixture of organic compounds that were synthesized abiotically in the presolar molecular cloud and in the early Solar System. Co-I Deamer and colleagues have established that certain components of the Murchison organics operate as surface-active amphiphiles and have been shown to assemble into membranous vesicles (Deamer 1985; Deamer & Pashley 1989) (Figure 5.5.1). Although the composition of the membrane-forming amphiphiles present in the Murchison organic mixture has not yet been established in detail, it is clear that monocarboxylic acids are present (Lawless & Yuen 1979; Shimoyama et al. 1989; Mautner et al. 1997). Monocarboxylic acids have also been synthesized under a variety of simulated prebiotic conditions (Huber & Wächtershäuser 1997; McCollom et al. 1999; Rushdi & Simoneit 2001; Dworkin et al. 2001; Cody et al. 2001, 2003b).

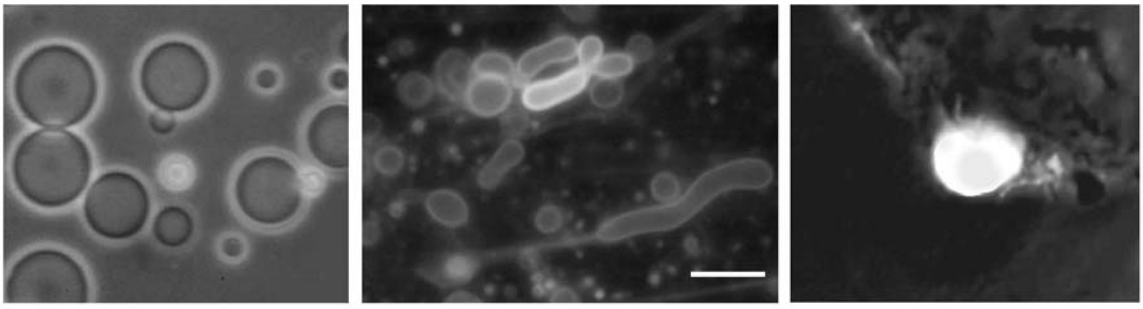

Figure 5.5.1. Membranous vesicles self-assemble from a variety of organic amphiphiles. Left: Murchison meteorite extracts contain amphihilic compounds that readily form membranes that are true permeability barriers to the flux of polar and ionic solutes. Center: Decanoic acid, a component of meteoritic organics, is one of the simplest amphiphilic compounds that can form stable vesicles. Right: Photoproduct from laboratory simulation of interstellar grain mantle ices (Dworkin et al. 2001). The photoproduct vesicle has captured pyranine, a fluorescent dye, which indicates the presence of a boundary membrane. The scale bar is 10 μm.

Research to Date. We have begun to investigate the physical properties of self-assembled structures produced by monocarboxylic acids of various chain lengths and of mixtures with other simple amphiphilic compounds. The bilayer-forming potential of fatty acids with shorter hydrocarbon chains (C8-C11) has been previously investigated (Hargreaves & Deamer 1978; Walde et al. 1994; Apel et al. 2002; Monnard et al. 2002). The length and the degree of

unsaturation of the hydrocarbon chains play important roles in bilayer-membrane properties such as permeability and stability, which would have been determining factors for primitive life forms.

In spite of our ability to demonstrate facile vesicle formation using pure fatty acids (Deamer & Barchfield 1982; Deamer & Pashley 1989), we have discovered significant limitations of this model system. The primary problem arises because fatty-acid bilayer membranes are stabilized by van der Waals interactions between their hydrocarbon chains and by hydrogen bonds formed between deprotonated and protonated acid molecules. Therefore, formation of bilayer vesicles is extremely sensitive to the pH of the medium. We have found that such vesicles are stable only at pH ranges at or near the pKa of the fatty acid, i.e., over a very narrow pH range. We have also discovered that stability is a strong function of chain length, and at least 10-carbon chains are required for reasonably stable vesicles. The Murchison meteorite contains fatty acids with hydrocarbon chain lengths from C8-C12, while reaction products of prebiotic Fischer-Tropsch type synthesis also produce fatty acids with hydrocarbon chains in the heptanoic, octanoic, and nonanoic acids (Rushdi & Simoneit 2001).

The stability and permeability of fatty acid vesicles is dependent on bilayer-intrinsic physical properties and environmental conditions, including the salinity or the ionic strength of the bulk aqueous phase. For example, if the emergence of life took place in a marine environment, sodium chloride in solution would have exerted osmotic pressure across membranes, and it has been established that fatty acid vesicles cannot withstand sodium chloride concentration in the range of today's oceans (Monnard et al. 2002). Furthermore, the presence of divalent cations in marine salts, probably at concentrations in the range of several millimolar or higher, causes fatty acid vesicles to precipitate (the "hard water" effect, Monnard et al. 2002). Thus, the presence of divalent cations in the primordial aqueous environments would have severely inhibited the self-assembly of simple fatty acid vesicles.

A possible solution to this conundrum is that early membranes were not composed of pure fatty acids, but were mixtures of amphiphilic compounds that included fatty acids, fatty alcohols, polycyclic aromatic derivatives, and perhaps even fatty acid esters with glycerol. It stands to reason that such mixtures are much more probable than pure compounds given the complexity of the prebiotic environment. We have begun to investigate binary mixtures and have discovered that the addition of a fatty alcohol to fatty acid membranes dramatically increases their robustness to pH effects. For example, pure fatty acid vesicles become micelles only above pH 7.5; however, addition of 20 mole percent of the equivalent-chain-length fatty alcohol results in vesicle formation from pH 7 to pH 11 (Apel et al. 2002). We have also discovered that a simple glycerol ester of a fatty acid (glycerolmonodecanoate) readily formed stable membranes at pH ranges and divalent ion concentrations that were prohibitive for the pure fatty acid system (Monnard et al. 2002).

Proposed Research. The preliminary results above reveal that a rich chemical phase space remains to be explored in the search for amphiphilic components that would form stable vesicles under plausible prebiotic conditions.

Co-Is Deamer and Hazen propose to explore vesicle formation systematically in mixed-amphiphile suites. Our previous studies of vesicle formation in pure water have relied on either pure lipid species, such as long-chain carboxylic acids and alcohols, or on chloroform-soluble extracts from natural or synthetic samples. We propose to extend these studies to more diverse mixed suites of amphiphiles, as well as to aqueous solutions that include realistic concentrations of ionic species. We will start with a mixture of decanoic acid, decanol, glycerol monodecanoate, and hydroxypyrene, all of which are expected to be present in meteoritic organic components, or to be products of simulated geochemical syntheses. Aliphatic chain length will be chosen to be sufficiently long for stability, yet still fluid at ordinary temperature

ranges. The head groups (-COOH, -OH, glycerol ester) are chosen for their ability to provide hydrophilicity as well as stabilizing effects of intermolecular hydrogen-bonding. Hydroxypyrene is chosen as a cholesterol analog, to serve as a stabilizing component of contemporary membranes through van der Waals interactions with non-polar lipid chains. The concentrations of components will be systematically varied over molar ratios ranging from 0 to 4, and the resulting structures will be analyzed microscopically. Analysis of membrane function will include measurement of permeability coefficients of the resulting membranes to amino acids (Chakrabarti & Deamer 1994), solute ions such as protons and potassium (Paula et al. 1996), and substrates such as nucleotides (Monnard & Deamer 2001). We will also establish the ability of the resulting vesicles to encapsulate macromolecules such as RNA and polymerase enzymes (Shew & Deamer 1983; Chakrabarti et al. 1994).

A second research focus involves the study of vesicle formation in model marine environments. We propose to examine suites of amphiphilic molecules described above as models for vesicle formation in plausible marine environments. As the concentrations of ionic solutes and the pH of early Earth sea water is still uncertain, we will vary the primary ionic species over plausible ranges in order to determine the extent to which a given amphiphilic mixture can form stable membrane structures. For example, NaCl will be varied from 0 to 1.0 M, divalent cations such as magnesium, calcium, and iron will be varied from 0 to 20 mM, and pH will be varied from 5 to 9. We expect that the results of these experiments will help to constrain the range of prebiotic aqueous environments and amphiphilic molecular species that could have promoted vesicle formation on the prebiotic Earth. A modern phospholipid, such as phosphatidylcholine, is able to form stable lipid bilayers over the entire range of environmental conditions described above. If we can discover a mixture of plausible prebiotic amphiphiles that matches this property in terms of stability and permeability, this result would provide an important stepping stone toward a better understanding of the origin of cellular life.

5.5.2 Molecular selection and organization on mineral surfaces (Astrobiology Roadmap Objective 3.2)

Background. Life's origin must have been preceded by the selection and concentration of monomers into macromolecules. Mineral surfaces are particularly interesting in this regard as it is well known that minerals provide sites for adsorption of key molecules in an aqueous environment (Bernal 1951). The potential effectiveness of crystalline surfaces in concentrating and organizing pure organic molecules has been demonstrated dramatically by the work of Sowerby et al. (1996, 1998a), who examined the adsorption of monolayers of the nucleic acid bases adenine and guanine on graphite and molybdenum disulfide surfaces. Such adsorption of pure molecules from solution results in highly ordered, two-dimensional organic structures, which might serve as templates for further organic selection and organization (Uchihashi et al. 1999).

Crystalline surfaces also may promote the emergence of one-dimensional molecular structures, in particular on surfaces that were subject to cycles of wetting and drying (Orgel 1998). Pioneering studies by Lahav and coworkers demonstrated peptide formation on clays subjected to cycles of wetting and evaporation (Lahav et al. 1978). Ferris and coworkers (Ferris et al. 1996; Ertem & Ferris 1996, 1997) and Orgel and coworkers (Hill et al. 1998; Liu & Orgel 1998) have demonstrated the oligimerization of amino acids on illite and hydroxyapatite and of nucleotides on montmorillonite. Other researchers have proposed similar roles for zeolites (Smith et al. 1998, 1999), hydroxides (Arrhenius et al. 1993), and calcite (Hazen et al. 2000), as well as for transition-metal sulfides, e.g., pyrite and pyrrhotite (Wächtershäuser 1988, 1992; Russell et al. 1993; Russell & Hall 1997; Brandes et al. 2000). Note, however, that all experiments on organic adsorption by minerals have been performed with relatively concentrated, pure solutions of the target organic species, rather than with a more realistic dilute and complex solution appropriate to the primordial Earth.

Hazen and Sholl (2002) have recently provided evidence that silica surfaces, quartz $(10\bar{1}1)$ and (001) faces, have the ability to select basic amino acids from a suite of acidic and neutral amino acids. This behavior is attributed to the difference between the point of zero charge of the mineral surface (pH ~ 3.5) compared with the amino acid isoelectric point.

The demonstrated ability of minerals to select and adsorb organic molecules has long been recognized as a possible mechanism for one of life's most distinctive biochemical signatures – its strong selectivity for L-amino acids and D-sugars. Almost all prebiotic synthesis reactions yield essentially equal amounts of L- and D-enantiomers (Lahav 1999; Mason 2000). Thus, to explain life's chiral excess two broad categories of symmetry-breaking phenomena have been invoked (Pályi et al. 1999; Bonner 1991, 1992, 1995). On the one hand, some researchers favor large-scale processes such as chirally selective photolysis by circularly polarized synchrotron radiation from a rapidly rotating neutron star (Clark 1999; Podlech 1999; Bailey et al. 1998), magnetochiral photochemistry (Rikken & Raupach 2000), or parity-violating weak interactions of nuclear particles (Salam 1991). It is possible that any of these processes may account for the small but significant excess of L-amino acids reported from some carbonaceous chondrite meteorites (Engel & Macko 1997; Cronin & Pizzarello 1997; Pizzarello & Cronin 2000).

On the other hand, many investigators have focused on chiral enhancement via local "asymmetric agents" (Popa 1997; Avetisov et al. 1991; Avetisov 1999; Cintas 2002). Some mechanisms rely on local amplification of slight chiral excesses, for example by Bose-Einstein condensation (Chela-Flores 1994) or by chiral self-assembly of polymers (Bolli et al. 1997; Lippmann & Dix 1999; Saghatelian et al. 2001) or crystals (Eckert et al. 1993; Lahav & Leiserowitz 1999). These and other chiral selection mechanisms (Bonner 1991) require further investigation, but the conceptually simple and geochemically relevant chiral selection mechanism of adsorption on chiral crystal growth surfaces of minerals, described above, has been largely overlooked.

Some authors argue that quartz and other minerals cannot contribute to the origin of biochemical homochirality (Bonner 1995) because left- and right-handed surfaces are present on Earth in equal abundance (Frondel 1978; Evgenii & Wolfram 2000). This argument is based on the assumption that the preponderance of L-amino acids in biology is evidence for a global prebiotic excess of L-amino acids rather than a chance selection in a local chiral environment. Nevertheless, both global- and local-scale chiral processes are deserving of further study.

Research to Date. Selective adsorption of a chiral molecule on a crystalline surface implies at least three non-colinear points of interaction between the molecule and an acentric solid surface (Davankov 1997). Consequently, chiral adsorption requires both a chiral molecule and a chiral crystalline surface (Hazen & Sholl 2003). The properties of chiral molecules have been studied for more than a century and a half (Pasteur 1848, 1851), and chiral chemistry is a thriving branch of organic chemistry (Stinson 2001; Rouhi 2002). The subject of chiral crystalline surfaces, however, has received relatively little attention, and many previous studies of chiral adsorption have failed to exploit these surfaces effectively.

Crystals that lack a center of symmetry provide the most obvious chiral surfaces for experimental study. Quartz (SiO_2; trigonal space group $P3_221$ or $P3_121$) is by far the most common acentric mineral in nature, and almost all experimental studies of chiral adsorption have thus focused on the symmetry-breaking effects of right- versus left-handed quartz (Tsuchida et al. 1935; Karagounis & Coumonlos 1938; Bonner et al. 1974, 1975; Soai et al. 1999). However, all of these studies employed quartz crystals that were powdered, a procedure that averages potentially strong adsorption effects on different faces, while destroying all structural information that might reveal adsorption mechanisms. Consequently, we have developed techniques to study adsorption on relatively large surfaces (~10 cm^2) of single crystals.

Centric crystals also provide a rich variety of chiral solid surfaces for study (Lahav 1999; McFadden et al. 1996; Hazen et al. 2001), because any crystal plane with a surface structure lacking mirror symmetry is intrinsically chiral. Most common rock-forming minerals display crystal growth faces that meet these conditions (Dana 1949; Smyth & Bish 1988). We have concentrated on the common and biologically relevant mineral calcite ($CaCO_3$; rhombohedral space group $R\bar{3}c$; Hazen et al. 2001). As a test of chiral selective adsorption, Hazen et al. (2001) immersed four calcite crystals for 24 hours in a 0.05-M solution of racemic aspartic acid. Enantiomeric faces equivalent to $(21\bar{3}1)$ and $(3\bar{1}\bar{2}1)$ displayed up to 10% preferential adsorption of D- and L-aspartic acid, respectively. By contrast, no selective adsorption was observed on rhombohedral $(10\bar{1}4)$ cleavage faces, which have centric surface structures and thus serve as an experimental control. This study of the calcite-aspartic acid system is the first experimental demonstration of significant chiral selectivity by a centric natural crystal.

Proposed Research. A focused program of study of molecular selection on calcite and quartz surfaces is now supported by a grant to Co-I Hazen from NSF. However, numerous promising aspects of the problem remain unstudied. With the support for an NAI Fellow, Co-I Hazen proposes to expand significantly on these developments with three complementary series of experiments: (1) differential adsorption of biomolecules, (2) origins of biochemical homochirality, and (3) condensation polymerization of homochiral peptides.

We plan to continue our studies of the relative adsorption of different amino acids, sugars, and other molecular species onto mineral surfaces, including new investigations of transition-metal oxide and sulfide minerals, exposed to equimolar suites of molecular species in aqueous solution. The objective is to determine the extent to which minerals might have selected and concentrated useful monomers from a complex prebiotic "soup." Such selective molecular adsorption on sulfide surfaces is an important yet untested mechanism in the origin models of Wächtershäuser (1988, 1992), Russell & Hall (1997), and Russell et al. (1993, 1998). This effort, which will employ the experimental and analytical procedures developed by Hazen et al. (2001), will tie in closely to our studies of sulfide mineral-mediated organic reactions (see **Section 5.4.2**).

We plan as well to expand our measurements of the relative adsorption of enantiomeric molecular species, including amino acids and sugars, onto prepared chiral surfaces of pyrite and magnetite. Pyrite and magnetite are centric minerals, but any arbitrary cut section is likely to offer a chiral surface structure for study (Hazen & Sholl 2003). Accordingly, we will prepare a variety of surfaces (> 5 cm^2) from natural single crystals. This effort will require modification of existing techniques to anneal and characterize specific crystallographic surfaces. We will expose these surfaces to equimolar mixtures of racemic amino acids and pentose sugars. For example, in one series of experiments we will employ six amino acids (GLU+D, L-ALA + D, L-GLU + D, L-ASP + D, L-TYR + D, L-LYS) that are easily resolved by GC techniques. After 24 hours of immersion, the surfaces will be washed in water and the absorbed amino acids will be recovered via HCl washing (Hazen et al. 2001). These experiments will then reveal the relative adsorption of different amino acids as well as chiral adsorption.

Finally, we plan to initiate new studies of polypeptide formation on calcite crystal surfaces, which have a demonstrated ability for chiral selection of amino acids (Hazen et al. 2001). In our initial effort, we will adsorb aspartic acid onto a $(21\bar{3}1)$ terraced growth surface of a scalenohedral calcite crystal. Hazen et al. (2001) interpreted the enhanced adsorption of aspartic acid to be the result of preferential adsorption along the step regions of terraced surfaces. We will apply N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDAC) as a condensation polymerization agent (Ferris et al. 1996; Liu & Orgel 1998) to such a prepared surface and desorb the resultant oligomers. Desorbed amino acid oligomers will be analyzed intact with a Surface Enhanced Desorption Ionization Time-of-Flight (SELDI-ToF) mass spectrometer in

collaboration with Co-I Fogel and High-Performance Liquid Chromatography (HPLC) with Co-Is Scott and Tuross. Once we have established that we can oligomerize amino acids, we will establish the extent to which the oligopeptides exhibit chiral excesses using chiral selective columns with either GC-MS (in the case of short oligomers) or HPLC.

5.6 Life in Extreme Environments

Does the adaptability of life in extreme environments on Earth yield clues to the potential for life existing elsewhere in the Solar System? Over the past quarter century there have been tremendous advances in our understanding of the versatility and adaptability of life on Earth. Life clearly exists at extremes of both heat and cold, at low and high pressures, and under extremes of chemical environment (e.g., acidic and hypersaline). Precisely establishing environmental boundaries to microbial viability has become harder. With this increased uncertainty comes the slightly increased probability of discovering life, or the vestiges of life, elsewhere in the Solar System. As part of its contribution to NAI, the CIW team will explore life under extremes of habitat and under extremes of adaptive response.

5.6.1 Life in deep-sea hydrothermal vents (Astrobiology Roadmap Objective 5.3)

Background. A general focus of the CIW NAI team is to understand better the earliest stages in organic chemical evolution from chiral selection of organic precursors to non-enzymatic catalytic systems for generating metabolic reactions and pathways (see **Sections 5.4.2** and **5.5**). Much of this work was derived and has evolved from theories that linked the properties inherent to submarine hydrothermal vent environments and the microbial communities they support. Recently, it has been shown that minerals can provide certain critical functions, both by selectively absorbing organic compounds (Hazen et al. 2001) and by providing catalytic surfaces for synthesis of organic compounds (Heinen & Lauer, 1996; Huber & Wachtershäuser, 1997; Cody et al. 2000, 2001, 2003b,c). These results help constrain models for the earliest stages in the evolution of metabolic pathways and the transition from inorganic-mediated catalytic reactions to protein-enzyme driven systems. These results also lead to the hypothesis that early microbial communities may have relied on minerals to provide nutrients for survival.

Research to Date. During the past five years of the CIW-NAI project, Co-I Baross and his students at the University of Washington have obtained considerable information regarding the microbiota that survive and thrive in the vent chimney walls at deep-sea spreading centers. Specifically, we have obtained evidence of an intimate link between minerals and microbial viability through microscopic and molecular phylogenetic analyses of microbial communities in hydrothermal vent environments, as well as detailed physiological characterization of the vent microorganisms (Figure 5.6.1). Our microscopic and molecular analyses of active sulfide chimneys reveal that microbes exist throughout the chimney structure and are found even in zones that have experienced temperatures considerably greater than 100°C based on mineral compositional constraints (Schrenk et al. 2003). Two observations from this study were particularly surprising. First, there are microbial communities, detected optically with DNA-specific fluorescent stains and RNA probes, which inhabit the hot interior zones of the sulfide chimney. All of the organisms belong to the Archaea and most could be detected with RNA probes indicating that they may be viable and active. Secondly, in all cases the microbial communities exist as biofilms. The prevalence of biofilms consisting of archaeal communities on minerals under the extreme conditions found in sulfide chimneys suggests several interesting hypotheses that we propose to test. These are:

1. Thermophilic and hyperthermophilic heterotrophic archaea from vent environments grow on carbon either absorbed on mineral surfaces or on organic material synthesized on mineral surfaces.

2. These microbes derive key inorganic nutrients, such as the electron acceptors Fe (III) and Mn (IV), phosphorus, and other essential minerals, from insoluble minerals, e.g., apatite.

3. Some non-enzymatic catalytic reactions mediated by hydrothermal vent chemistry may substitute for enzymatic catalytic reactions in some thermophilic and hyperthermophilic archaea.

4. The microorganisms associated with biofilms on minerals show enhanced survival under extreme conditions of elevated temperature and pressure, desiccation, high salt, and high levels of radiation.

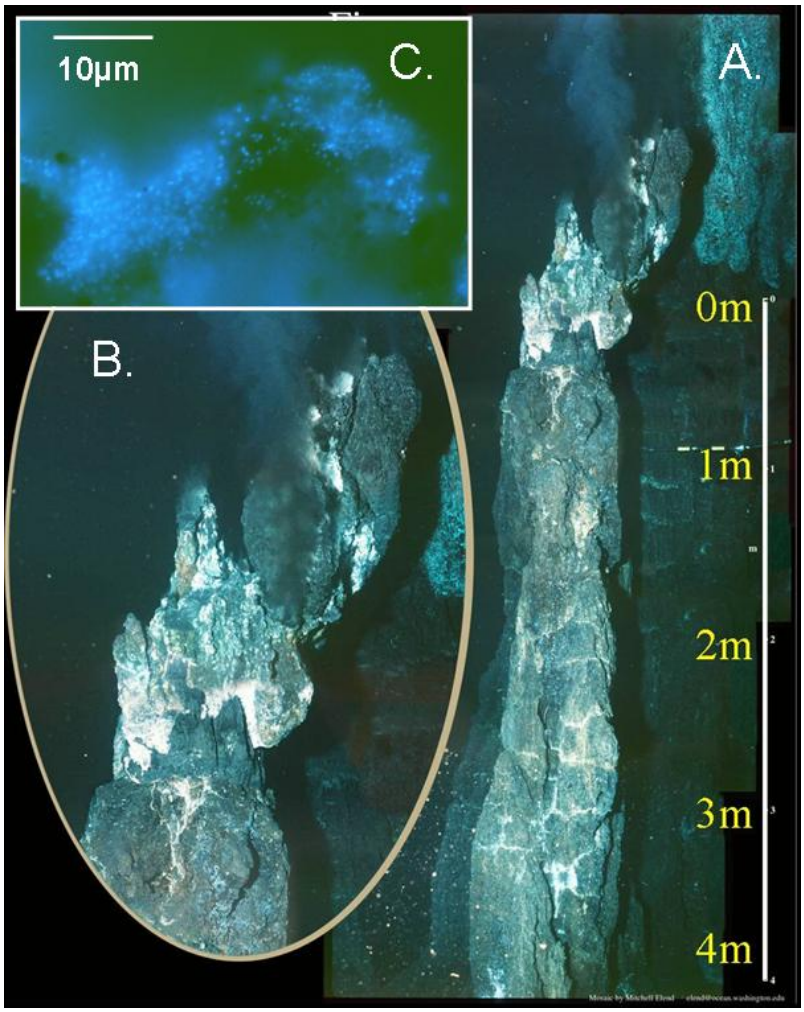

Figure 5.6.1. The black smoker sulfide chimney Finn (A) was recovered from the Mothra Vent Field on the Endeavour Segment of the Juan de Fuca Ridge in July 1998. Finn was venting 302°C fluid upon recovery (B, close-up of Finn) and contained complex mineralogical gradients within its walls. The structure was sampled by Co-I Baross for co-registered microbiological and petrological studies. Attached microbial communities, some of which formed 10-µm-thick biofilms (C), were observed, by use of fluorescent probes, throughout the structure, including high-temperature regions near the central vent conduit.

Proposed Research - Testing Hypotheses 1 and 2. Organic compounds can be abiotically synthesized utilizing a variety of energy sources, including heat, ultraviolet light, electrical discharges, meteor impacts, and radionuclide decay. Some of the compounds produced include

hydrocarbons, amino acids, organic acids, and insoluble polymers of unknown composition. Recently, both acetate (Huber & Wächtershäuser 1997) and pyruvate (Cody et al. 2000) were synthesized abiotically in the presence of metal sulfur compounds under conditions that coarsely mimic those at hydrothermal vents. We have isolated a group of hyperthermophilic archaea from both crustal and deep hot sediment environments that can grow anaerobically on acetate using Fe (III) as the electron acceptor. Based on 16S rRNA sequence analysis, these subseafloor isolates are new genera within the Family *Thermococcales*, and they are also the deepest-rooted organisms within this group (Summit & Baross, 1998). They appear to be using acetate and Fe (III) in classic dissimilatory iron-reduction fashion (Lovley & Phillips, 1988). Unlike many organisms that can reduce soluble iron-organic compounds, these isolates reduce Fe (III) from insoluble minerals. They accomplish this by first attaching to the minerals where they generate organic polymers and biofilm. Recently, a new genus within the *Geobacteraceae* was isolated from a hydrothermal vent environment (Kashefi et al. 2003). Unlike other members of the *Geobacteraceae* that are mesophilic and can use a very limited number of carbon sources, this new isolate is thermophilic and can grow anaerobically on a wide range of organic compounds. These organisms would be excellent candidates for testing hypotheses 1 and 2.

We propose to test these isolates for their ability to oxidize carbon sources commonly synthesized abiotically using Fe (III) as the electron acceptor. In this regard, this proposed research complements some of the work outlined in **Section 5.4.2**. The carbon sources will include low-molecular-weight acids, hydrocarbons, ketones, and other compounds shown to be synthesized abiotically (e.g., Heinen & Lauwer 1996; Huber & Wächtershäuser 1997; Cody et al. 2000, 2001, 2003b,c).

We will also continue our efforts to isolate new hyperthermophilic microorganisms that use other transition metals as electron acceptors and determine the mineral products formed from microbial reduction. Such mineral phases may constitute important biosignatures for detecting life (see **Section 5.7**). Emphasis will be on isolating organisms that obtain all of their nutrients from organic material synthesized on mineral surfaces (see **Section 5.4.2**). One of our isolates uses acetate and Fe (III), attaches to insoluble iron oxides, and precipitates magnetite. Acetate utilization will be followed, along with the production of magnetite. This organism is also motile when not forming biofilms on minerals.

Proposed Research - Testing Hypothesis 3. There are a number of enzyme reactions that can be replaced by inorganic chemical catalysis (see, for example, Cody et al. 2001, 2003b). It is intriguing to consider whether there are extant microorganisms that take advantage of these catalytic reactions. If there are, from a prebiotic point of view did these reactions precede the advent of protein-mediated catalysis? There is evidence that some of the most ancient enzymes found in microorganisms involve catalytic reactions that can be accomplished without protein enzymes (e.g., Huber & Wächtershäuser 1997; Brandes et al. 2000; Cody et al. 2003b). Some examples include carbonic anhydrase, formate dehydrogenase, hydrogenase, nitrogenase, and acetyl CoA synthetase. All of these enzymes involve a metal-sulfur core and are thought to be ancient (e.g., Pereto et al. 1999).

The fixation of molecular nitrogen is particularly important and thought to be one of the most ancient of enzymes (Fani et al. 2000). The enzyme is also not specific for reducing N_2 to ammonia but can reduce most triple-bonded compounds including acetylene and cyanide (Silver & Postgate 1973). We propose a study focusing on nitrogenase and the search for this enzyme within archaea that populate vent environments in which sources of nitrogen other than N_2 are either limited or absent.

The conversion of atmospheric nitrogen to ammonia is widespread in microorganisms. Nitrogen fixation (*nif*) genes have been sequenced and analyzed from a large number of microorganisms (Ohkuma et al. 1999; Zehr & Capone, 1996). The *nif* genes are highly

conserved in both bacteria and archaea. One of the interpretations from these data is that nitrogen fixation is an ancient trait and may have been present in the common ancestor before the separation of bacteria from archaea (Fani et al. 2000). Very little is known about the nitrogen cycle at deep sea hydrothermal vents. Ammonia has not been detected in subsurface vent fluids except at sites where there is overlying or buried sediments (Lilley et al. 1993). The dominant source of nitrogen to the hot subseafloor biosphere is N_2; however, nothing is known about nitrogen fixation by hyperthermophiles at vents. We propose to use molecular methods to search for nitrogen fixing genes (*nif*) in both an existing culture collection of hyperthermophilic archaea and in archaeal communities from subseafloor environments and to identify the groups of organisms, if any, that are actively fixing nitrogen. We will compare the sequence of these archaeal *nif* genes with other sequences in the database and determine if they show ancient lineages.

5.6.2 Stress adaption on microorganisms and expansion of habitability (Astrobiology Roadmap Objective 5.3)

Background. An interesting question regarding habitability is the nature of adaption as a means of expanding habitability. Recently Co-I Scott and Collaborator Sharma have shown that organisms that are not "adapted" for survival at high pressures are able to survive pressures approaching 2.0 GPa (20,000 atm) (Sharma et al. 2002). (For reference, this pressure is encountered at 60 km depth in the Earth.) Our approach uses a modified hydrothermal diamond-anvil cell to provide a "windowed" high-pressure environment. We have been able to show that these prokaryotes are able to carry out biochemical reactions at pressures up to 1 GPa (Figure 5.6.2). While we have not yet shown that replication occurs at these elevated pressures (a focus of current work), we have shown that when the pressure is released back down to approximately 1 atm the cells remain viable and are able to replicate.

Proposed Research. Co-I Scott proposes to investigate the mechanism(s) that allow cells to adapt to pressures well above those usually encountered by microorganisms such as *Shewanella oneidensis* and *Escherichia coli.* Pressure provides a model system to study stress adaption. In the natural world, stress involved with increased pressure will likely be linked to thermal and chemo-osmotic stresses. Three sub-tasks will be pursued: (1) proteomics and genomics of stress adaptation, (2) subsurface microbiology, and (3) microbial interaction with hydrates.

In the first sub-task, Co-I Scott and Collaborator Sharma will apply molecular biological analysis coupled with further refinement of high-pressure experimental methodologies (in collaboration with Co-I Hemley) at CIW. We seek to identify specific proteins and genes that are involved in the adaptation of these two ubiquitous pressure-sensitive bacteria. In this regard we are well situated at CIW, because of the unusual mix of scientific expertise and instrumentation available. For example, in addition to state-of-the-art diamond-anvil cell technology available in the high-pressure laboratory, we have a SELDI-ToF mass spectrometer obtained by Co-I Fogel with partial financial support from the NAI. This instrument is well suited to allow us to build fatty acid and protein profiles for organisms that have grown under extreme conditions (e.g., elevated pressures). Such profiles then can be compared with those of the organisms grown at ambient pressures, which will enable us to target specific classes of proteins that signature stress adaptation. Analysis of the lipid fractions will allow us to follow changes in membrane composition. We also have a DNA chip maker and reader (in collaboration with Co-I Steele) that will allow us to exploit the copious genome data that reside in public gene databases (e.g., TIGR and/or GeneBank).

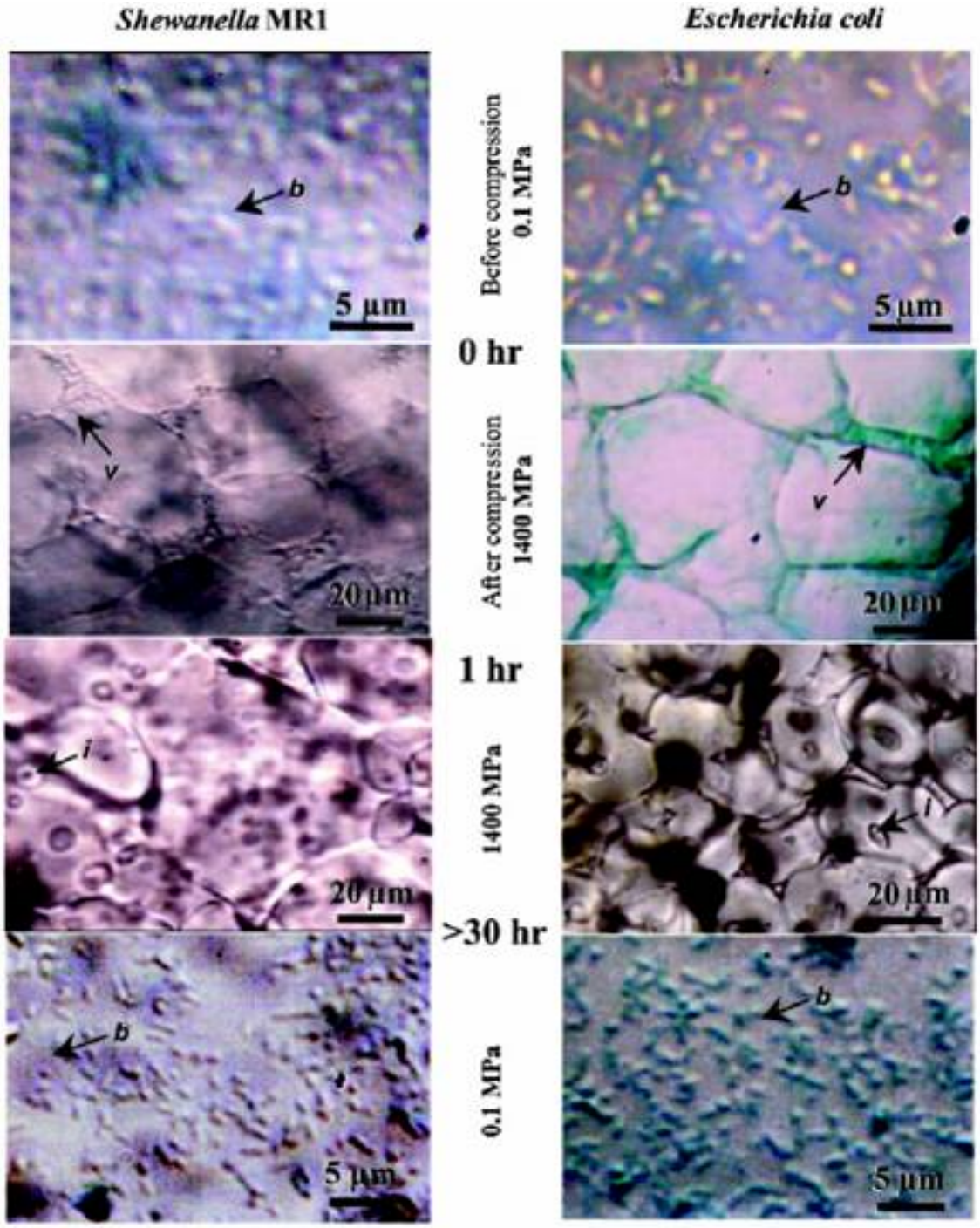

Figure 5.6.2. Microbial activity and viability in ice-VI (1400 MPa). Upon ice nucleation (0 hr, 1400 MPa), organic fluid veins (v) filled with bacteria (*Shewanella* MR1 on the left, *E. coli* stained with methylene blue on the right) appear. After ~ 1 hr, textural changes occur in the ice, defined by the formation of organic-rich inclusions (i) containing motile bacteria. Viable and countable bacteria were observed upon subsequent lowering of pressure (Sharma et al. 2001).

In concert with the proteomics work, genome analysis will provide us with an overview of how the organisms turn on genetic regulons in response to pressure. It will also provide clues to how the cells sense when pressures elevate to stressful levels. We plan to establish a genetic database and tools to ascertain whether the response in *Shewanella* and *E. coli* are unique or are ubiquitous in prokaryotes, without having to repeat the procedures carried out in the hydrothermal diamond anvil cell with hundreds or thousands of bacteria. The answer to that question bears on whether the response to elevated pressures is tied to a general stress response to the environment and whether all life on Earth still has the history of these survival mechanisms encoded within their genes.

It has become clear that a large portion of the biomass of the planet is located in the subsurface. Current estimates vary from 30% to 70%. Very little is known, however, about the geochemistry and biology of these ecosystems. There are a number of deep drilling programs being launched in the U.S., Japan, and elsewhere. However, the cost benefits and sampling difficulties will make any progress in this area slow. We have considerable experience in high-pressure experiments. We will apply and refine our methods to test bacterial growth models. These efforts will be directed at determining the best means for isolation of organisms from the subsurface. We are particularly well situated for working with samples from deep drilling expeditions to isolate subsurface microbes. The deep subsurface, sheltered from global events that have episodically altered the evolution of life on the surface and in the oceans, may provide the best environment for finding the closest extant relatives to a common ancestor. For this reason our NAI research into laboratory simulations of extreme environments will benefit from the collaborative field component with Co-I Baross (see **Section 5.6.1**). Combined, these efforts will provide valuable guidance to fundamental questions on the evolution of life and benefit the design of experiments aimed to search for life on Mars and, perhaps later, Europa.

Enormous stores of gas hydrates are located on the deep continental shelves of our planet. How these hydrates were formed and whether they constitute a viable environment for microbial communities remains uncertain. Our ability to create in the laboratory methane, CO_2, and other geochemically-relevant hydrates under realistic conditions will allow us to determine, *in situ*, whether microbes or microbial consortia can utilize such hydrates to support essential life processes. Toward this end Co-Is Scott and Hemley and Collaborator Sharma propose to study how pure microbial cultures as well as microbial consortia are able to interact with hydrates and elucidate metabolic mechanisms by obtaining kinetic data via microscopic and spectroscopic probing (for example, we can apply micro Raman spectroscopy to follow the loss or gain of a given substrate accompanying metabolism, as was used by Sharma et al. 2001).

Further, we will investigate whether microorganisms influence the generation of methane hydrates, as there is still a great deal of debate regarding whether natural hydrates might have biological origins (Paull et al. 1994; Brooks et al. 1994). Our work on hydrates has implications for examining the geochemistry of the outer planets, because much of the volatile inventory in the outer planets may be in the form of hydrates. Hydrates could thus be an important source of energy and carbon for non-Earth ecosystems. Hydrates may also sequester organic-rich fluids that might provide a low-temperature aqueous environment for life in the subsurfaces of cold "icy" environments, such as Europa and the polar caps on Mars.

5.6.3 Iron-based metabolic strategies for microbial life (Astrobiology Roadmap Objective 5.1)

Background. Iron is the fourth most abundant element in the Earth's crust and one of the most abundant redox elements in the Solar System. Oxidation and reduction reactions involving this element provide energy for the growth of some groups of microorganisms, and it is postulated that Fe-metabolism is among the most ancient forms of microbial metabolism. Among microbes that obtain fuel from the oxidation or reduction of Fe, circumneutral Fe-

oxidizing bacteria (FeOB) are the least understood. For many years, pure cultures of this group of bacteria were not available, and basic physiological studies were thus not possible. Now that a number of freshwater and marine isolates are available, crucial questions regarding carbon and nitrogen metabolism can be answered (Emerson and Moyer 1997).

Proposed Research. In the context of this proposal Co-Is Emerson, Fogel, Hauri, and Scott, and Collaborator Kehm propose to apply C and Fe isotopic methods to investigate several fundamental questions about this group of organisms. We will investigate autotrophy using ^{13}C tracer studies, and we will track the fractionation of stable isotopes in the metabolic intermediates as a consequence of anabolic synthesis. We will study Fe-isotope systematics to pursue some intriguing preliminary findings that FeOB are capable of causing a detectable mass fractionation of Fe isotopes and assess whether such organisms impart a robust biosignature (see **Section 5.7**).

Our initial studies will be directed toward providing evidence that our model pure culture organism, *Siderooxidans lithotrophicum* ES-1, is an autotroph. We propose to grow gradient tube cultures of ES-1 with $NaHCO_3$ that is enriched in ^{13}C (2.1 atom %). Although the carbon source could be enriched in ^{13}C by as much as 99 atom %, new cellular material will be readily apparent above the natural abundance level of ^{13}C (1.1 atom %) using isotope ratio monitoring mass spectrometery (IRMS). In order to concentrate the cells, the iron oxides must be removed by a low-speed centrifugation followed by centrifugation of the supernatant. The $^{13}C/^{12}C$ composition of the cellular material will be analyzed by standard mass spectrometric stable isotopic techniques in collaboration with Co-I Fogel. Preliminary calculations indicate that the yield of cells from a typical gradient tube culture will provide sufficient carbon necessary for high-precision stable isotopic analysis. Enrichments in ^{13}C of the ES-1 cells will provide confirmation of $NaH^{13}CO_3$ assimilation and autotrophic carbon fixation. In addition to ^{13}C, a parallel ^{14}C experiment will be conducted to confirm uptake of the radiolabel and a concomitant decrease in the dissolved inorganic carbon concentration.

In addition to this tracer approach, the isotopic fractionation factor during carbon fixation by ES-1 will be determined in laboratory experiments. The isotopic fractionation during carbon fixation by the Calvin cycle is approximately –20 ‰ (Madigan et al. 1989). The fractionation factor calculated in the study will provide further proof that the Calvin cycle is the carbon fixation mechanism for ES-1, or else it will point to the need to investigate other metabolic strategies. In addition, we will use the technique pioneered by Scott and Fogel (Scott et al. 2003) to analyze the fractionation of $\delta^{13}C$ in the individual amino acids (Figure 5.6.3) of these organisms to discern metabolic pathways for carbon incorporation.

Our preliminary studies of the iron isotopes in Fe-oxides collected from the Loihi hydrothermal vent site indicate that there is a significant fractionation toward heavier isotopes that may be indicative of a biological fractionation. Co-I Hauri and former NAI Postdoctoral Fellow Karl Kehm have developed new methods in iron isotope analysis using a multi-collector inductively coupled plasma mass spectrometer (ICP-MS) that are particularly well suited for these studies (Kehm et al. 2003). We have found that as bacterially precipitated oxides age, the degree of iron isotope fractionation approaches that of the surrounding basalt. We have observed that biogenically formed Fe-oxides are excellent substrates for the products of abiotic Fe-oxidation. These observations lead us to consider the following simple model to explain the evolution in iron isotopic fractionation recorded in iron oxides. First, we envision that microbes initially catalyze biological iron oxidation, precipitating oxides that record an isotopic shift of $\delta^{56}Fe \cong 0.5$ ‰; these oxides are precipitated in an organic matrix, and the initial ratio of biotic to abiotic FeOOH precipiation is > 1. As the FeOOH-organic matrix ages, the precipitation of additional Fe-oxides by purely chemical oxidation proceeds to drive the ratio of biotic to abiotic oxidation products toward zero and drives the bulk iron isotopic signature toward that of the

surrounding basalt. If this phenomenon is general, it poses serious problems for the application of iron isotope systematics as potential biosignatures.

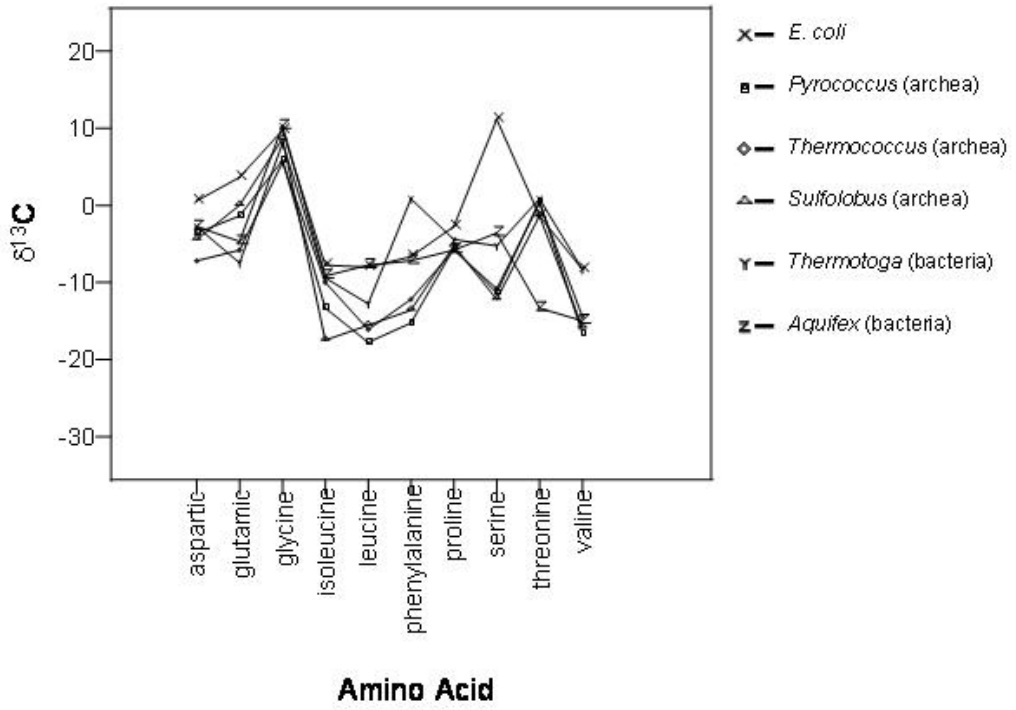

Figure 5.6.3. A graphical comparison of the stable isotopic composition of individual amino acids, normalized to the ^{13}C isotopic abundance of alanine, for a number of prokaryotes including *E. coli, Pyrococcus, Thermococcus, Sulfolobus, Thermotoga,* and *Aquifex*. The significant deviations in $\delta^{13}C$ clearly evident among many of the amino acids are indicative of differences in intermediary metabolism. These data, obtained as part of NAI-sponsored research of Co-Is Scott, Fogel, and Emerson, provide unique biosignatures of discrete metabolic pathways used by different prokaryotes.

We propose to explore this aging phenomena in a considerably different environment than that of the Loihi seamount, yet one where iron oxidation provides a key source of biologically useful energy. We will focus our studies on a local (to CIW and ATTC) field site that has Fe-rich ground water flowing through a small wetland. On the basis of preliminary observations, we recognize within this environment zones of very active growth of FeOB and other zones either at depth or on the edge of the flow regimen where the Fe-oxides appear much more aged. At this site, we will carry out a more complete iron isotopic survey to confirm and extend the results obtained from the Loihi seamount. In addition, we will perform aging experiments at the site by placing freshly collected oxidized material that has been poisoned with sodium azide to kill the microbes. These samples will be placed on site into mesh bags that have a pore size of < 0.2 µm that will exclude bacteria but will enable diffusion and flow of the Fe(II)-rich water, allowing continued abiotic oxidation to occur *in situ*. Parallel experiments will be conducted under more controlled conditions in the laboratory, where freshly collected material will also be

subjected to different aging regimens in sterile laboratory media. We will apply multi-collector ICP-MS methodologies (e.g., Kehm et al. 2003) to use iron isotope fractionation to track the ratios of biotic to abiotic oxides. As a third component of these studies we will use pure cultures of lithotrophic FeOB isolated by Co-I Emerson to carry out similar aging experiments. Finally we will compare the isotopic signatures in Fe oxides formed by these lithotrophic organisms that oxidize Fe(II) as their energy source with heterotrophic Fe-oxidizers *L. discophora* and *S. natans* that grow using organic compounds as substrates yet precipitate Fe oxides biologically.

Finally Co-Is Emerson, Fogel, and Scott propose to continue their investigations into identifying metabolic pathways of extremophiles through isotopic analysis of individual amino acids (Figure 5.6.3). This work will involve specifically grown organisms both in pure culture and in mixed cultures to determine the efficacy of this method for use as a biosignature technique on mixed samples (see **Section 5.7**). Co-I Emerson will also continue to provide cultures and advice on S-isotopes using methods for analyzing the complete suite of S isotopes as pioneered by Co-I Rumble. In particular we will apply these molecular-isotopic approaches to our local field studies.

5.7 Biosignatures and Abiosignatures

What criteria reveal whether a suite of organic compounds are the result of microbial activities or non-biological processes? The difficulties associated with recognizing unambiguous biosignatures are underscored by recent scientific controversies over the quality of evidence for fossil life in Earth's earliest rocks (e.g., Schopf 1993; Mojzsis et al. 1996; Brasier et al. 2002; Fedo & Whitehouse 2002; Schopf et al. 2002; van Zuilen et al. 2002) and within the Martian meteorite, ALH84001 (e.g., McKay et al. 1996; Jull et al. 1998; Golden et al. 2000; Steele et al. 2000; Thomas-Keprta et al. 2001). The common denominator of these debates is the underlying difficulty of demonstrating conclusively the biogenicity of the respective evidence. A consensus has emerged from these discussions: Molecular evidence is critical, in addition to morphological evidence, to support extraordinary claims.

In this context, we argue that the convincing demonstration of an abiotic origin in any ancient terrestrial or Martian specimen with carbon-based molecules would also constitute an extraordinary finding, with possible implications for both the timing of life's origin and its subsequent radiation. Thus consideration should also be given to the search for abiosignatures – i.e., various chemical and physical characteristics that may point to likely abiological processes.

A principal difficulty in the search for unambiguous biosignatures and abiosignatures is that billions of years of diagenesis inevitably degrade physical structures and alter suites of organic molecules (whether biotic or abiotic) to assemblages of molecular fragments that rarely retain morphological information. Our challenge, therefore, is to identify diagnostic suites of ubiquitous and stable molecules and to develop analytical techniques for their reliable detection. We propose to tackle this objective by developing sensitive new analytical methods and applying these methods to relevant natural molecular suites from both biogenic and abiogenic processes.

5.7.1 Preservation of molecular biosignatures (Astrobiology Roadmap Objective 7.1)

Background. Organic geochemists have made considerable progress in the isolation, identification, and taxonomic attribution of biologically informative organic compounds in sediments. Several distinctive classes of molecules point unambiguously to the presence of life millions of years ago (e.g., Peters & Moldowan 1993). These compounds include pentacyclic triterpane hydrocarbons (so called hopanes), which are fossil remnants of biosynthesized hopanoids produced exclusively by bacteria as membrane components; sterane, which is derived from eukaryotic sterols; acyclic isoprenoids from various precursors, but particularly from lipids of archaea; and certain hydrocarbons derived from carotenoid precursors (Summons & Walter

1990). Other classes of compounds include porphyrin-based molecules from the diagenesis of chlorophyll and cytochromes and the associated isoprenoids. Apart from the porphyrins, these biomarker classes are collectively known as terpenoid hydrocarbon and are derived from isoprene monomer precursors. Hopanes, which are ubiquitous on Earth due to their resistant carbon skeleton (Innes et al. 1997), are generally regarded as unambiguous bacterial biomarkers (although a small number of eukaryotes also produce hopanoids, they are chemically distinct). Recent research reveals the presence of a specific cyanobacterial hopane (2-methyl hopane) in 2.7-Ga sediments (Brocks et al. 1999). This research shows that the diagenetic products of bacteria can be detected on Earth dating from a time near the end of the first half of Earth history, rendering this class of bacterial biosignature an interesting target molecule for planetary exploration.

The survivability of a biomolecule depends not only on its refractory properties, but also on the diagenetic environment and the burial conditions (e.g., pressure and temperature, pH, and redox potential). It appears likely that theoretically less refractory compounds such as squalene can be preserved up to at least 1.7 Ga (Summons & Walter 1990). Consequently, a main thrust of our research has been aimed at understanding the processes involved in the geochemical preservation of bacteria and methods to identify their fossil biochemical traces.

Proposed Research. In continuation of this research effort, Co-Is Steele, Tuross, and Vicenzi propose a program of analytical instrument development coupled with laboratory studies of fossilization, field studies, and analysis of relevant natural samples.

Co-I Steele and Collaborator Toporski propose to continue studies of morphological and biochemical characteristics of 25-Ma old bacterial biofilms from lacustrine sediments in Germany – an extension of previous work that led to the unambiguous identification of bacterial fossils (Toporski et al. 2002a). We have gathered a substantial amount of new sample material during the 2002 summer field season. We will further characterize the provenance of the organic substances contained in these samples, and we aim to establish chemical pathways by which biomolecules converst to fossil biosignatures. We will apply several analytical techniques, including Gas Chromatography-Mass Spectroscopy (GC-MS) and pyrolysis GC-MS in collaboration with Co-I Cody, HPLC with Co-I Scott, and *in situ* measurements using ToF-SIMS with Co-I Vicenzi, to identify comprehensively the organic inventory of the bacterial fossils. Surface Enhanced Laser Desorption Ionization-Time of Flight (SELDI-ToF) mass spectrometry and Protein Chip technology, in collaboration with Co-I Fogel, will be applied to test the samples for the possibility of protein/peptide preservation, as initial analyses have shown the presence of 6% N contained in the bacterial fossils.

Co-Is Steele, Tuross, and Vicenzi, working with Collaborator Toporski, propose to leverage the sub-micrometer localization of atomic and molecular ions offered by a Time of Flight-Secondary Ion Mass Spectrometer (ToF-SIMS) to help unravel chemical modifications that accompany the fossilization of simple organisms. This task will encompass two principal modes of operation: inorganic/organic molecular imaging (mass range is 1 – 10,000 AMU) on fresh fracture surfaces of known fossil specimens such as those from the Enspel Formation of Germany (Toporski et al. 2002a), followed by shallow 3D inorganic depth profiling of conventional thin sections.

The samples targeted for these investigations span ages from 14 million to 3.5 billion years and are in the possession of the investigators. These include laboratory fossilized bacteria and viruses; Enspel, Willershausen, and Messel oil shales (see Toporski 2002a,b); and Gunflint, Bitter Springs, and Apex cherts. Other techniques will be a valuable complement to ToF-SIMS and serve to provide ground truth for these investigations during both the imaging and chemical characterization of natural and experimental fossils. These include light microscopy with 3D rendering capabilities, high-resolution electron microscopy (FEG-SEM and TEM) (CIW, NRL),

focused ion beam (FIB) (SI), scanning probe microscopy (CIW), and SELDI-ToF and GC-MS (CIW).

Co-I Tuross will use modern stromatolites as a model for long-term degradation of biochemicals and will focus on the types of cross links formed in extractable organic matter using multiple chaotropic agents. Chaotropic agents are compounds that disrupt the hydrogen bonding structure of water and enhance the solubility of non-polar organic compounds. The bioavailability of the organic matter in the stromatolites will be determined in a range of sequential enzymatic digestions, and the quantitative systematics of the cross-links in both enzymatically accessible and refractory material will be determined. Both reducible and nonreducible cross-links will be characterized and quantified using chemical, enzymatic and chromatographic (primarily LC) methods.

The characterizations of organic matter obtained via rigorous extraction with chaotropic agents will be compared with pyrolysate patterns (GC-MS) in order to assess the production of compounds in high-temperature conditions. Finally, a third preparation that involves removal of the mineral surround under the most gentle chemical conditions possible will be compared with pyrolysate and chaotropic extractions.

Co-I Steele proposes to amplify these studies through molecular characterization using a Protein Chip reader. Specifically, he will test whether the complex higher molecular weight compounds found in living organisms can be preserved and identified in the fossil record.

5.7.2 Studies of organic compounds and silicified microbial remains associated with the Kamchatka geothermal region (Astrobiology Roadmap Objective 7.1)

Background. There is considerable astrobiological interest in the geothermal hot springs of the Kamchatka Peninsula, situated in the far eastern portion of Russia. In September 2001, Co-I Deamer helped organize and attended an NAI-sponsored field expedition to Kamchatka (led by Sherry Cady, Portland State University), where Deamer and the other participants discovered the remarkable potential of this unique environment for research in astrobiology. The region spans a geographical area of approximately 400 by 1200 km and contains nearly ten percent of the world's active volcanoes as well as over 150 surface-exposed hydrothermal sites. The primary attraction of Kamchatka for the work proposed here is its isolation from potential sources of contaminants related to human activities. Furthermore, much of the volcanism occurs at high latitudes and altitudes, so that geothermal areas are available in which biological processes are entirely microbial. Numerous microbiologists (mostly Russians but including such Americans as Frank Robb of the University of Maryland, Jonathan Trent of NASA Ames, who also participated in the Kamchatka field expedition, Ken Stedmen of Portland State University, Juergen Wiegel of the University of Georgia, and many others) have investigated these hot springs in order to catalog the diversity of microorganisms present. It has recently been shown that some of the Kamchatka hot springs provide a home for an interesting group of anaerobic carbon-monoxide-oxidizing bacteria and archaea (Sokolava et al. 2002).

Astrobiologists find Kamchatka interesting not only for its intriguing microbial ecosystems but also because this locale may provide a modern example of the types of hydrothermal ecosystems that were active early in Earth's history. Similar environments were likely (and might still exist) at or near the surface of Mars. It is well known that rapid silicification provides an excellent means for the preservation of biomolecules, hence microbial biosignatures (e.g., Schultze-Lam et al. 1995; Cady & Farmer 1996; Toporski et al. 2002) (Figure 5.7.1). In the case of the Kamchatka, or Mars, any microbial communities trapped and encased in precipitating silica will likely retain a substantial amount of biochemical information for a considerable length of time. The unresolved question is whether the process of initial silicification is moderately or highly deleterious to biomacromolecules (Toporski et al 2002).

Co-I Deamer, in collaboration with Co-I Steele and Collaborator Toporski, propose to use the Kamchatka territory as a model environment to study possible geochemical synthesis of organic compounds and the extent of preservation of microbial biosignatures contained within mineral precipitates derived from the hot springs. It is well known that the numerous hydrothermal fields are highly variable in their fluid and volatile chemistry and can be divided roughly into five geochemical types with respect to the primary gases present: (1) H_2S - CO_2; (2) N_2 - CO_2; (3) CO_2; (4) N_2; (5) CH_4 and N_2 - CH_4. These differences in chemistry manifest differences in microbial biodiversity, as well as the types of minerals precipitated. Thus, the types of fossilized biosignatures preserved in each type of hydrothermal system will likely vary considerably, a circumstance that will allow us to explore the effects of natural variables such as precipitation chemistry and precipitation temperatures on the preservation of recognizable microbial biosignatures.

The Kamchatka region offers many pristine sites for astrobiological exploration. For example, Craternaya Bay on Jankicha Island, in the Kurile Archipelago and situated several hundred kilometers from Kamchatka, features unique hot-spring ecosystems that have been only cursorily explored (Tarosov et al. 1989). The bay, approximately 1.5 km in diameter and up to 60 m deep, is connected to the ocean by a narrow and shallow (less than 1-m deep) strait. Numerous hydrothermal vents are distributed from the floor of the bay all the way up to the surrounding mineralized shoreline of the bay. Other promising sampling sites include high-altitude geothermal springs on accessible volcanos such as Mt. Mutnovskiy, which is located approximately 40 km from Petropavlovsk. Even more accessible are numerous boreholes (associated with geothermal energy production) located near the city of Petropavlovsk and other settlements. These boreholes permit direct measurement of chemical and physical properties of deeper geothermal aquifers. For this proposed research we are particularly interested in samples of hydrothermal deposits that formed at known depths within the boreholes and that are available from drilling. A valuable component of the proposed research is the establishment of a collaborative relationship between the Russian Astrobiological Center and the CIW NAI team.

Proposed Research. The wealth of unique hydrothermal samples from Kamchatka provides the focus for a promising research program of sample collection and analysis. As a primary objective, Co-I Deamer proposes to organize a field expedition to Kamchatka during the summer of 2004 along the lines of the NASA-sponsored field trip he helped organize and participated in during the summer of 2001. Ideally, investigators from throughout the NAI as well as other interested parties would undertake further collaborative study of this extremely interesting environment. During this expedition, Co-I Deamer, in collaboration with Collaborator Toporski, would collect water samples for organic analysis and samples of silicified deposits at a variety of geothermal springs. Co-I Steele and Collaborator Toporski have been studying the preservation of microbial biomarkers and biosignatures by laboratory silicification (Toporski et al. 2002a). The collection and thorough analysis of natural silicified samples from the Kamchatka hot springs will therefore provide an excellent opportunity to test the predictions derived from their experiments.

The silicified samples will be returned to CIW where they will be analyzed using a number techniques, including quasi *in situ* TEM studies in collaboration with Co-I Stroud, pyrolysis-GC-MS and STXM (see below) with Co-I Cody, and an array of novel yet powerful bianalytical techniques, including antibody-based assays to specific cell components (e.g., lipo-polysaccharides, extracellular polysaccharides, purins, peptidoglycan), wherein fluorescent labels are used to report quantitatively on the concentration of a given target biomolecule. In order to observe and analyze intact proteins (if present) as well as detect any thermal breakdown products that may have accompanied silicification, Co-I Deamer will collaborate with Co-I Fogel to apply SELDI-ToF on demineralized samples (using the mild fluorination method derived by Fouad Tera (CIW) and outlined in Cody et al. 2002 and adopting the novel solubilization

strategies derived by Co-I Tuross) to observe whether there are intact proteins or substantial protein fragments. These data will be correlated with the chemical characteristics of the hot springs from which the samples were obtained to see whether the preservation of biomolecular signatures of microorganisms is ubiquitous to silicification.

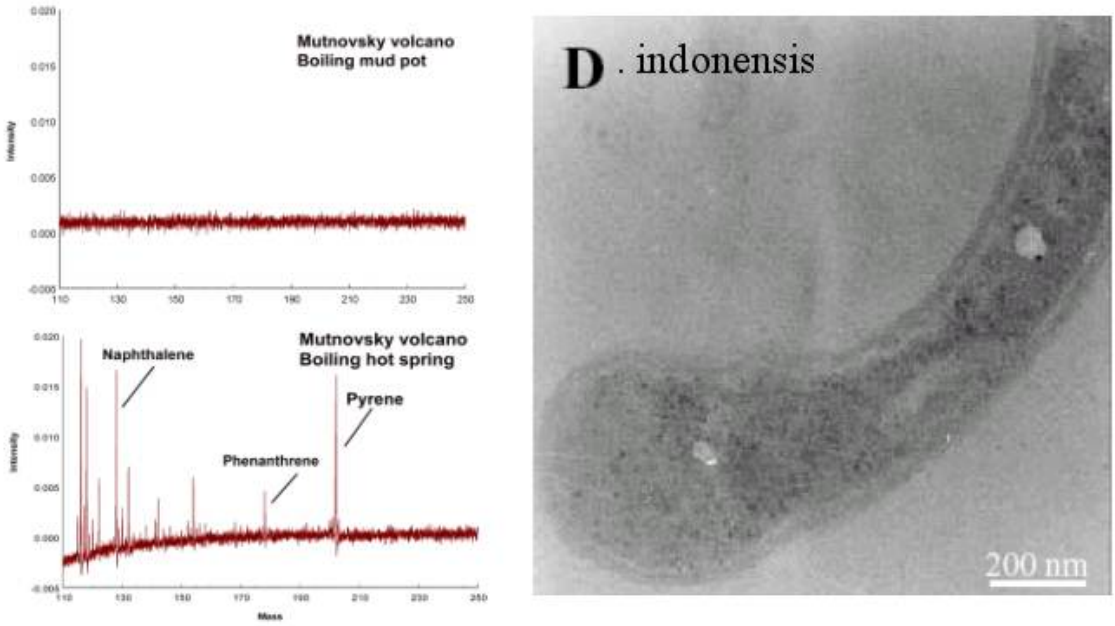

Figure 5.7.1. Left: laser desorption mass spectra of solute from Kamchatka hot spring waters. Note that only the spring contains organic compounds. Right: TEM micrograph of a cell of *D. indonensis* that had been exposed to mineralizing solutions of silica (1000 ppm Si concentration) for one week (Toporski et al. 2002). EDS spectra reveal considerable silicification around the cell membrane but minimal alteration of the cell structure. Experiments such as this will provide a basis for comparison with natural silicified samples retrieved from the proposed expedition to Kamchatka.

We further propose engage in research into the presence of DNA at the study site as well as in silicified materials. Co-I Steele and Collaborator Toporski would conduct field PCR (polymerase chain reaction) studies to allow the identification of target DNA, e.g., of archea and/or bacteria in the field. PCR products produced in the field can be returned to the laboratory for sequencing. The use of the field PCR reduces transportation issues, as original material does not have to be kept frozen after sampling until returned to the laboratory and further avoids sample contamination during transport and storage. Sequencing would help identify species present in the Kamchatka hot spring ecosystems. Silicified materials containing microfossils would be tested in the laboratory for the presence of DNA. Extraction of DNA from deposits of different ages and chemical environments would help us to understand the factors controlling DNA survivability in silicified deposits.

Co-I Deamer will collect water samples from each of the hot springs investigated. Total soluble carbon and nitrogen as well as bulk stable isotopic measurements will be assayed at CIW by combustion and mass spectrometry in collaboration with Co-I Fogel. Amino acid content and distribution will be determined in an amino acid analyzer. A freeze-dried aliquot of each sample will be derivatized by alkylation and reaction with trifluoroacetic anhydride in order to analyze for organic acids (including amino acids) via GC-MS. Co-Is Scott and Fogel will apply compound-specific isotope (nitrogen and carbon) ratio monitoring mass spectrometry to see whether these isotopic biosignatures can yield information on the prokaryotic sources and diversity based on their previous analysis of such isotopic signatures in a broad suite of prokaryotes (see **Section 5.6** for details and Figure 5.6.3 for example).

Finally, Co-I Deamer will explore whether free fatty acids and fatty alcohols are present in these hydrothermal systems. As discussed in **Section 5.5**, self-organizing, lipid-like compounds may have provided the mode of encapsulation for the first cells. Because it is unlikely that complex lipid biosynthesis pathways co-evolved with the first forms of life, it seems reasonable that the first cellular organisms used lipid-like molecules available in the environment. For example, suites of alcohols and monocarboxylic acids ranging up to 30 carbons in length have been produced under plausible prebiotic hydrothermal conditions by a Fischer-Tropsch reaction mechanism (McCollum et al. 1999). However, there have only been a few reports of unambiguous signatures of abiotic organic synthesis in nature (e.g., Sherwood Lollar et al. 2002). The paucity of reported abiotically synthesized organic carbon results, in part, from heterotrophic microbial activity. Heterotrophic microbes would rapidly remove all but the most recalcitrant abiotic organic species from most environments. Furthermore, there have been very few studies that have specifically sought to establish (or discriminate) between organic compounds synthesized abiotically from otherwise expected biological sources. The chemistry of the fluids from some of these hot springs is sufficiently reduced to support, at least theoretically, the abiotic synthesis of such lipids (e.g., Sokolava et al. 2002). Significantly, Isidorov et al. (1992) reported a series of organic compounds to be present in steam-gas outflows from Mutnovskiy. These included hydrocarbons up to 12 carbons in length. Remarkably, a variety of fluorinated and chlorinated hydrocarbons were also present, including difluorodichloro-methane, fluorotrichloromethane, chloroform, and tetrachloroethylene. The halogens are presumably derived from mineral sources deep in the volcanic rock matrix. If these observations are verified, then it is clear that some very interesting organic geochemical reactions are occurring in Kamchatka geothermal settings. For these reasons, Co-I Deamer, working in collaboration with Co-Is Fogel and Hazen, will seek to isolate such hydrocarbon derivatives and other soluble organic compounds from geothermal springs and use compound-specific isotopic analysis to ascertain whether the stable isotopic distributions can distinguish between abiotic synthesis and a biological origin of the organics.

5.7.3 Scanning transmission microscopy and *in situ* chemical analysis of organic fossils (Astrobiology Roadmap Objective 7.1)

Background. In **Section 5.3.1** we propose to apply scanning transmission X-ray spectro-microscopy (STXM) as a probe of extraterrestrial organic matter in meteorites with a focus on ultimately providing a robust means for analyzing extraterrestrial carbon, nitrogen, oxygen, and sulfur functional groups in IDPs and any organics contained within particles collected in the Stardust mission. STXM is also a very powerful tool to extract molecular biosignatures in ancient fossiliferous rocks.

It is well known that permineralization preserves plant and even microbial fossils in three-dimensional cellular detail (e.g., Boyce et al. 2001). The exquisite preservation of organic matter by silicification has long afforded opportunities for the study of anatomical evolution. Such preservation also raises the possibility that cell- and tissue-specific chemical analyses might also

be possible, thus providing insights into biochemical and physiological evolution. The problem is that the amount of carbon preserved in permineralized fossils is generally very low (Boyce et al. 2001). Conventional, albeit powerful, analysis using either ^{13}C solid-state NMR or pyrolysis GC-MS require relatively large samples to be demineralized first to obtain sufficient material for these standard organic geochemical analyses (Collinson et al. 1998). While the quality of the chemical information from NMR or pyrolysis GC-MS is excellent, the demineralization unfortunately destroys the spatial-anatomical context of the organic matter that had been "locked" into place via silicification. When analyzing ancient fossils, this destruction becomes a serious problem as the majority of fossil organic matter has been altered substantially during diagenesis. In some cases this alteration is so severe that discrete molecular biosignatures no longer exist. This stated, there may still remain chemical differentiation at a very fine scale related to the initial presence of different biopolymers that can be used for biomolecular reconstruction.

Research to Date. In collaboration with Kevin Boyce and Andrew Knoll (Harvard-NAI; Boyce is a currently an NAI-NRC Fellow who divides his time between Harvard and CIW), Co-I Cody has recently developed methods that exploit the classic preparative techniques employed by paleontologists and paleobotanists coupled with a state-of-the-art X-ray synchrotron-based scanning transmission microscope (see **Section 5.3.1** for details). Recent advances in X-ray micro-focusing techniques coupled with brilliant, synchrotron-derived X-ray sources have led to the development of soft X-ray spectromicroscopy (Jacobsen & Kirz 1998) that allows for the analysis of functional group distributions in ancient bio-organic structures at spatial resolutions approaching 30 nm (Cody et al. 1995a,b, 1996, 2000; Boyce et al. 2002).

Recently Boyce and Cody applied STXM to analyze the signature of ancient biochemistry in 40- and 400-Ma fossils. Specifically, we applied carbon (1s) X-ray absorption near edge spectroscopy (C-XANES) to characterize the organic chemistry stored within structurally differentiated regions of tracheid cell walls in fossil plants (see Figure 5.7.2). For the purpose of comparison we included analysis of a modern extant tracheid cell wall from re d cedar *Juniperus virginianan*. Our selection of fossils included *Metasequoia milleri*, a conifer from the Middle Eocene (ca. 40 Ma) preserved in the Princeton Chert (Basinger 1981) and the stem lycopod *Asteroxylon mackiei* from the Early Devonian (ca. 400 Ma) preserved in the Rhynie Chert (Kidston & Lang 1920).

The power of soft X-ray microscopy is that we obtain image contrast through variations in carbon chemistry using a high-resolution monochromator (~ 0.03 eV) (Jacobsen et al. 1998)) tuned to the characteristic energy of specific core-level (1s) to bound-state (e.g., π^*) electronic transitions. Using this instrumentation, we have been able to observe chemically differentiated cell wall, even in the 400-million-year-old sample. Using C-XANES obtained from sub-micron analysis regions, the STXM reveals that carbon derived from both lignin and structural polysaccharides has been preserved within individual tracheids, albeit in a diagenetically transformed state (Boyce et al. 2002).

This result is remarkable for two reasons. First, this result constitutes an analytical "record" for the chemical detection of biomacromolecular differentiation within fossil cell walls. While individual molecular biomarkers have been detected in samples as old as 2.7 Ga (Brock et al. 1999), our observations reveal the oldest preserved <u>chemical</u> evidence of cellular differentiation, at 400 Ma. Second, and more importantly, detecting the presence of lignin in *Asteroxylon* may help explain the critical biomolecular evolutionary step that allowed early plants to colonize the continents.

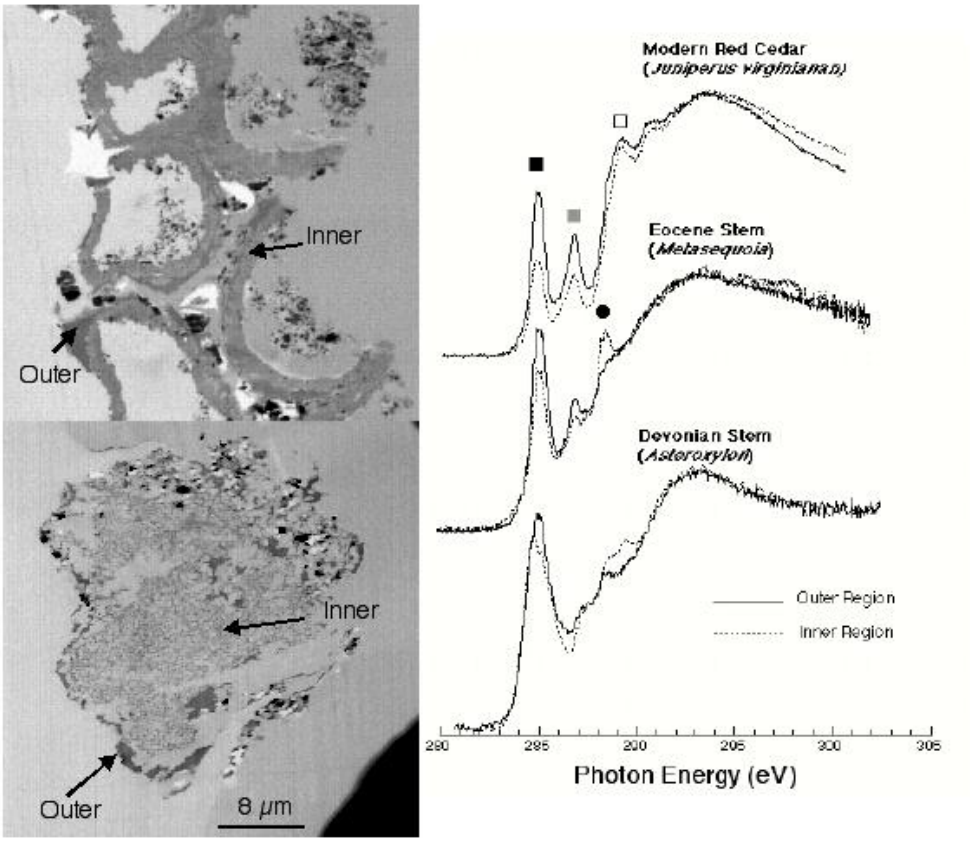

Figure 5.7.2. High-resolution STXM images (left) of preserved tracheid cells of ancient vascular plants, *Metasequoia milleri* (top), preserved in the ~ 40-Ma Princeton Chert and *Asteroxylon mackiei* (bottom), preserved in the ~ 400-Ma Rhynie Chert. These high-resolution (40-nm spot size) X-ray images (with the photon energy tuned to 286 eV) reveal a mechanically resistant, strongly absorbing outer region and a weakly absorbing inner region. Comparison of the C-XANES spectra from each region (shown on the right) clearly reveals significant differences in the carbon chemistry distinguishing the differentiated regions of the cell membrane even in the 400-Ma fossil (Boyce et al. 2002). The squares and circles highlight specific absorption bands corresponding to organic functional groups diagnostic of ligno-cellulosic and altered ligno-cellulosic material.

The global significance of such an evolutionary gain need not be expanded upon here. It is sufficient to note that the current global carbon, oxygen, and weathering cycles are strongly controlled by the presence of continental flora. The eventual rise of sentient vertebrates may well owe their existence to the first shallow marine plants that developed the ability to colonize the continents.

Proposed Research. The methods described in Boyce et al. (2002) are applicable to any sample, terrestrial or extraterrestrial, subject to the chemical constraints that the matrix material exhibits different solubility characteristics from the fossil carbonaceous material. As part of our commitment to the NAI as a whole, we offer our considerable experience in soft X-ray spectromicroscopy to any affiliate or external collaborator who has astrobiological science questions that would benefit from this unique analytical technology.

Co-I Cody plans to continue the development and refinement of methods for STXM analysis of ancient permineralized fossils. He will focus on analyzing microbial assemblages in the relatively young Enspel Formation with Co-I Steele and Collaborator Toporski. Co-I Cody will collaborate with Co-Is Deamer and Steele to apply STXM to analyze microbial material encased in silicified deposits collected from the expedition to Kamchatka. He will also focus on the analysis of organic fossils preserved in Proterozoic cherts from Western Australia with Co-I Rumble and Collaborator Ono. In each case the goal will be to derive molecular signatures from altered biomolecules, *in situ*, and provide more robust evidence for the existence of life in the early Earth's fossil record. One of the best means of assessing the provenance of organic matter is to analyze it *in situ* using technology such as STXM.

5.8 Astrobiotechnology (Astrobiology Roadmap Objective 7.1)

How can advances in biotechnology be used to further NASA's objectives to find and characterize life on other worlds? Over the last few years, enormous advances have been made in our understanding of the extent to which life can survive in apparently hostile and otherwise improbable environments. The significance of these revelations to NASA's Astrobiology Program lies in the fact that these observations increase the probability that life may exist elsewhere, e.g., Mars. The problem remains, however, that even if life does exist on certain extraterrestrial bodies, will we be able to detect it? Also, given the recent debate on the authenticity of the earliest fossil life on Earth (Brasier et al. 2002), it is important to utilize new techniques and philosophies to tackle these issues.

The principal driving force for the recent advances in molecular biology has been the advent of extraordinarily powerful and sensitive bioanalytical tools. The majority of these biotechnological developments have been fueled by medical considerations, and it is only recently (within the last decade and largely in response to NASA initiatives) that environmental microbiology (and the study of multi-species interactions) has risen as a viable scientific endeavor. There currently remain numerous poorly surveyed environments. For example, it is only recently that studies in Antarctica have begun to show the breadth and adaptation of life that exists there (Ward & Priscu 1997).

Clearly one of the limiting factors in microbial surveys has been accessibility to plausibly habitable environments. Surface and shallow subsurface environments, for example, are readily accessible; biological surveys of photoautotrophic microorganisms, e.g., cyanobacteria, are consequently comprehensive. On the other hand, studies of autotrophic, chemolithotrophic, and heterotrophic organisms, such as those that thrive at hot springs, at kilometer-plus depths in the oceans, or within deep crustal environments on the continents, remain unevenly investigated. From an astrobiological perspective, the development of strategies to investigate largely inaccessible (at least to manned scientific teams) environments on and within the Earth will lead to technology that benefits surveys on similarly inaccessible extraterrestrial environments, e.g., Mars.

The key to developing critical life-detection instrumentation for future NASA missions lies in the transfer of state-of-the-art biotechnology from medicine and biosciences to mission planning and its integration with other established techniques. However, the growth of biotechnology in the last 20 years has been staggering, and the sheer number of new techniques and procedures can be disorientating. The question arises as to which of the many methods currently available provide the greatest opportunities while at the same time achieve technical feasibility as spacecraft instrumentation packages. NASA's Astrobiology Program provides the natural interface between biotechnology and planetary science to address this question.

Why use biotechnology? The answer is simple; the techniques used for Solar System exploration have provided ambiguous conclusions or failed completely to detect life. In the case

of the debate on relic biogenic activity in the Martian meteorite ALH84001, many traditional techniques such as amino acid analysis, C-isotope analysis, and gas chromatography - mass spectrometry (GC-MS) all yield the conclusion that no life was contained within the meteorite (McKay et al. 1996). However, some if not all of this entire meteorite has been colonized by terrestrial microbiota (Steele et al. 2000). A further example is the ambiguity associated with the Viking Lander results (Mancinelli 1998). Recent re-evaluations of these experiments have revealed that the sensitivity of the GC-MS was simply inadequate for the task and would have been unable to detect organic material produced by approximately 10^7 bacteria per gram of Martian regolith (Glavin et al. 2001). This same group points out that a newer instrument (Mars Organic Detector) is 100 times more sensitive than the Viking GC-MS, which presumably means that 10^5 bacteria/gram will remain undetected.

Current immunoassays, such as enzyme-linked immunosorbent assay (ELISA), are known to be 150 times more sensitive and a great deal more specific than the best laboratory-based GC-MS instruments (Li et al. 1999). For example, ELISA has been used in environmental waste water studies to detect specific estrogen hormones at levels as low as 0.05 ng/L (0.05 parts-per-trillion), and there is evidence to show that miniaturization of these instruments increases sensitivity (Huang et al. 1999). A wealth of microbiological, molecular, and genetic techniques is now being refined as biotechnology approaches its golden age. Nanotechnology has been integrated with biotechnology, resulting in exciting new fields of research with the development of robust "lab-on-a-chip" commercial instrumentation (see Auroux 2002 and Reyes et al. 2002 for extensive reviews). These include the Agilent bioanalyser 2100, Perkin Elmer's protein array workstation, the Biosite™ Triage system, and the Charles River Food Spoilage Detector, using Limulus Amebocyte Lysate (LAL) assay (which has single-cell detection sensitivity, Wainwright 1999). These instruments are extremely sensitive to target molecules, sufficiently robust to be used in hospitals and the food industry, and becoming tailored to environmental samples such as detection of agents of biological warfare.

In the case of the Agilent bioanalyser 2100, a range of microfluidic chips performs sample processing and capillary electrophoresis, and the resulting DNA/RNA/protein separation is detected using laser-induced fluorescence (www.agilent.com/lab-on-a-chip). Each of the other instruments contains a probe or probes (tagged with either an enzyme or fluorochrome) specific to a target. By simply changing the probe, one can make an instrument shift from detecting a food spoilage organism (using a probe to bacterial LPS) to detecting bacterial diagenetic products such as hopanes (using an anti-hopane antibody, recently developed and under testing at CIW and JSC, Maule et al. 2003). These techniques have already been developed to answer the same questions on Earth that we wish to address in the rest of the Solar System: is there or was there once microbial life present, what are its characteristics, how can we detect it in small quantities, and what effect is it having on the environment in which it resides (and vice versa). Whether it is a novel microbe in a black smoker, an unknown infection, or a cancer cell in the human body, the task, goals, methodology, and philosophy are the same. To the question, "Why use biotechnology for astrobiology to search for life?", the answer becomes, "Biotechnology was independently, and successfully, developed for the detection of trace levels of biomarkers on Earth, and need only be modified and applied to Solar System exploration" (Steele and Toporski 2003).

Originally designed for high throughput data mining of genomes, microarrays can fit up to several million separate tests per glass slide (Hoheisel 1997; Schena 2000). This technology is growing at an unprecedented rate and will revolutionize environmental microbiology (including issues relevant in astrobiology and geobiology). Although these tests comprise mostly DNA- and protein-based probes, their use can be diversified to tests using any reagents that produce a color change (either chromophore or fluorophore) during the detection of a primary molecule. The technology is commercially available and is being utilized to produce miniaturized, highly specific test kits for a range of organic compounds linked to life processes and indeed has been

highlighted for further development by a number of agencies (Gibson 2002). These test kits could be tailored for use on all of the potential targets listed in Table 5.8.1. A further interesting possibility for the detection of extraterrestrial life arises if one considers integrating combinatorial chemistry with microarray technology. It would then be possible to produce millions of random probes per glass slide. For example, one could incorporate all the possible combinations of purine and pyrimidine bases that can combine into an information-storing system sufficiently complex to code for an organism. This vast coverage of plausible bases then reduces the assumptions needed for the detection of an extraterrestrial organism from DNA-based life to that of nucleic-acid-based life. These techniques may be further refined for use in experiments on the formation of life on Earth by screening large numbers of prebiotic reactions printed either onto glass slides or mineral interfaces.

It is essential to assess any biological-contamination background signal for organic analysis instruments to be used in any landed mission on another planet. Unambiguous detection and elimination of any terran contaminant signal is critical to show that any residual signal is indigenous to the Solar System body being explored.

In the case of astrobiological bioanalytical instrumentation packages for missions, it may be advantageous to "de-tune" specific bioanalytical instruments to the point that one obtains sensitive detection of discrete classes of biomarkers. This procedure would constitute a search strategy leading from a general terran signature to specific metabolisms, not only to identify life but potentially to define its nature. General classes of target materials of astrobiological interest are listed in Table 5.8.1.

Table 5.8.1. Classes of organic molecules of astrobiologic interest

Prebiotic/protobiotic molecules	Amino acids, purines and pyrimidines, polycyclic aromatic hydrocarbons, chain hydrocarbons, fatty acids, sugars and sugar derivatives, isoprenoids.
Terrestrial contaminating organisms	Whole cells, cell components (LPS, DNA, proteins, cytochromes).
Terrestrial contaminating organics	Condensation products derived from rocket exhaust, lubricants, plasticizers, atmospheric contaminants.
Terrestrial-like organisms	Transferred from Earth to an extraterrestrial body or have evolved on the extraterrestrial body in a manner similar to that by which life evolved on Earth. This conclusion could be reached only if procedural blanks had removed the possibility of hitchhiker contamination. Target molecules could include individual genes, membrane constituents, specific enzymes, and co-enzymes that would be expected to be over-expressed or adapted in ambient conditions.
Non-terrestrial-like organisms	Utilizes an array of molecules for information storage, information transfer, compartmentalization, and enzymatic activity that differ from those used by extant terrestrial life. An example would be the use of novel amino acids or nucleotides.
Fossil biomarkers	Detection of established terrestrial fossil biomarkers such as hopanes, archaeal lipids, and steranes, for the detection of the diagenetic remains of terrestrial-based life.

Proposed Research. Obviously the above discussion covers a greater scope than can be addressed by a single group. Co-Is Steele and Vicenzi propose the following tasks:

Co-I Steele will use a variety of state-of-the-art bioanalytical techniques to investigate detection-sensitivity issues for a range of terrestrial microbiota biomarkers obtained from so-called "hostile" environments. The philosophy is not to characterize the microbial ecology in these sites, but rather to detect biomolecules and remnants of life. It therefore becomes irrelevant what DNA is being detected (as long as it can be differentiated from a known contaminant); detection of DNA, RNA, proteins, and ATP are an indicator of life in these environments. Specifically, Steele will assay samples using the techniques listed below, from Yellowstone hot springs, USA (in collaboration with M. Schweitzer, Montana State University), the acidic Rio Tinto, Spain (in collaboration with V. Parro Garcia and C. Briones at the Centro de Astrobiología, Spain), wall rock from deep-sea hydrothermal vent chimneys on the Juan de Fuca Ridge (in collaboration with Co-I Baross and D. Kelly, University of Washington), Mars desert research station, Haughton crater (in collaboration with C. Cockell, British Antarctic Survey), and Lake Tanganika sediments, a site of continental sub-aquatic hydrothermal vents (with Co-I Scott). Many of these samples are already in our possession, and other sample sets of relevance to Solar System exploration will be sought.

Co-Is Steele and Vicenzi and Collaborator Toporski will also explore whether bioanalytical techniques can be applied to detect evidence of ancient life from various fossil localities (see **Section 5.7**). Some of the best-preserved organic fossils (including those of bacterial mats) are from the Enspel Formation in Germany. These ~ 25-Ma old rocks contain fossils spanning a wide range of mono- and multi-cellular organisms. Preliminary analyses (with ToF-SIMS, GC-MS and DNA extraction, SELDI-ToF antihopane and PAH antibodies) reveals a considerable array of well-preserved biomarkers. Thus the Enspel Formation may be an optimum fossil locality (along with the Messel and Wilershausen oil shales) to test bioanalytic strategies for the detection of ancient life (Toporski et al. 2002a). Also of considerable interest to the astrobiological community are organic fossils contained within some of the most ancient terrestrial sediments, i.e., the Archaean deposits and red sandstones of Western Australia. These extremely ancient rocks will likely prove to be the most difficult samples in which to detect *bona fide* chemical biosignatures. In collaboration with M. Brasier at Oxford University we will seek to address the ambiguity of the reported microfossils within the Warrawoona Group using the suite of techniques outlined below.

A suite of Martian and carbonaceous meteorites in Co-I Steele's possession will be assayed for any terrestrial contamination that may have occurred over many years of storage. Co-I Steele will apply site-specific molecular probes and imaging techniques to identify and distinguish terrestrial contamination from endogenous organics in this suite along with new material in complementary experiments including those of Co-Is Alexander, Cody, and Nittler. These experiments will bear on future curatorial strategies as well as help us to assess whether there exists a substantial fraction of terrestrial organic matter that could complicate our evaluation of cosmochemical organic inventories.

Each sample locality or experiment chosen poses a unique set of scientific challenges. This research task is designed to begin to set terrestrial baseline signatures for instruments that are currently being developed for Mars exploration for detection of both viable and fossil life. For this study we will apply a broad array of analytical techniques to augment and refine the bioanalytical methods currently used. Although most of these methods will not be part of any spacecraft science instrumentation package for the foreseeable future, all will serve to optimize the select subset of instruments that are ultimately chosen for the purpose of extraterrestrial life detection or sample return. The methods we will employ include the following:

Imaging. We will make use of fluorescence and light microscopy, scanning electron microscopy (CIW), soft X-ray (C, N, O) chemical imaging (in collaboration with Co-I Cody and Collaborator Ade at the Advanced Light Source, ALS), atomic force microscopy (AFM,

Montana State University), and chemical force microscopy (Montana State University). This last technique uses an antibody attached to the silicon nitride tip of an atomic-force-microscopy cantilever to detect an antigen to the antibody binding (Mazzola et al. 1999). This information is combined with a 3D topographic image of the surface afforded by standard AFM protocols. We will explore the use of nano-size gold particles attached to secondary marker antibodies to localize the initial antibody reaction (available from Molecular Probes, Inc.) and image these reactions using field emission SEM and TEM (Vali et al. 2001). Finally, we will explore the utility of near-field scanning optical microscopy that will allow for direct visualization of target objects with spatial resolutions well below the diffraction limits of conventional optics (under development in collaboration with Co-I Nittler as part of a project funded by the NASA Sample Return Laboratory Instruments and Data Analysis Program).

Spectroscopy. We will apply micro-Raman (CIW) and synchrotron-based micro-infrared spectroscopy (National Synchrotron Light Source, NSLS), XANES of carbon, nitrogen, and sulfur) (NSLS and ALS), and ^{13}C solid-state nuclear magnetic resonance spectroscopy (CIW) (of demineralized samples) for quantitative analysis of total organic carbon functionality.

Spectrometry. We will apply time-of-flight secondary ion mass spectrometry (ToF-SIMS, see **Section 5.7**), surface enhanced laser desorption ionization (SELDI) mass spectrometry (CIW), as well as conventional GC-MS (CIW).

Bioanalysis. At CIW we will apply real-time polymerase chain reaction (RT-PCR) using common primers to study the effect of mineralization on the amount of DNA that can be recovered from viral and bacterial species that are deliberately fossilized and that are recovered from both recent and ancient materials. The Cepheid system will yield semi-quantitative PCR, providing an assessment of the decay in detection rates of DNA with diagenesis. We will employ enzyme assays, both LAL and detection of ATP. We will explore the use of capillary electrophoresis coupled with laser fluorescence detection for extractible DNA, RNA, and proteins. We will design and test protein and DNA microarrays, as well as use commercially available DNA chips, to assay changes in bacteria during fossilization. In addition we will test our limits of detection and selectivity using flow cytometry and enzyme-linked immunosorbant assays (ELISA).

Completion of the experiments described above will provide results that can optimize the choice of techniques for a bioanalytical life-detection science package designed for future lander missions to Mars and other appropriate Solar System bodies. From the outset we envision a versatile, small-footprint instrument based on the design principle of the Modular Assay for Solar System Exploration (MASSE), under development in collaboration with D. McKay at JSC and colleagues at the Centro de Astrobiología, Spain. Specifically, the MASSE instrument concept uses microchip-based microfluidics to accept, extract, concentrate, filter, buffer, and process organic molecules of interest from a single, small sample-handling chamber. The prototype MASSE study was initially funded by NASA (2005 Mars opportunity), and it has been recommended for further development by ESA. In its current stage of development this instrument has achieved its essential proof-of-concept goals, but considerable refinement is required before this instrument can fly. Many of the analytical refinements are best done under the auspices of NASA's Astrobiology Program, to ensure the greatest input from the large community of biologists, chemists, physicists, and engineers with a commitment toward missions designed to search for extraterrestrial life.

5.9 Management Plan

A key feature of our proposed NAI research plan is a broadly integrated approach that draws on the expertise of a team of researchers in astronomy, biology, chemistry, and Earth sciences. This section describes how the activities of researchers from these different disciplines and

several widely-separated geographic locations will be integrated, both within our team and into the larger arena of the NAI virtual institute.

5.9.1 Executive Committee

Our team will be managed by an eight-member Executive Committee, consisting of Co-Investigator representatives from the consortium's institutional partners and chaired by the Principal Investigator. The initial membership will consist of John Baross from the School of Oceanography at the University of Washington, David Deamer from the Department of Biochemistry at the University of California, Santa Cruz, Alan Boss from the Department of Terrestrial Magnetism of the Carnegie Institution of Washington, Noreen Tuross from the Smithsonian Institution, George Cody from the Geophysical Laboratory of the Carnegie Institution, James Farquhar from the Department of Geology, University of Maryland, Jay Brandes from the Department of Marine Sciences, the University of Texas, and Sean Solomon from the Department of Terrestrial Magnetism of the Carnegie Institution, who will act as committee chair. Over the five-year lifetime of the project, other team members will rotate onto the Executive Committee at regular intervals. The Executive Committee will meet on a monthly basis, in face-to-face sessions where possible by virtue of appropriately timed workshops, symposia, and other multi-purpose travel opportunities, and via teleconference otherwise.

Our team benefits not only from CIW's record as an NAI lead institution for the past five years, but also from having members with management experience appropriate for guiding and coordinating such an endeavor. Principal Investigator Sean Solomon is the Director of the Carnegie Institution's Department of Terrestrial Magnetism. A past President of the American Geophysical Union, he has led multidisciplinary scientific endeavors ranging from oceanographic expeditions to explore mid-ocean ridges to the MESSENGER mission to orbit the planet Mercury.

5.9.2 Plan for multi-institutional cooperation

While most of the research described in this proposal will be conducted at the Carnegie Institution's Washington, D.C., campus, the site of both the Geophysical Laboratory and the Department of Terrestrial Magnetism, the contributions of Co-Investigators at George Mason University, the Naval Research Laboratory, the Smithsonian Institution, the University of California at Santa Cruz, the University of Maryland at College Park, the University of Texas at Austin, and the University of Washington are critical to the success in this endeavor. Our challenge, therefore, is to coordinate work at eight institutional locations. Experience has taught us that regular electronic interactions among all consortium members, as well as frequent multi-day intersite visits and longer personnel exchanges, are essential to achieve our objectives. To this end, our plan for multi-institutional cooperation consists of the following elements:

NAI Team Symposia. The Carnegie Institution's Washington, D.C., campus is centrally located in the nation's capital. We are, for example, within a few miles of NASA Headquarters, the NASA Goddard Space Flight Center, the Naval Research Laboratory, the Smithsonian Institution National Museum of Natural History and Air and Space Museum, the headquarters of the United States Geological Survey, the National Science Foundation, the National Institutes of Health, the National Academy of Sciences, and the headquarters of numerous other government agencies and scientific societies, including the American Geophysical Union, the American Chemical Society, the American Physical Society, and the American Association for the Advancement of Science. George Mason University and the University of Maryland are short drives from the Carnegie Institution campus, enabling frequent visits. Co-Investigators Deamer from the University of California, Baross from the University of Washington, and Brandes from the University of Texas all plan to make several trips per year to the area, and all have visited CIW laboratories at least once during the past 12 months.

In order to take full advantage of Carnegie's convenient location and to reduce the costs of transportation and lodgings, we will hold most of our consortium's symposia and Executive Committee meetings at Carnegie Institution facilities. (Committees of NASA, AGU, and other organizations frequently take advantage of the Broad Branch Road facility for their organizational meetings.)

In the first year, as we define and integrate our new and larger NAI team, we propose to sponsor one research and planning symposium for all of our consortium participants. The meeting will be open to others, including representatives of other NAI teams, but the focus of the symposium will be our own team's research agenda. We anticipate that this meeting will result in new collaborative links within the NAI structure. The first symposium will be held at the Carnegie Institution.

In each succeeding year we plan at least one dedicated symposium for all members of the team, held either at CIW or at another partner institution (and tied to an appropriate national meeting, if possible, to reduce travel costs). All of our symposia will be conducted in the style and spirit of Gordon Conferences, with time divided between lecture-style presentations, group discussions, and one-on-one interactions.

Intersite visits. In addition to the above symposia, we propose to sponsor numerous intersite visits during the five years of this program. These intersite visits will be of three types:

a. Lecture visits. We will sponsor an active lecture program. Each Co-Investigator, NAI Postdoctoral Fellow (in his or her second year), and NAI predoctoral fellow (near completion of the Ph.D.) will be encouraged to deliver scientific research lectures at the other partner institutions.

b. Senior staff research exchanges. When appropriate to accomplish our team's research objectives, we will sponsor site exchanges for research collaboration. Several such exchanges are mentioned in connection with specific research tasks above.

c. Junior staff exchanges. All NAI Postdoctoral and Predoctoral Fellows will be encouraged to spend at least one month visiting and engaging in research at one of the other NAI institutions. For example, a Postdoctoral Fellow in organic geochemistry at the Carnegie Institution might visit the University of Washington to collect and study samples from an ocean vent system. We believe that such exchanges will greatly enhance the professional breadth of our young colleagues, while also strengthening ties within the team.

Electronic interactions. Members of the team are already in frequent electronic contact. Information exchange will be facilitated by the new CIW NAI Website (see **Section 7.8**). A major goal of our team's IT initiative (**Section 7.8**) will be to develop the tools that best meet the interactive needs of team members for each of our collaborative research tasks.

5.9.3 Roles and responsibilities of each participant

Following are the key participants in our project and their roles in this consortium effort. Each of these individuals has endorsed the objectives and research plan described herein, and each has committed to taking on the responsibilities given below. Further details on the background and expertise of each of these participants can be found in **Section 9**. Copies of the letters of commitment from all Co-Investigators and a number of Collaborators are in **Section 13**. In the list below, the Geophysical Laboratory and the Department of Terrestrial Magnetism of the Carnegie Institution of Washington are denoted by the abbreviations GL-CIW and DTM-CIW, respectively.

- **Conel M. O'D. Alexander** (Research Staff Member, DTM-CIW) is a cosmochemist and is a supervisor of the Carnegie ion microprobe, with which he studies meteorites. Alexander will coordinate research on meteorite organic material, as well the characteristics of hydrothermal activity in meteorite parent bodies.
- **John A. Baross** (Professor, School of Oceanography, University of Washington) is a microbiologist who studies the physical and chemical environment and microbial biology of ocean hydrothermal vent systems. He will provide critical expertise on natural hydrothermal environments.
- **Nabil Boctor** (Consultant, GL-CIW) is geochemist and an expert of transition-metal sulfides. He provides a theoretical understanding of sulfide formation and reactivity, and he provides well-characterized synthetic sulfides for our experiments.
- **Alan P. Boss** (Research Staff Member, DTM-CIW) is an astrophysicist who models the collapse of molecular cloud cores and the formation of stars and planets. He will study the origins and distribution of volatiles in planetary systems.
- **R. Paul Butler** (Research Staff Member, DTM-CIW) is an astronomer who collaborates closely with Co-Is Boss, Seager, and Weinberger in assessing the modes of giant planet distributions and the probable influence of such distributions on the galactic distribution of habitable worlds.
- **Jay A. Brandes** (Co-I, University of Texas, Austin) is a marine chemist who is conducting experiments on the hydrothermal origins and isotopic signatures of abiotically synthesized reduced nitrogen and amino acids.
- **George D. Cody** (Research Staff Member, GL-CIW) is an organic geochemist who is studying rates and pathways of hydrothermal organic synthesis related to protometabolism. He will continue to guide this experimental program as well as contribute to the work on biosignatures and extraterrestrial materials using scanning transmission X-ray microscopy.
- **David Emerson** (Research Scientist, American Type Culture Collection, George Mason University) is a microbiologist who is investigating Fe-oxidizing lithotrophic procaryotes. He will continue to study metabolic processes in these organisms, while coordinating the sampling, archiving, and distribution of type microbial specimens to NAI members.
- **David W. Deamer** (University of California, Santa Cruz) is a biochemist who will coordinate sampling of hot springs and subsequent analysis at CIW. He will collaborate with prebiotic chemistry initiatives at CIW in lipid encapsulation experiments.
- **James Farquhar** (University of Maryland, Department of Geology) is a stable isotope geochemist and will participate in the research related to the Earth's earliest sulfur cycle as well as provide expertise in sulfur isotopic analysis.
- **Marilyn L. Fogel** (Research Staff Member, GL-CIW) is a biologist and will collaborate in the analysis of stable isotopic fractionation in biotic and abiotic processes and the application of SELDI-ToF for proteomics in collaboration with studies of life in extreme environments.
- **Erik H. Hauri** (Research Staff Member, DTM-CIW) is a geochemist who will provide measurements of Fe isotopes to integrate with the studies on iron-oxidizing bacteria with Co-I Emerson.
- **Robert M. Hazen** (Research Staff Member, GL-CIW) is an experimental mineralogist with expertise in high-temperature, high-pressure apparatus. He will participate in research related to the origins of chirality, biosignatures, and mineral-mediated organic synthesis, and he will engage in extensive public outreach and education activities.
- **Russell J. Hemley** (Research Staff Member, GL-CIW) is a physical chemist who has developed spectroscopic methods for high-pressure research. He will coordinate studies that employ hydrothermal diamond-window devices for real-time spectroscopic analysis of organic synthesis and bacterial metabolism under extreme conditions.
- **Wesley T. Huntress** (Director, GL-CIW) is a chemical physicist and will collaborate with the extraterrestrial materials research group by providing insight into chemical dynamics and ion-molecule reactions.

- **Karl Kehm** (Collaborator, Washington College) is a geochemist and will measure iron isotopes in natural and laboratory samples in an effort to characterize and understand the various processes, both biotic and abiotic, that can induce iron isotopic mass fractionation. These studies will help determine whether iron isotopes are a useful biomarker.
- **Timothy J. McCoy** (Smithsonian Institution) is a cosmochemist and will collaborate on studies of the fate of carbon during planetary differentiation and the relationship between such processes and the chemistry of the early Earth.
- **Larry R. Nittler** (Research Staff Member, DTM-CIW) is a cosmochemist who will explore the organic chemistry of IDPs as part of the extraterrestrial materials research initiative.
- **Shuei Ono** (Postdoctoral Fellow, GL-CIW) is a geochemist and will participate in the research related to the Earth's earliest sulfur cycle providing expertise in sulfur isotopic analysis. He will focus on the development of ultra-low blank methods and methods for the analysis of organic sulfur.
- **Douglas Rumble III** (Research Staff Member, GL-CIW) is a stable isotope geochemist and will lead the research on the Earth's earliest sulfur cycle. He provides expertise in sulfur isotopic analysis and will conduct field studies in the Archean basins of Western Australia.
- **Sara Seager** (Research Staff Member, DTM-CIW) is an astronomer and will model carbon and water in extrasolar planetary atmospheres. Specifically she will investigate the temperature-diagnostic potential of carbon in giant planet atmospheres as well as develop giant planet metallicity indicators based on carbon and water atmospheric spectral features.
- **James H. Scott** (Research Staff Member, GL-CIW) is a microbiologist and will focus on stress adaption of microorganisms under extreme conditions. He will develop and use molecular biological methods to ascertain global regulation in response to pressure.
- **Anurag Sharma** (Postdoctoral Fellow, GL-CIW) is a geochemist and will collaborate on experiments involving life in extreme environments as well as provide expertise in hydrothermal diamond-anvil-cell studies related to life in extreme environments.
- **Sean C. Solomon** (Director, DTM-CIW) is a planetary scientist who has spent more than 30 years investigating mid-ocean ridges in the Earth's oceans and the thermal and magmatic evolution of planets and satellites. In addition to providing overall leadership to the consortium, he will explore the range of possible Solar System environments of present and past hydrothermal systems.
- **Andrew Steele** (Research Staff Member, GL-CIW) is a molecular biologist who will develop and apply methods in biotechnology to address astrobiological questions related to biosignatures and life in extreme environments.
- **Rhonda Stroud** (Research Staff Member, Naval Research Laboratory) is a physicist who will conduct high-resolution analytical transmission electron microscopy studies at the NRL of carbon-bearing phases in interstellar dust particles and meteorites and of fossilized bacterial and viral material in collaborations with Co-Is Alexander, Nittler, and Steele.
- **Jan Toporski** (Postdoctoral Fellow, GL-CIW) is a microbiologist who will participate in biomineralization studies of microbes and viruses as part of the biosignatures research effort. He will continue studies of the ancient bacterial mats in the Enspel Formation, Germany.
- **Noreen C. Tuross** (Smithsonian Institution) is a biochemist who will provide expertise in bioanalytical methods for the detection of biosignatures in the Enspel Formation, Germany. She will also study the preservation of molecular biosignatures in stromatolites.
- **Edward P. Vicenzi** (Smithsonian Institution) is a geochemist who will provide expertise and analytical collaboration in the use of time-of-flight secondary ion mass spectrometer (ToF-SIMS) for applications in biosignatures research and extraterrestrial materials.
- **Alycia J. Weinberger** (Research Staff Member, DTM-CIW) is an infrared astronomer who will integrate observations of extrasolar disks to bridge to models of planet formation made by our astronomical theorists as well as make observational connections to our research on extraterrestrial materials.
- **George W. Wetherill** (Director Emeritus, DTM-CIW) is a planetary scientist who performs numerical simulations of planet formation. He will continue his investigation of the origin

and distribution of Earth-like planets and implications for volatiles on other planets and satellites.

- **Hatten S. Yoder, Jr.** (Director Emeritus, GL-CIW) is an experimental petrologist who supervises the gas-media high-pressure laboratory used for hydrothermal synthesis experiments.

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7. Plan for Strengthening the Astrobiology Community

7.1 Education and Public Outreach

Background

The Carnegie Institution of Washington (CIW) has been working since 1989 to improve science, mathematics, and technology education for students nationally and locally in the Washington, D.C., public schools. The Carnegie Institution of Washington's Education and Public Outreach (E/PO) programs are dedicated to the increase and diffusion of knowledge among children, teachers, and the general public about the natural world. To accomplish this goal, the institution develops, presents, and promotes educational programs and materials so that science can be understood, valued, and enjoyed.

The E/PO plan for the CIW NAI team blends new and traditional approaches to bring teachers, children, and the general public relevant astrobiology-related content and experiences. Just as the institution's astrobiology efforts engage the science community in highly focused research, so the E/PO program engages the public in a highly-leveraged learning adventure that elevates the understanding of the nature of science. The broad and deeply connected field of astrobiology presents unusually clear opportunities not only to teach about the science but also about the nature of science itself, an important dimension of the National Science Education Standards.

Accomplishments to Date

Over the past five years, the Carnegie Institution has involved educators and scientists to produce a variety of unique materials and experiences for elementary students and teachers. Highlights of our E/PO accomplishments include the following:

1. Involved all lead scientists in astrobiology-related E/PO activities that last year reached 8,000 teachers, students, and members of the general public.

2. Produced and distributed 10,000 copies of the booklet *Astrobiology: The Search for Water*.

3. Developed significant collaborations with outside partners, such as the Smithsonian Institution, to produce over 75 book/activity backpacks for Pre-K-2 students that developmentally explored some of the fundamental ideas related to astrobiology. The packs are being used in a pilot program in over 25 schools in the District of Columbia. Backpack topics included Water, Life on Earth, Small Life, Past Life, and Fossils.

4. Trained over 300 District of Columbia teachers as part of the five-week Carnegie Academy for Science Education (CASE) Summer Science Institute. For three years, CASE has used astrobiology as a way to organize thematically instruction and content. CASE programs require the active involvement of lead scientists.

5. Produced and distributed over 22,000 copies of a poster and associated educational materials entitled *Astrobiology: Discovering New Worlds of Life*. This poster and an accompanying article were published in the November-December 2001 issue of *Science Scope*, the journal of the National Science Teachers Association (NSTA).

6. Collaborated with NAI on the creation of activities for a series of educational posters for grades K-12.

7. Collaborated with NAI partners (JSC and JPL) on the production of a comprehensive set of astrobiology materials for the elementary level entitled *Ice in the Solar System*.

8. Featured the science of astrobiology in over a dozen Capital Science Lectures, each attended by an average of 200 local high-school students and interested citizens of the District.

E/PO Goals for the Next Five Years

We propose several new projects that over the course of the coming five years will sustain contact with millions of youngsters, teachers, and the general public in order to promote concepts and ideas related to astrobiology. Over the next five years the Carnegie Institution's E/PO effort, under the leadership of Julie Edmonds, will:

1. Involve all lead scientists in direct participation in the institution's E/PO efforts;

2. Place special emphasis on reaching local underserved urban communities;

3. Place strong emphasis on K-8 education, including educational technology components, educator workshops, and curriculum support activities;

4. Place strong emphasis on informal science learning through participation of museums and libraries;

5. Leverage partnerships such as TIME For Kids, NSTA, Smithsonian Institution, NOVA (WGBH-TV), Platypus Media, and others, in order to reach a national audience;

6. Team with the Minority Institution Astrobiology Collaborative on E/PO projects.

TIME For Kids (No-cost leverage)

Six years ago, Time Magazine, Inc., initiated a new division called *TIME For Kids*. *TIME For Kids* (TFK) is a weekly news publication for youngsters that presents a context for science, history, art, and culture. The publication reaches over 4 million children through classroom subscriptions and another 200,000 through *Time* magazine subscriptions.

TIME For Kids is already well known in America's classrooms as the nation's fastest growing educational news magazine, but TFK is much more than a magazine. Whether experienced online, in print, or through a vast array of educational products and resources, TFK delivers a vital tool for teachers and a unique avenue for K-6 students to connect with the world around them and feel empowered to play a role in it. The voice of TFK features the expertise of educators, scientists, publishers, veteran journalists, content experts, as well as one of the premier cadres of young reporters in the country.

Science forms a significant core of content for *TIME For Kids*. Over the course of the next five years, CIW and *TIME For Kids* will begin a partnership to promote astrobiology through content, feature articles, interactive web components, and teacher resources. CIW will support *TIME For Kids* in the following ways:

1. Access to scientists for interviews by *TIME For Kids* young reporters.

2. Press releases, content summaries, and activity outlines related to new and cutting-edge research in the field of astrobiology.

3. Supplementary print and visual materials published by CIW, NASA, and NAI that will enhance both the print and web medium.

Carnegie Frontiers – a Collaborative Effort to Create a Children's Book About Microbes

As part of CIW's E/PO efforts, a series of publications entitled *Carnegie Frontiers* was created to highlight the institution's related research in various aspects of the field of astrobiology. Over the course of five years, three publications were created and distributed to teachers across the country through NSTA, direct mail, and regional distribution by cooperating museums and institutions. The titles were *Astrobiology: The Search for Water* (a 15-page booklet), *Astrobiology: New Worlds of Life* (a poster was featured in the NSTA publication *Science Scope* along with an article about astrobiology), and *Astrobiology: Ice in the Solar System*, an instructive CD with accompanying activities information and visuals about ice in our Solar System. (This CD is now in production).

Plans for the next five years include two new additions to the *Carnegie Frontiers* series. The first new release in the series will emerge from collaboration between CIW, other NAI members, and a private publishing house that specializes in science-related books for youngsters. The collaborative will create and publish a 64 page, full-color children's book about the microbial world with over 70 original illustrations and high-quality photographs. This trade book about microbes will introduce children (8-12 years of age) to the science of astrobiology through a connected and engaging story line of scientific content about life and the natural world. The resulting book with accompanying teacher's guide will be distributed to libraries, schools, NAI partners, and bookstores nationwide.

Carnegie Academy for Science Education (No-cost leverage)

The Carnegie Academy for Science Education (CASE) was established in 1993 as a professional development program for pre-Kindergarten through 6th-grade public school teachers in Washington, D.C. The program's mission is to train teachers to align instruction with national science and math standards and integrate interactive activities, assessment, and content into their classroom curriculum.

Over the last three years a centerpiece of CASE has been the DC ACTS initiative, a collaborative project based on the premise that systemic reform of science, mathematics, and technology education can support positive changes for all parts of the educational system. The Carnegie Institution of Washington, the American Association for the Advancement of Science (AAAS), and the District of Columbia Public School system are collaborators on this project. CASE provides the elementary-school component. The middle-school and high-school teacher training is the responsibility of the AAAS. Other CASE programs include the following:

• *Summer Science Institute*. The only long-term professional development program in science for District of Columbia Public School teachers.

• *Yearlong series of six-week courses*. These yearlong sessions train some 200 additional teachers in District schools. As with the Summer Institutes, teachers receive graduate credits upon completion of the courses.

• *Mentor Teachers*. Outstanding CASE teachers are selected each year to receive additional training and return the following summer as members of the teaching staff of the Science and Mathematics Institutes.

• *Model Schools*. CASE helps 15 D.C. public elementary schools develop coherent, standards-based, pre-K to 6th-grade science and mathematics programs. Model Schools

receive additional curriculum materials and workshops. Citywide expansion of this component of the program is planned for September 2004.

• *First Light*. Since 1989, First Light continues as a free Saturday science school and also serves as a "laboratory" for testing CASE's instructional methodologies. The free, 5-hour classes attract some 30 neighborhood children on a first-come, first-served basis every school year. In addition to participating in hands-on science activities, students go on weekly field trips and receive free lunches.

CASE participants, young and old, develop their scientific sensibilities through the incorporation of topics such as astrobiology that serve as points of connection for understanding biology, astronomy, geology, and chemistry. Astrobiology themes are embedded during the CASE Summer Science Institutes, monthly trainings, and workshops. Thematic topics of instruction include microbial life, extremophiles, hydrothermal vent morphology, evolution of life on Earth, NASA mission design, planetary geology, and the discovery of extrasolar planets. Classroom activities, readings and regular instructional dialogues by staff scientists combine to create a professional development experience for teachers that is vibrant and provides the necessary content and instructional models for classroom use.

Smithsonian Institution Display

The Smithsonian Institution's National Museum of Natural History (NMNH) is host to nearly 7 million visitors annually. As part of this proposal's E/PO effort, NMNH and CIW will jointly oversee the production of a display in the Constitution Avenue lobby that will focus visitors' attention on the science of astrobiology. The display will allow for printed material for the public as well as a display of artifacts and images. Such an exhibit will feature the joint research of the Smithsonian and the Carnegie Institution, as well as the NAI overall.

The Minority Institution Astrobiology Collaborative

The Minority Institution Astrobiology Collaborative (MIAC) is a national initiative, based at Tennessee State University, that aims to advance the cause of education and research in astrobiology among minority communities in the United States. The goals of MIAC are to bring together participating members and other organizations to share expertise and resources in order to create an environment for faculty and students of minority institutions in which significant contributions to the field of astrobiology in education and research are made; to provide support and professional development for college and university faculty and K-12 teachers to teach astrobiology content; and to collaborate with NAI lead teams and in 5-to-10 years to become a NASA Astrobiology lead team. MIAC will assist CIW in the implementation of the following of its E/PO efforts.

1. Minority faculty and student research opportunities;

1. Student internships;

2. CASE instructional support;

3. Development of the trade book for children on microbes;

4. Professional development opportunities for Pre-K-12 teachers.

Summary of Astrobiology-related Education and Public Outreach Activities by the Carnegie Institution of Washington

Activity	Product	Description	Budget	Lead organization(s)
2003-2004 Development of trade book for children on microbes	Nationally distributed trade book	The proposed project would be the creation of a 64-page, full color children's book about the microbial world with over 70 original illustrations and high-quality photographs. This trade book will introduce children (8-12 years of age) to the science of astrobiology through connected and engaging scientific content about microbes, life, and the natural world.	Year 1. $45,000 Year 2. $45,000	CIW, NAI partners, Platypus Media
2003-2008 *TIME For Kids* articles and content	Articles, interviews and web-features as part of a nationally distributed periodical.	Science forms a significant core of content for the publication *TIME For Kids*. Over the course of the next 5 years, CIW and *TIME For Kids* will begin a partnership to promote astrobiology through content, feature articles, interactive web components, and teacher resources.	No cost	CIW, *TIME For Kids*, NAI members
2003-2008 CASE instructional support	Professional development for teachers, workshops, instructional support, and materials for classrooms.	The DC ACTS initiative is a collaborative professional-development project based on the premise that systemic reform of science, mathematics, and technology education can support positive changes for all parts of the educational system. The Carnegie Institution of Washington, the American Association for the Advancement of Science, and the District of Columbia Public School system are collaborators on this project. CASE provides the support and training for the elementary school component.	No cost - funded by CIW, NSF, HHMI	CIW, AAAS, DCPS
2003-2008 Capital Science Lectures	Series of public lectures	Free public talks designed to help non-scientists understand scientific thinking and to appreciate the importance of basic research in our lives today. Each year, a selection of speakers has been chosen to focus on the institution's interest in astrobiology.	No cost - funded by CIW and other partners	CIW, NAI partners
2003-2008 Website	Website	Maintain CIW astrobiology website with E/PO components that include activities, a section for students and teachers, as well as content and a calendar of activities.	No cost - funded by CIW	CIW
2006-2008 Informal science learning	Museum exhibits	A dedicated astrobiology-related display in the Constitution Ave. lobby of the Smithsonian Natural History Museum	Year 3. $16,000 Year 4. $7,000	Smithsonian NAI partners
2003-2008 Involvement of minority institutions	Minority Institution Astrobiology Collaborative	The involvement of MIAC in the following E/PO efforts: 1. Minority faculty and student research opportunities 2. Student internships 3. CASE instructional support 4. Development of trade book for children on microbes 5. Professional development of Pre-K-12 teachers	Year 1. $4,000 Year 2-5. $3,000/year	MIAC and CIW

7.2 Professional Community

Members of the Carnegie Institution NAI team have played a large role in helping the field of astrobiology develop, through their service on advisory groups, review panels, conference organizing committees, and other efforts that strengthen the professional astrobiology community. We provide here a selection among the activities that CIW NAI Co-Is have undertaken in support of astrobiology during the last five years, activities of the type that we intend to continue to pursue over the coming five years as well.

Advisory Groups. Co-Is have served on the NASA Astrobiology Roadmap team (Boss, Deamer), the NRC Committee on the Origin and Evolution of Life (Baross as Co-chair and Deamer and Fogel as members), the NASA Solar System Exploration Subcommittee (Solomon), the NASA Astronomical Search for Origins Subcommittee (Weinberger), the NRC Task Group on Sample Return from Small Solar System Bodies (Baross), the NRC Steering Committee on Size Limits of Very Small Microorganisms (Baross), the NASA Space Science Advisory Committee (Deamer), the Mars Exploration Payload Assessment Group (Baross, Farquhar), the NASA Terrestrial Planet Finder Science Working Group (Seager), and the NASA Navigator Program Independent Review Team (Boss). Solomon chaired and Deamer served on a Review Panel for NASA Specialized Centers of Research and Training (NSCORTs) in Astrobiology.

Editorial Positions. Boss serves as Series Co-Editor for the Cambridge University Press *Astrobiology Series* and is on the scientific advisory board for the book *Meteorites and the Early Solar System II*; Baross, Deamer, Fogel, Hazen, Solomon, and Steele serve on the Editorial Board for the journal *Astrobiology*, and Baross serves as a Co-Editor for the Springer-Verlag Press *Advances in Astrobiology and Biogeophysics Series* and for *Planets and Life: The Emerging Science of Astrobiology*.

NAI Focus Groups. Members of the Carnegie Institution NAI team have been active on NAI Focus Groups, including the current focus groups on Mars (Alexander, Hauri, Solomon), Mission to Early Earth (Ono, Solomon), Europa (Solomon), Astromaterials (Alexander, Nittler), and Titan (Baross, Hazen). Seager is chairing an effort that will culminate in a formal proposal for an Astronomy Focus Group (to also include Boss, Butler, and Weinberger), and Steele is leading an effort that will result in a proposal for a Biotechnology Focus Group.

Hosting Laboratory Visits. The Carnegie Institution has hosted laboratory visits by more than 30 astrobiologists from other institutions, including six NAI teams, two NAI foreign associate or affiliate organizations, one of the two NSCORTs in Astrobiology, and half a dozen other institutions in the U.S. and abroad.

Scientific Conference Organization. The Carnegie Institution hosted the Second NAI General Meeting in 2001, and team members served on the scientific organizing committees (SOCs) for that meeting (Boss, chair), the Second Astrobiology Science Conference in 2002 (Baross, Boss), and the Third NAI General Meeting in 2003 (Baross, Brandes). The Carnegie Institution also hosted the Conference on Scientific Frontiers in Research on Extrasolar Planets in 2002 (Seager served as SOC chair and Boss was a SOC member) and a workshop on Water on Mars in 2002 (Co-chairs Alexander and Boctor). Boss chaired the Gordon Research Conference on Origins of Solar Systems in 1999, and this year's conference features four team members as speakers and discussion leaders (Alexander, Desch, Seager, Weinberger). Cody is the vice-chair of this year's Gordon Conference on the Origin of Life, and he will chair the conference on that topic in 2005. Baross co-chaired the NRC Workshop on Life Detection in 2000 and chaired the University of Washington Origin of Life Conference in 2001. Boss serves on the SOCs for the Bioastronomy 2004 conference and the Protostars and Planets V Conference in 2005.

Departmental Seminar Series. Both DTM and the GL host a weekly seminar series. A large fraction of those seminars, typically attended by scientists from throughout the Washington, D.C., area, focus on astrobiological topics. In addition, NAI team members at the Carnegie Institution participate via teleconference in a weekly Astrobiology Journal Club hosted by the NSCORT in Astrobiology at the Rensselaer Polytechnic Institution.

International Organizations. Boss chairs and Butler serves on the International Astronomical Union Working Group on Extrasolar Planets. Deamer serves as Second Vice President for the International Society for the Study of the Origin of Life and is the Editor of their online newsletter.

7. 3 Training

The CIW-led NAI team has been actively engaged in training the next generation of astrobiologists through collaboration with neighboring universities, through research training of competitively selected undergraduate and high-school students, and through the recruitment and training of predoctoral and postdoctoral scientists. We plan to continue these efforts throughout the next 5 years of participation in NAI.

Teaming with Neighboring Universities

Although CIW is not a degree-granting institution, there is strong interest among universities in the region to develop instructional programs in astrobiology, and discussions have been initiated regarding the involvement of CIW staff in teaching programs at those universities.

In the spring of 2002 the Virginia State Council for Higher Education approved a new Ph.D. degree track in Astrobiology as part of the Biosciences Department at George Mason University. This degree program was developed in close collaboration among scientists at George Mason University, NASA, the Carnegie Institution, and the American Type Culture Collection. Several members of the CIW NAI team, including Co-Investigators Emerson, Hazen, and Huntress, will teach courses as part of this program. During the 2002-03 academic year Hazen developed and taught the first new offering in this program – a 2-semester graduate seminar entitled "The Literature of Astrobiology." The first student to enroll in this Ph.D. program, Ms. Mary Ewell, will begin her thesis research at the Carnegie Institution during the summer of 2003.

At the four university partners on this NAI team, the Co-Investigators from those institutions are committed to the involvement of students in their astrobiological research within NAI. Budget requests from those four institutions all include funds for student support.

Carnegie Institution Summer Intern Program

Beginning in 1997 the Carnegie Institution initiated a dynamic summer research program for high-school and undergraduate student interns. During the 10-week summer program, sponsored in part by the NSF Research Experiences for Undergraduates Program and the Camille and Henry Dreyfus Foundation, each student works on his or her own research project with one or more of scientific staff at the Broad Branch Road Campus (encompassing the Department of Terrestrial Magnetism and the Geophysical Laboratory). Ninety-one students have participated during the past six summers; they were mentored by a total of 54 Carnegie scientists. This nationally advertised program has proven especially successful in attracting women and minority students; last year's group of 20 interns, for example, included 12 women and 3 minority students. For the past three years the Director of the summer intern program has been Dr. William Minarik. Additional information on this program may be found on our web site: http://www.gl.ciw.edu/interns/.

In addition to working on their own research projects, the interns meet as a group at least weekly for an informal lunch with one or more of our scientific staff. During these lunches the staff share their own experiences of "becoming a scientist" with the students, describe their present research interests with a tour of their labs, and answer questions about scientific careers. A group of predoctoral and postdoctoral fellows also meet with the interns for lunch, during which the main topic of discussion centers on graduate school selection and job opportunities. The interns are given tours to local scientific organizations, for example the Gem, Mineral and Meteorite Collection at the Smithsonian Institution, and laboratories at the United States Geological Survey and the NASA Goddard Space Flight Center. The interns also spent one afternoon participating in Carnegie's CASE Summer Science Institute, the science teacher education program also sponsored by the NSF (see above). Another component of the intern program is a biweekly afternoon scientific ethics seminar on topics such as treatment of coworkers, laboratory misconduct, whistle blowing, and co-authorship.

Each intern's primary effort centers on a research project that is supervised by one or more Carnegie staff scientists. The character of the student-mentor interaction ranges from scenarios in which a student works closely with his or her mentor every day, to instances in which the students receive input from senior personnel once or twice a week. The nature of these interactions also changes with time: At the beginning of the program the students are very dependent on their advisors for guidance, but as the program progresses most acquire sufficient laboratory skills to work more independently. At three points during the program, as interns prepare research proposals, abstracts, and final research papers, advisors are required to review their student's written work and discuss their progress. The program director also monitors student progress and is available throughout the summer to address any concerns the students or advisors might have on a daily basis. At the end of the summer the students present the results of their work at a symposium attended by their advisors as well as by other staff.

On the basis of the experience of students from the last six summers, the intern program opens doors for other scientific opportunities. Several of the students have presented their work at national scientific meetings, several have coauthored papers on the research carried out during their internship, and a few have won recognition for their work. Lora Armstrong (2000) and Jean Li (2001) were a semi-finalist and finalist, respectively, in the Intel Science Talent Search, and last year intern Kevin Gan won a $50,000 scholarship from the Siemens Westinghouse Competition in Math, Science, and Technology. Nearly all of the students in our program expressed an interest during their internship in applying to graduate school in the sciences, and many are now pursing graduate degrees at top universities.

Participation in the NAI has provided exciting opportunities for our summer interns, expanding both the number of student applicants and the breadth of their experience. Training of interns in topics related to astrobiology — notably planetary formation, high-pressure methods, and organic chemistry — has played, and will continue to play, an important role in our team's mission within NAI. During the 2002 summer intern program 7 of the 20 interns pursued research topics of astrobiological interest, ranging from circumstellar disks and organic materials in meteorites to prebiotic chemical reactions and the preservation of biomarkers in fossil materials. To continue to involve interns in astrobiological research projects, we seek a modest contribution to the continuation of the summer intern program from NAI.

Predoctoral and Postdoctoral Fellow Programs

For more than half a century the Carnegie Institution has sponsored an internationally recognized program for predoctoral and postdoctoral fellows. A hallmark of these training programs at the Carnegie Institution's Broad Branch Road Campus is the close collaborative ties among staff scientists with backgrounds in astronomy, biology, chemistry, geology, and physics.

All fellows (as well as summer research interns) are encouraged to participate in the full range of campus activities, including seminars, workshops, group meetings, and collaborative research.

The Broad Branch Road campus of the Carnegie Institution currently supports approximately 15 predoctoral and 50 postdoctoral fellows (typically two- or three-year positions) annually. All positions are advertised internationally, and candidates are subject to a rigorous selection process. Prospective candidates are required to submit a research proposal and supporting documents. Most candidates who pass a first screening are invited to present a campus seminar prior to selection.

For each of the past five years, six Carnegie postdoctoral fellows have been engaged in research in some area of astrobiology and have been supported half time by NAI, with the remainder of their support provided by Carnegie Institution funds. We propose to raise that number to 7 postdoctoral fellows per year in years 2 and 3 of this project and 8 per year in years 4 and 5, with the same stipulation that each of these individuals would be supported in equal measure by NAI and the Carnegie Institution. NAI fellows are encouraged to spend a portion of their time at one or more of the partner institutions of our team or at an institution of one of the other NAI teams, and each will be expected to attend at least one NAI or related conference each year.

During the past five years, 17 individuals have been supported as NAI Fellows at the Carnegie Institution. While some of these individuals are still at a postdoctoral stage in their career, others have gone on to academic and research positions where they have initiated astrobiological research programs of their own. Three are Co-Investigators on this proposal. Those individuals, and their current positions, are

Jay Brandes	Assistant Professor, University of Texas (Co-I, this proposal)
Joakim Bebie	Now pursuing a career in art
Kenneth Chick	Research Staff, Dynamics Technology, Inc.
Steven Desch	Carnegie NAI Fellow
Andrew Dombard	Research Staff, Washington University
Timothy Filley	Assistant Professor, Purdue University
Nader Haghighipour	Carnegie NAI Fellow
Karl Kehm	Assistant Professor, Washington College
Michelle Minitti	NAI/NRC Postdoctoral Fellow, Arizona State University
Sujoy Mukhopadhyay	Carnegie NAI Fellow (will accept an Assistant Professor position at Harvard University this fall)
Larry Nittler	Staff Member, Carnegie Institution (Co-I, this proposal)
Eugenio Rivera	Carnegie NAI Fellow
Anurag Sharma	Carnegie NAI Fellow
James Scott	Staff Member, Carnegie Institution (Co-I, this proposal)
Sarah Stewart-Mukhopadhyay	Carnegie NAI Fellow (will accept an Assistant Professor position at Harvard University this fall)
Jan Toporski	Carnegie NAI Fellow
Susan Ziegler	Assistant Professor, University of Arkansas

We regard it as noteworthy as well that in the first three years of the NAI/National Research Council Postdoctoral Fellowship program, two of the 18 individuals awarded fellowships to date (Henry Scott and Michael Smoliar) have chosen to accept their fellowship at the Carnegie Institution. A third fellow (Michelle Minitti) was a Carnegie Fellow (supported half time by NAI) at the time of her selection, and a fourth (Kevin Boyce) is collaborating with members of the Carnegie NAI team in the course of his postdoctoral research.

7.4 Teaming with Minority Institutions

The Carnegie Institution is committed to expanding the involvement of individuals from underrepresented minorities in the science of astrobiology, from students at all levels of education to active scientific professionals. As described in **Section 7.1**, CIW is partnering with the Minority Institution Astrobiology Collaborative (MIAC) at Tennessee State University on research opportunities for minority faculty and students, on providing internships for minority students, on providing instructional support from the Carnegie Academy for Science Education for schools in underserved neighborhoods, and on providing development opportunities for Pre-K-12 teachers in such schools. MIAC will also serve as a partner with CIW on the development of a trade book on microbes for children that will feature astrobiological themes.

CIW was also one of three NAI institutions that hosted a participant in the NAI Minority Institution Faculty Sabbatical Program last year. Through that program, Prof. M. F. Mahmood from Howard University spent the summer of 2002 in the laboratory of Co-Investigator Andrew Steele. Prof. Mahmood, an expert on optical devices, worked on the application of advanced laser and microfluidic concepts for bioassays on Mars. The collaboration has been a fruitful one; Prof. Mahmood has continued his visits on a weekly basis since the summer, and he and Steele have identified several follow-on projects of mutual interest. A proposal naming Prof. Mahmood as a collaborator has been written, and discussions have been initiated on inviting Howard University graduate students to carry out a portion of their Ph.D. research working at the Carnegie Institution under the supervision of NAI Co-Investigators.

7.5 Staff

In the first five years of its membership in NAI, the Carnegie Institution has made a major commitment to astrobiological research through its appointment of new members of the Research Staff. On the same day that the initial selection of the first NAI teams was made, Wesley Huntress agreed to join the Carnegie Institution as Director of the Geophysical Laboratory (GL). In his previous position as the NASA Associate Administrator for Space Science, Huntress had fostered both the discipline of astrobiology and the creation of NAI. As GL Director one of his early goals was strengthening the department's staff in microbiological aspects of astrobiology. As a result, Andrew Steele and James Scott were recruited to the staff in 2001 and 2002, respectively.

At the Carnegie Institution's Department of Terrestrial Magnetism, the last four additions to the Research Staff are all now Co-Investigators on our NAI team. These include astronomers Paul Butler (who joined DTM in 1999), Alycia Weinberger (2001), and Sara Seager (2002), and cosmochemist Larry Nittler (2001).

The Carnegie Institution's Postdoctoral Fellow program constitutes a less direct contribution to staffing in astrobiology. In particular, a number of the individuals who have carried out research in astrobiology while at the Carnegie Institution now have permanent faculty or research positions where they are continuing to make scientific contributions to the field (**Section 7.3**). Carnegie staff members and Co-Investigators Nittler and Scott were formerly Postdoctoral Fellows supported in part by NAI. Two other graduates of the institution's postdoctoral program, Jay Brandes and James Farquhar, now have university faculty positions and are Co-Investigators on this proposal.

7.6 Facilities

The institutional partners of this consortium collectively offer an extraordinarily rich diversity of laboratories, analytical capabilities, and other facilities to the NASA Astrobiology

Institute. All of these facilities are currently in operation, and all will be open to the full membership of NAI.

Carnegie Institution of Washington

At the Carnegie Institution's Geophysical Laboratory, there are several well-equipped laboratories that will be utilized in support of NAI research. A Stable Isotope Laboratory has the capability to provide high-precision, high-accuracy stable isotopic analyses of a broad range of materials including gases, solutions, and solids and inorganic and organic materials. We support a Thermo-Finnigan MAT 252 Isotope Ratio Mass Spectrometer (IRMS) dedicated to the analysis of the three isotopes of oxygen; and a Finnegan Delta plus Excel IRMS with a variety of interfaces, including an elemental analyzer (EA), a thermal conductivity elemental analyzer (TCEA), and a gas chromatograph (GC). This instrument is capable of providing stable isotopic data for hydrogen, carbon, nitrogen, oxygen, and sulfur in both organic and inorganic solids. The fully automated EA and TCEA interfaces provide fast throughput analysis of bulk samples, the GC provides for compound specific analysis. We recently installed a new Thermo-Finnigan MAT 253 IRMS dedicated to simultaneous analysis of the four isotopes of sulfur (in the form of SF_6 derivatives). Our laboratory can accommodate both in-line and off-line fluorination chemistry, necessary to measure three isotope fractionations in the oxygen and sulfur systems. Toward this end we support a UV excimer laser — Lambda-Physik for use with KrF (248 nm) and ArF (193nm) fill gases — for *in situ* "spot" analysis and multiple CO_2 IR lasers for analysis of mineral chips and powders.

A Molecular Organic Analysis Laboratory is equipped with a broad range of analytical instrumentation for the analysis of complex organic materials. For the analysis of soluble organic compounds seven gas chromatographs are available with different injector and detector options. For example, we have two quadrupole mass spectrometer detectors with both electron impact (EI) and chemical ionization (CI) sources. Other detectors include nitrogen-phosphorus detectors, sulfur-selective electron-capture detectors, and numerous flame ionization detectors (FIDs) and thermal conductivity detectors (TCDs). Available injectors include standard split/splitless and temperature-programmable. We also have two pyroprobe interfaces for pyrolysis GC and GC-MS studies. For the analysis of non-derivatized or less volatile samples we have an HPLC with both UV-visible and fluorescence detection.

For the analysis of organic solids the laboratory is equipped with a three-channel solid-state nuclear magnet resonance (NMR) spectrometer (Varian-Chemagnetics CMX Infinity 300) bundled with three double resonance probes, ^{1}H and ^{19}F and X (^{15}N-^{31}P) maximum Magic Angle Spinning (MAS) speeds of 7, 12, and 30 kHz, respectively; one triple-resonance probe ^{1}H or ^{19}F and X + Y (^{15}N-^{31}P) with a maximum spinning speed of 18 kHz, and one single-resonance combined rotation and multipulse spectroscopy (CRAMPS) probe for ^{1}H or ^{19}F. Supporting these probes our NMR has one narrow-band high-power RF amplifier (^{1}H or ^{19}F) and two broad-band high-power RF amplifiers.

For the measurement of vibrational and optical properties of organic molecules and biological matter at high pressures, a custom-built laser micro-optical system for precise work in diamond anvil cells permits high-pressure Raman spectroscopic studies. This system contains Spectra Physics 165 and 171 ion lasers, a Spex 1877 spectrograph, a Dilor XY spectrograph, and a small spectrometer for blackbody and ruby fluorescence measurements (for pressure determination). We also have both a Fourier Transform Infra Red (FTIR) spectrometer as well as an FTIR microscope.

The Molecular Biology Laboratory includes a Surface Enhanced Laser Desorption Ionization-Time of Flight (SELDI-ToF) mass spectrometer (Ciphergen protein chip reader); a Genepix microarray reader, for both nucleic acid and protein arrays; and an Agilent bioanalyzer,

with DNA, RNA, and protein electrophoresis and flow cytometry. Also available are an Olympus BX61 fluorescence microscope with SIS image analysis software; PCR and real-time PCR; denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE); ELISA; single nuecleotide polymorphisms (SNP); and ATP luminometry analytical instrumentation. A portable PCR machine is available for field studies. Finally we also have stereo, biological, atomic force, and hand-held digital microscopes to support microbiological research.

The Carnegie Institution's Department of Terrestrial Magnetism (DTM) has several state-of-the-art instruments for trace element and high-precision isotope analysis. The DTM Cameca 6f ion microprobe is equipped with both oxygen and cesium primary ion sources and an electron flood gun for charge compensation. The 6f offers significant improvements in such areas as primary ion beam current densities, secondary ion transmission, and computer automation over earlier models. Among other applications for tasks described in this proposal, the ion microprobe will be used for *in situ* analysis of alteration features in meteorites to understand the conditions during alteration and the origin of the fluids. We are also developing a second high-transmission, multi-collector (5 faradays/multipliers) secondary beamline for the 6f using a large magnet from an accelerator mass spectrometer. It will have capabilities that are similar to or better than the Cameca 1270 ion microprobe. We expect to obtain our first data from the new beamline by June 2003.

A JEOL 6500F thermal field-emission scanning electron microscope (SEM) has been recently installed at CIW. This instrument allows secondary and backscatter electron imaging with a routine spatial resolution of better than 5 nm. Elemental mapping can be performed with a 0.5-•m spatial resolution using an energy-dispersive and a wavelength-dispersive spectrometer (EDS and WDS, respectively). The WDS is optimized for light elements providing a >10-fold increase in C count rates over EDS. Also, an electron backscatter diffraction camera and associated software allows crystallographic information to be obtained in the SEM.

DTM has two inductively-coupled plasma (ICP) mass spectrometers, a VG P54 and a VG Axiom. Both are used for high-precision isotopic analysis. The Axiom is now equipped with a multiple faraday/multiplier detector array, enabling analysis of samples with very large variations in isotopic abundances. The fast switching capability of the Axiom magnet means it can also be used for major/trace element analysis. However, we have recently acquired a VG PQ2 ICP that will be used exclusively for trace element analysis. We also have a CETAC LSX-200 UV laser-ablation system for *in situ* microanalyses of trace elements and isotopes. The department operates two thermal-ionization mass spectrometers for radiogenic isotope analysis. Also available are labs for mineral separation, as well as binocular and petrographic microscopes with digital image acquisition and processing.

The computing environment at DTM includes a wide range of workstations. The theoretical calculations of the DTM astronomy group are enabled by the Carnegie Alpha Cluster, a cluster of over 40 Alpha chip processors running Red Hat Linux. This cluster, supported in large part by the NSF, provides Co-I Boss and others with supercomputer-class computer power at a small fraction of the cost. A second cluster of 16 Intel Xeon 2.2 GHz machines is used to support orbital dynamics calculations. Two high-speed, broadband T1 lines to nearby NASA Goddard Space Flight Center connect the local DTM and GL Ethernet to the Internet.

The astrometric search for Solar System analogues by Co-Investigators Boss and Weinberger will utilize the Carnegie Institution's Las Campanas Observatory, located at an elevation of 7,200 feet in the Chilean Andes. NSF support and Carnegie matching funds have been requested in order to build a new astrometric camera for use at the Cassegrain focus of the DuPont 2.5-m telescope. With the new camera, the group expects to be able to achieve astrometric accuracies

of 0.25 milliarcseconds, sufficient for the detection with a signal-to-noise ratio of 4 of a Jupiter-analogue planet orbiting a solar-mass star 5 parsecs distant from the Sun.

American Type Culture Collection, George Mason University

The American Type Culture Collection (ATCC) is a private not-for-profit institution founded by scientists in 1925 to serve as a national repository and distribution center for cultures of microorganisms. Although the ATCC now takes on special contracts through its Applied Sciences Group and offers a variety of workshops, the principal business of the ATCC remains the acquisition, maintenance, and distribution of microorganisms and related biological materials in support of the scientific community's needs. The ATCC is on 26 acres adjacent to the George Mason University Prince William Campus. The custom-designed, 104,000 ft^2 headquarters building houses the administration, state-of-the-art microbiology laboratories, and an operations area. The operations area, where the actual collection resides, has a media preparation area and a preservation lab for lyophilization and programmed freezing of cultures. The culture repository provides 8,200 ft^2 of storage area, including a 2,500-ft^2 cold room for storage at 4°C, 55 ultra-low mechanical freezers (-70°C), and space for 65 vapor-phase liquid-nitrogen freezers (-170°C). All freezers are equipped with dual, independent temperature monitoring and recording systems. Both systems trigger facility alarms that are monitored on-site continuously and are backed by third-party, central station monitoring.

Co-Investigator David Emerson's 800-ft^2 laboratory is well-equipped for routine microbiological work, including centrifuges, biosafety cabinets, and incubators. He has complete facilities for doing anaerobic microbiology, including a COY anaerobic glove box. He also has an Olympus BX-60 epifluoresence microscope equipped with an Optronics CCD camera for low-light imaging. Other equipment in his lab includes an Applikon 3-l bioreactor, a gas chromatograph, a Bio-Rad Biologic HPLC system, a PCR machine, a gell electrophoresis apparatus and gel documentation systems, -80°C freezers and a liquid-N_2 storage tank, centrifuges, a walk-in cold room, a UV/visible spectrophotometer, peristaltic pumps and other equipment for development of microcosm studies, a French press, and a variety of incubators. In addition Emerson's lab has a variety of workstations with software that includes the complete Bionumerics suite, S-Plus, MacVector, and the GCG package.

State-of-the-art facilities exist on site for doing molecular biology and genomics. These include two ABI 377 DNA sequencers, multiple PCR machines, Diversilab lab-on-a-chip genotyping system using rep-PCR, Micromass-Waters MALDI-TOF-MS, a MIDI FAME system, a Biolog system for phenotypic characterization of bacteria, and a Qualicon Riboprinting system for genotyping of bacteria.

Naval Research Laboratory

The Naval Research Laboratory (NRL) has extensive facilities for electron microscopy studies, including two 300-kV conventional transmission electron microscopes (TEMs) and one 200-kV field-emission transmission electron microscope, a field emission scanning electron microscope, and a focused ion beam (FIB) instrument, as well the standard array of sample preparation tools, e.g., argon ion mill, dimpler, and metallurgical microscopes. The field-emission TEM is a high-resolution analytical instrument, with atomic-resolution Z-contrast imaging, sub-nanometer probe size for energy dispersive X-ray spectroscopy, and energy loss spectroscopy. It should remain an important facility for the analysis of returned samples over the next decade. Co-Investigator Stroud is in charge of the operation of this microscope. The FEI200 FIB at the NRL will be available for preparing thin membrane sections of interstellar dust particles, meteorites, and fossilized materials for *in situ* and *ex situ* characterization, as well as for extracting grains from ion or electron probe mounts after automated analysis. A new dual-

beam FIB, scheduled for purchase by NRL in 2004, will improve the speed and quality with which these sections can be prepared.

Smithsonian Institution

The combined facilities of the Smithsonian Institution's Laboratories of Analytical Biology encompass approximately 20,000 ft^2 in three Washington, D.C., locations: The genetics laboratory of the National Zoological Park, a large molecular biology and biochemistry facility in Suitland, Maryland, and a small multipurpose laboratory plus an SEM facility on the mall in the Natural History Building (NHB). As part of the NAI, the facilities unique to the Smithsonian at Suitland and the NHB will be available to NAI members on a cost-recovery basis. The facilities and equipment include complete pre-PCR facilities (separate from the PCR facilities) for sample extraction with all necessary grinding and extraction devices, centrifuges, 8-ft laminar flow hood, incubators, shakers and low-temperature storage. A fully-equipped PCR facility includes two MJ Research Tetrads, a Robocycler, multiple Perkin Elmer PCR units, an Opticon real-time PCR unit, multiple centrifuges, gel rigs, and an Eagle Eye unit for visualization. There is also a fully equipped dedicated cloning area containing a DNA sequencing service on an ABI 3100 for both fragment and sequence determination, sequencing analysis software, and multiple phylogenetic software packages.

A recently acquired state-of-the-art ToF SIMS IV has been installed in the Department of Mineral Sciences. It is equipped with a high-spatial-resolution (> 100 nm) liquid Ga ion source and a dual-source sputter column (Cs^+, Ar^+, and O_2^+). ToF-SIMS is capable of virtually simultaneous collection of all secondary ions of a given polarity, which allows us to image major, minor, and many trace element species in a specimen. Additionally, molecular species up to 10,000 AMU can be detected, allowing for localization of organic compounds on unprepared surfaces. Operated in a dual-ion mode, a sample can be imaged in 2D in one pass and then the surface sputtered in another. This allows for shallow depth profiling (100s of nm) and the creation of 3D secondary ion images of inorganic substrates.

A new full-spectrum imaging Energy Dispersive Spectrometer (EDS) system (Thermonoram Vantage 3) has been mounted on a conventional tungsten-source Scanning Electron Microscope (JEOL 840A). The solid-state detector processes X-ray pulses digitally, allowing for high-speed collection. Moreover, X-rays of all energies are collected at each electron beam position during mapping, which enables one to reconstruct energy spectra.

A LaB_6 FEI XL30 Environmental Scanning Electron Microscope (ESEM) located in the Laboratory for Analytical Biology is available to examine specimens in their native state (uncoated). Even fully hydrated samples can be imaged as the water pressures ranging up to 20 torr can be achieved. The instrument is equipped with several high-pressure gaseous secondary electron (GSE) detectors, a large area GSE for low-magnification imaging, as well as a solid-state backscattered electron detector.

An Electron Probe MicroAnalyzer (EPMA) is a five-wavelength spectrometer microprobe with a sixth integrated solid-state detector (JEOL 8900R). It is used routinely for quantitative analyses, stage X-ray imaging of large areas, and offline data reduction, and it has been kept under service contract since its installation 6 years ago. A cathodoluminescence (CL) microscope and spectrometer consists of a high-sensitivity true-color CCD camera (Optronics MagnaFire) mounted on a beam-regulated benchtop luminoscope (SpectruMedix ELM-3R). Light spectra (UV-VIS-NIR) are collected with a solid-state detector (Santa Barbara Group ST) and a McPherson 272 monachrometer. A new set of high-transmission long-working-distance lens have recently improved the luninoscope optics and allow for higher magnifications, which benefits both the imaging of fine CL features and the spectra collection.

The institution maintains several controlled-atmosphere experimental facilities, including two Deltech one-atmosphere, gas-mixing (CO-CO_2) furnaces with oxygen sensors for precise measurement of oxygen fugacity and programmable temperature controllers with maximum operating temperatures of ~1650°C. There is a vacuum oven for storage of starting materials.

The Smithsonian holds the National Collections of Meteorites, Rocks & Ores, and Minerals, all of which are invaluable resources for the study of prebiotic chemistry and mineral-microbe interactions. Co-Investigator McCoy is Curator-in-Charge of the Meteorite Collection.

University of California, Santa Cruz

Co-I Deamer's laboratory will make use of, and make available to other NAI investigators, a $50,000 Zeiss digital microscope to perform microscopic examination of self-assembled molecular systems and to record images. Additional instrumentation includes matrix assisted laser desorption ionization (MALDI)-ToF and electrospray mass spectrometry to analyze samples. Co-I Deamer plans to continue his collaboration with Richard Zare at Stanford University, whose laser-desorption laser-ionization mass spectrometer (L^2MS) instrument will be used to identify PAHs in Kamchatka samples.

University of Maryland

Laboratory facilities at the University of Maryland include wet chemical and gas-source mass spectrometry capabilities for analysis of $\delta^{17}O$, $\delta^{18}O$, $\delta^{33}S$, $\delta^{34}S$, and $\delta^{36}S$. A Finnigan MAT Delta Plus mass spectrometer with continuous-flow and dual inlet capabilities was installed in October 2001, and a four collector, oil-free Finnigan MAT 253 with continuous-flow and dual inlet capabilities was installed in June 2002. The Finnigan MAT 253 is used for high-precision measurements of $\Delta^{33}S$ and $\Delta^{36}S$.

The laboratory also has an Excimer Compex 110 UV laser and New Wave beam delivery system and two IR CO_2 lasers with home-made beam delivery systems. These lasers are used for fluorination of sulfur and oxygen compounds for isotopic analysis and also as light sources for photochemical experiments. In addition to the laser fluorination capabilities, there are extraction manifolds for on-line pyrolysis of oxygen-bearing compounds and on-line fluorination of silver sulfide. There is a 10-bomb reaction manifold that will allow for rapid throughput of silver sulfide samples that are difficult to handle with laser fluorination techniques. Experience with bomb fluorinations of silver sulfide and a Finnigan MAT 252 at UCSD indicates that accuracy and precision of $\Delta^{33}S$ and $\Delta^{36}S$ can be significantly better than +/- 0.02 and 0.3 per mil, respectively. The present system yields uncertainties significantly better than this (0.007 and 0.10 per mil, respectively).

Wet chemical facilities have been built for extraction and analysis of sulfur in trace sulfate, organic sulfur compounds, elemental sulfur, monosulfide, and disseminated pyrite. Facilities for oxygen analysis will include a focus on analysis of oxygen in salts, silicates, oxides, and organic compounds. The availability of these techniques will allow for a variety of analytical questions to be addressed that are different from the *in situ* and mineral separate work that is presently being undertaken at CIW.

The laboratory has a high-power deuterium lamp and grating monochrometer for photochemical experiments chosen specifically because it radiates at different wavelengths than the high-pressure mercury arc lamp at GL, and it could be used for complementary experiments. We have a solar UV chamber that would be available for other experiments that require UV. Maryland also houses two additional gas-source mass spectrometers (directed by J. Kaufman), two magnetic-sector ICP instruments with UV laser ablation capabilities at 213 nm and 293 nm

(directed by R. Ash and W. McDonough), two thermal-ionization mass spectrometers, clean-lab facilities (directed by R. Walker and P. Tomascak), and a JEOL EPMA (directed by P. Piccoli).

University of Texas

The University of Texas Marine Science Institute has a variety of instruments and facilities available for the proposed NAI study. Major instrumentation includes a ThermoFinnigan Delta Plus Isotope Ratio mass spectrometer coupled to a Carlo Erba NC2500 elemental analyzer sample-preparation unit available for analysis of up to 100 samples per day for C and N isotopic values. An additional compound-specific interface consisting of a TRACE GC, ThermoFinnigan MAT Precon unit, and GC PAL Autosampler provides the capability to analyze compounds from gasses (N_2, CH_4, CO_2, N_2O) to high-molecular-weight hydrocarbons, fatty acids, and amino acids (with derivitization). In addition a Waters/Hewlett-Packard gradient HPLC with UV/visible and fluorescent detectors is available for analyses of amino acids, amines, fatty acids, and other compounds.

University of Washington

Located in the Oceanography Research Building, three laboratories totaling approximately 1200 ft^2 of space are equipped with fume hoods, Milli-Q water, gas and air, and most of the standard equipment for culturing anaerobic hyperthermophiles and for routine biochemical and molecular biological work. One of the laboratories is a designated high-temperature, high-pressure facility. Shared equipment available in the School of Oceanography includes four cold rooms, three autoclaves, sonicators, lyophilizers, coulter counters, and a dark room. There is also a dedicated molecular biology laboratory in the College of Ocean and Fisheries Sciences equipped with DNA sequencers, PCR equipment, and a Pharmacia FPLC system for protein purification, and the university has facilities for electron microscopy, peptide synthesis, and protein microsequencing.

Major items of equipment available include a Packard Tri-carb 4000 scintillation counter, a Sorvall RC5C high-speed centrifuge, a Zeiss UEM photomicroscope equipped with epifluorencence capabilities, a Perkin Elmer UV/VIS LAMBDA 25 spectrometer equipped with a Peltier Temperature Programmer (PTP-6), a Forma Scientific anaerobic hood with a built-in anaerobic high-temperature incubator, five high-temperature oil baths, four constant high-temperature air incubators, a Varian 3400 gas chromatograph, an IBM LC/9533 HPLC, a Packard Picolite Luminometer for measuring bio- and chemi-luminescence, a Amicon RA2000 ultrafiltration system, three Eppendorf microcentrifuges, electrophoretic equipment, a PCR system, and an ultracold freezer. The pressure laboratory is equipped with 30 stainless-steel pressure vessels designed for use to 200°C and 0.2 GPa, a computer-controlled high-temperature/high-pressure incubator, and a rocking high-temperature/high-pressure incubator designed for subsampling during incubation without loss of pressure and temperature and for continuous culture studies.

Advanced Light Source

The Scanning Transmission X-ray Microscope (STXM) resides at beamline 5.3.2 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory. The instrument is dedicated to the analysis of organic polymers by X-ray Absorption Near Edge Spectro (XANES)-microscopy at the K edges of carbon, nitrogen, and oxygen. A bending magnet is used to provide a sufficiently brilliant X-ray beam (1×10^6 photons/second at the sample) in energy range spanning the range 150 to 600 eV. The nominal resolving power is 3000 across this energy range with the use of a spherical grating monochromator. X-ray focusing is achieved with a Fresnel-zone plate objective providing a 40-nm spot size.

7.7 Flight Missions

A number of the investigators on this proposal are involved in spacecraft missions, including flight missions, missions under development, and missions now in the planning stages.

PI Solomon is a member of the Mars Orbiting Laser Altimeter Team on the Mars Global Surveyor mission and is the Principal Investigator for the MESSENGER (MErcury Surface, Space ENvironment, GEochemistry, and Ranging) mission. The MESSENGER spacecraft, selected for flight under NASA's Discovery Program, is scheduled to launch in 2004 and will fly by Venus and Mercury twice each en route to one year of orbital observations at Mercury in 2009-2010.

Co-Is Alexander and Cody are developing two microanalytical techniques, XANES and STXM, for the analysis of organic matter in small samples, including the cometary particles that will be returned by the Stardust mission. Alexander and Co-I Hauri are also involved in the development of a large-radius, high-transmission, multicollector ion microprobe that will be used for the analysis of samples to be returned by the Stardust and Genesis missions.

Co-I Boss is on the Science Team for NASA's Kepler mission, scheduled for launch in 2007. Kepler's photometric observations will permit the detection of numerous Earth-like planets in Earth-like orbits around stars in the disk of our galaxy, offering the first estimate of the frequency of Earth-like planets. Boss is also a member of the Navigator Program Independent Review Team (NP IRT), which reports to NASA Headquarters and has oversight responsibility for all elements of the Navigator Program, including the Keck Interferometer, the Large Binocular Telescope Interferometer, the Space Interferometry Mission, and the Terrestrial Planet Finder (TPF). During 2003, Boss will chair a sub-committee of the NP IRT that will be charged with reviewing the Science Plan for the TPF mission.

Co-I Deamer has proposed, in collaboration with scientists at NASA Ames Research Center, the development of a particle detector and analyzer for a future Mars polar lander.

Co-I Farquhar has been involved in first-phase analytical development and testing of protocols for analysis of oxygen isotopes in diamond as a member of the Genesis Science Team.

Co-I Huntress is presently involved in a concept study for the Comet Sample Return mission under NASA's New Frontiers Program, and he is leading a proposal to NASA's Discovery Program for a Venus Orbiter and Probe mission.

Co-I McCoy is a collaborator on the Gamma Ray Spectrometer team on the Mars Odyssey mission, and he serves on a team preparing a proposal to NASA's Discovery Program for a mission to return a sample from 433 Eros. McCoy is also part of a science team for a neutron generator gamma-ray spectrometer designed for astrobiological prospecting. The instrument, currently under consideration for support by NASA's Astrobiology Science and Technology Instrument Development (ASTID) Program, is intended for the solicitation for the next rover mission to Mars.

The Carnegie Institution has recently purchased, with funding from NASA's Sample Return Laboratory Instrument and Data Analysis Program, a field-emission scanning electron microscope that will be used by Co-Is Nittler and Alexander to examine cometary and interstellar dust returned by the Stardust mission. Nittler and Alexander are also collaborating with Kenneth Grabowski at the Naval Research Laboratory to improve an accelerator mass spectrometer there to enable high-sensitivity elemental analyses of solar-wind particles returned by the Genesis mission.

Co-I Seager is a Co-Investigator for the Legacy Program and the Galactic Plane Survey on the Space Infrared Telescope Facility (SIRTF). The SIRTF mission is scheduled to launch in April. Seager is also a member of the Terrestrial Planet Finder Scientific Working Group and serves as a Support Scientist for the Microvariability and Oscillations of Stars (MOST) Microsatellite, to be launched by the Canadian Space Agency in June.

Co-I Steele serves as science team leader on a space flight concept to use biosensors to detect life, microbial contamination, and organic materials on Mars. Initially entitled Mars Immunoassay Life Detection Instrument (MILDI) and funded by NASA under the 2005 fundamental biology AO, the instrument concept has been renamed Modular Assays for Solar System Exploration (MASSE). This instrument utilizes technology currently available in laboratories to extract liquid samples, processes these samples, and inoculates both enzyme- and microarray-based detection systems. Positive reactions are monitored by a change in fluorescence. Current microarray technology allows the interrogation of up to 6 million probes in a space the size of a normal glass slide. Originally developed for the human genome project, this technology is being modified with minimal engineering to contain antibodies to a range of terrestrial compounds that indicate viable and fossil life, contamination, and prebiotic organic material and may be further tailored to look for exotic chemistries indicative of non-Earth-centric life. The instrument concept has been developed to a demonstration stage by collaborators in Spain, and Steele's group is currently designing a breadboard version containing a micro-fluidic sample handling system. The work is being carried out in collaboration with Johnson Space Center, Jet Propulsion Laboratory, Lockheed Martin, Montana State University, and the Centro de Astrobiología.

7.8 Information Technology

Background. A successful virtual institute needs not only technical expertise but also a clear understanding of how technology must be implemented to support the institution. By necessity NAI requires broad-band internet and state-of-the-art technology to access a wide range of distributed environments, including both traditional units where non-traditional work environments have been implemented and those that exist across organizational boundaries — such as distributed scientific teams, ad hoc project teams or focus groups of limited duration, and a super-group of inter-organizational teams and institutes. Virtual team members therefore can work across space, or organizational boundaries, or both. The needs of a true virtual institute can be met only with non-traditional work-environments, such as telecommuting, satellite work centers, and telemetry. These obvious goals notwithstanding, the history of information systems is laden with many expensive technological failures, mainly attributable to ignorance of social and scientific knowledge about the groups, organizations, and behavioral systems for which they were to be used. Currently CIW scientists participate actively in the virtual collaboration projects led by the Collaborative Research Support Group at NAI Central. These projects include the NAI member Needs Assessment Survey, the Collaborative Tools Comparison Study pilots, and NAI video seminar series. The IT point of contact for the CIW NAI team, Gotthard Sághi-Szabó, is an active member of the NAI IT Working Group, which meets periodically via videoconference to discuss issues associated with the development of the virtual institute. Sághi-Szabó has attended face-to-face IT meetings during a 5-day NAI IT conference held at Ames Research Center in April 2002, and he presented a "live" poster at the 2003 NAI General Meeting. The poster introduced the CIW astrobiology website (http://astrobiology.ciw.edu/), a collaborative and educational tool developed in-house by the CIW IT group. This group has also participated in the development of a website and collaboration tool for the Modular Assays for Solar System Exploration (MASSE) project and designed and built an online survey tool to support the decision-making process of the Mars Exploration Program Analysis Group (MEPAG) community (http://mepag.gl.ciw.edu/). Last year, the CIW IT group began to introduce social science methodologies to the analysis of social, psychological, and scientific

interaction among CIW team elements and team members as a model for the future development of the NAI virtual institute.

Proposed Work. As part of our proposed work, we will develop a comprehensive conceptual framework that has the necessary embedded constructs and variables to define properly teams and organizations, including virtual entities. This framework will be deployed for the study of the CIW astrobiology team and NAI overall as working examples of highly diverse virtual groups. The first question we seek to address is whether NAI and the virtual teams were correctly set up with traditional organizational practices in mind. In other words, does the current approach meet good management and organizational effectiveness criteria? As an integral part of the NAI institute we plan to study the critical components of a virtual institute, including organizational stability, security of membership, reward and recognition systems, clarity of objectives, directions, project plans, motivation and influence processes, leadership methods, the quality of personnel, team involvement and project visibility, stability of goals and priorities, built-in organizational control systems, conflict management, trust-building methods, decision making, and creative problem-solving processes. We will focus on devising mechanisms to prevent conformity, group-think, and negative organizational politics, all possible attributes of a highly connected virtual institute. We will consider means for increasing entity attractiveness and cohesiveness.

We further propose to analyze specific factors and challenges to virtual entities. The major challenges to virtual team effectiveness originate from the lack of face-to-face interactions, indirect communication, and coordination difficulties. Due to the limited time-span and intrinsic multi-disciplinary, cross-functional, cross-organizational nature of many interactions, virtual organizations may be unable to reach a “critical mass” of communication and information sharing that is fundamental to the group's or organization's success. Therefore, periodic face-to-face meetings are still necessary for teams involved in communication-intensive tasks. Virtual teams might also experience reduced effectiveness due to confusion arising from a lack of social context cues during electronic interaction, the difficulty of enforcing group norms and roles, and ambiguity in the communication process further amplified by physical-temporal distances and socio-cultural-technical differences. Communication and collaboration patterns and methods, trust relations, and managerial methods should be analyzed and optimized if the concept of the virtual institute is to be realized. An ethnographic approach (Twidale et al. 1994), wherein the behavior and attitudes of the team members can be observed in the context of their work, will be used to gather information during meetings and the scientific collaboration process. Electronic data and information exchange will be collected and analyzed using the Social Network Analysis approach (Wasserman & Faust 1994; Haythornthwaite 1996; Wellman 1988). This latter method focuses on patterns of relations among people and organizations and will provide us with invaluable results regarding the extent and effectiveness of our virtual entities. The overall measure of an entity's performance will be defined by the innovative ideas produced, goals accomplished, project outcomes achieved, adaptability to change, personnel and team commitment, and stakeholders' satisfaction.

Because of the limited time-span of virtual entities, and their multi-disciplinary, cross-functional, cross-organizational nature, traditional system design life cycles may be too slow to help groups to reach a “critical mass” of communication and information sharing. Time is obviously a critical factor for IT support. In order to accelerate the information system lifecycle process, we propose to investigate the feasibility of implementing modern system development and deployment methods, including disruptive technologies, prototyping, joint application design, nominal group techniques, rapid application deployment, and group support systems.

Information on the technological requirements associated with the varied activities of the virtual entities will be collected. We propose to test tools and methodologies that will best suit the needs of intra-, and inter-group communications, managerial, control, and administrative

functions, decision-support functions, scientific collaboration, problem solving, and knowledge management processes. The NAI Collaborative Research Support Group has identified several technical challenges, including hardware and software incompatibilities, differences in available network bandwidth, uneven access to collaboration tools, issues regarding ease of use, uneven local IT support, and differences in individual learning curves among participating scientists regarding this new technology. NAI Central has identified the most desired electronic tools by current NAI members, e.g., internet presentations and real-time meeting tools, desktop videoconferencing tools, document- and data-sharing tools, and knowledge management tools. This assessment clarified current NAI member user requirements with regard to available technology. Particularly important objectives recognized during this assessment include cross-platform compatibility, ease of use, desktop accessibility of tools, web-based access, high speed, reliability, security, and low cost.

CIW's IT team proposes to follow the important groundwork laid by NAI Central with a proactive strategy to try to match the growth in demand for software and hardware that can be used to manage and coordinate scientific projects, people, and activities. Apart from traditional information system support tools, such as websites, mailing lists, instant messaging, video conferencing, and teleconferencing, we are testing and developing collaborative *Knowledge Base*, *Incident-reporting* or *Issue-tracking*, *Bug Tracking*, *Resource & Asset Management*, and *Project Management* systems. Distance remains the principal barrier to managers and scientists in the virtual world. Carefully chosen information and communication technologies can play an instrumental role in helping managers and team members to "stay close" to each other. Within the CIW NAI team we hope to work with NAI Central to provide fast, geographically independent, secure access to diverse resources ranging from data warehousing to desktop sharing and to scientific instrument control using Virtual Private Networks (VPNs).

Computational collaboration has been enhanced by employing collaborative software development platforms with key features such as enhanced monitoring and reporting capabilities, improved source control and issue tracking integration, flexible workflow capabilities, as well as improved performance through database integration. Over the past five years the CIW IT team has gained significant experience with clustering, distributed computing, and large-scale massively parallel environments, and we recently started experimenting with spanning over Wide Area Networks (WAN), metacomputing systems, and computational grids. This summer, three NAI interns will work with Sághi-Szabó to explore the possible use of Extensible Markup Language (XML) and relational and object-oriented Data Base Management Systems (DBMSs) in scientific knowledge management, organization, and archiving.

7.9 Linkages to Other Agencies

While the NASA Astrobiology Institute provides the primary source of support for research in astrobiology at the Carnegie Institution and for most of the Co-Investigators at partner institutions, there are a number of programs funded by agencies other than NASA or by private sources that support either work on complementary research projects within astrobiology or laboratory instrumentation that will be utilized in NAI-supported measurements (see **Section 10**). Our team's astronomers, for instance, receive now or are currently requesting support from the National Science Foundation (NSF) for extrasolar planet searches (Boss, Butler, and Seager). Co-I Baross derives support from NSF for his oceanographic expeditions to mid-ocean ridge hydrothermal vent sites. Co-Is Emerson, Farquhar, and Hazen are receiving NSF support for research projects distinct from but related to those they are carrying out as part of NAI. The NSF has contributed as well to instrumentation in cosmochemistry, astronomy, and computation that will be utilized on NAI-supported projects. Many of those items of instrumentation were purchased in part with funds from private foundations and individual donors, as well as with institutional funds.

Indiana University
Bloomington, IN

Indiana-Princeton-Tennessee Astrobiology Institute (IPTAI): Detection of Biosustainable Energy and Nutrient Cycling in the Deep Subsurface of Earth and Mars

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EXECUTIVE SUMMARY

Our decision to seek membership in NASA's Astrobiology Institute is motivated by a desire to bring our expertise in deep subsurface ecosystems to bear on the scientific and technological difficulties that will be encountered during the exploration of life beneath the surface of Mars. Our center will be a consortium composed of senior level investigators representing Indiana University, Princeton University, University of Tennessee, Pacific Northwest National Laboratory, Oak Ridge National Laboratory, Lawrence Berkeley National laboratory, University of Toronto, and the Lunar and Planetary Institute. We believe our expertise in subsurface ecosystems and our access to extraordinary analytical facilities and field sites will enable development of synergistic relationships with other biological, geological and planetary research in the NAI.

Geochemists, chemists, microbiologists, and hydrologists on our team seek to collaboratively investigate physical and chemical limitations on life deep beneath the subsurface of Earth in order to design life detection methods for subsurface exploration of Mars. We have recovered indigenous microbes (Bacteria and Archaea) from ten- to hundred-million year old, highly saline fracture water at depths up to 3200 meters below ground in South African gold mines. Most of these microbes are sulfate reducing bacteria, but indigenous species utilizing other pathways of electron transfer can be detected by ribosomal sequencing. The magnitude of and ergonomic constraints on microbial activity and growth in these environments are poorly understood. The relationship between abiogenic chemical processes, such as radiolysis and Fischer-Tropsch reactions, and biogenic processes that sustain life remain largely enigmatic in the deep subsurface. The identity of specific genes that are critical to the survival of subsurface microorganisms inhabiting these environments has not been established. The evolutionary relationship of such genes to those of surface dwelling microorganisms is not known, but could provide clues at to whether microbial life on Earth originated in the subsurface. Life-forms in the subsurface of other planets presumably concentrate energy from geological sources like terran subsurface ecosystems, but their cellular machinery could be radically different. The fundamental elements and behavior common to all subsurface life forms need to be established in order to design life detection instruments for subsurface planetary probes.

A combination of field and laboratory experiments will be utilized to address the geochemical and genomic signatures of subsurface microbial ecosystems. In addition to continuing work in South African mines, an Arctic field site will be established where brine-containing fractures exist beneath a permafrost cap and provide an environment believed to be analogous to that of Mars. Levels of nutrients and metabolites will be quantified using stable-isotopic and molecular data on gaseous and aqueous species and on cellular and mineral materials collected from boreholes drilled into tunnel walls. Geochemical profiles from these boreholes will be combined with information on the genomic diversity in order to assess potential limiting factors for microbial survival. These results will be compared to results obtained from much warmer South African subsurface sites to determine elements common to microbial life at depths. The development of geophysical and chemical sensors for detecting life will be field tested at these sites.

Laboratory studies will make use of several strains of anaerobic Bacteria and Archaea from deep-subsurface sites including sulfate reducers, thiosulfate/sulfur disproportionators, Fe reducers, Mn reducers, and methanogens. We will design experiments to differentiate between microbial and abiogenic processes that control energy and nutrient cycles in the deep subsurface. These experiments will focus on process response to inferred environmental parameters in the

Martian subsurface, such as low water activity, high salinity, dessication, radiolysis, high CO_2 partial pressure, and clathrate formation. Based upon the results of these microbial experiments, we will develop and deploy borehole instruments to image fractures and biofilms, monitor the expression of genes, detect the presence of metabolites, and identify cellular activity.

The proposed research will address several goals identified in the Astrobiology Roadmap. Recovery of uncontaminated samples from deep-subsurface ecosystems on Earth is directly relevant to exploration for extant life in the subsurface of Mars, Objective 2.1 of Goal 2--Life in Our Solar System. We have accessed deep-subsurface ecosystems at depths greater than 3 kilometers below the ground surface in South African gold mines. The gold bearing conglomerates are late Archean in age and, consequently, characterization of the geochemical, lipid and isotopic signatures preserved in these rocks will enable us to contribute to Goal 4--Earth's Early Biosphere and its Environment. We will explore the evolution, environment and limit of life, Goal 5, by examining the community composition of subsurface ecosystems in different geochemical venues and by performing in situ experiments to see how the community evolves in response to environmental changes. Given high contents of uraninite in the gold-bearing conglomerates, our research is relevant to Objective 5.3--the determination of survival strategies that permit organisms to maintain viability in a radioactive environment for millennia. Finally, our previous research identified isotopic signatures that derive from the presence of subsurface bacterial life, Objective 7.1 of Goal 7--Signature of Life. Our future research plans will evaluate the preservation potential for these bio-signatures in clastic and chemical sediments analogous to regolith deposits on Mars.

Education and Public Outreach (EPO) activities in the proposed Indiana-Princeton-Tennessee Bio-Sustaining Cycles Team are designed around three areas of emphasis. First: educational workshops for undergraduates and high school teachers where participants actively collect and interpret data from laboratory and field experiments. Second: public outreach through a web site with premiere-quality digital media including animations and video that illustrate how and why scientists conduct microbiological research in extreme environments on Earth in preparation for exploration of Mars. Third: mentoring undergraduate and graduate research at Indiana, Princeton, and Tennessee universities. Inclusion of collaborators from the School of Fine Arts, IU Instructional Support Services, and University Information Technology Services at Indiana University is an unusual aspect of this proposal. High-resolution digital video/audio materials will be collected during field experiments and will be use in both research and educational components of the IPTAI. Videos produced by scientists will document research methods in a substantially different way from conventional commercial films. We hope to capture examples of both set-backs and advances in research resulting from unanticipated and challenging conditions in deep mines. Given severe time and logistical constraints in deep mines, digital documentation of physical conditions and instrumental configurations are essential for later interpretation of experimental results.

The proposed collaboration to study Bio-sustainable Energy and Nutrient Cycles in the Deep Sub-surface of Earth and Mars offers unusually high levels of institutional commitment from Indiana University, Princeton University, and the University of Tennessee. The total matching funds from these three institutions is in excess of $1,000,000. The match includes technical staff, graduate student stipends and tuition, academic salary, renovated space for an institutional office, and travel and supplies for E/PO.

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RESEARCH PLAN

INTRODUCTION

Our NAI would focus upon subsurface microbial communities that have been sequestered from the surface photosphere for tens to hundreds of millions of years and the environments that support their in situ activities. These terran ecosystems would represent the closest analogy to what might exist beneath the cryosphere of Mars. We seek to characterize the microbial, mineralogical and geochemical interactions, the isotopic signatures of the organic and inorganic gaseous, aqueous and metallic species, the interspecies and interkingdom communications and interactions, the genomic diversity and capabilities, the proteins expressed and their origin and the metabolites created and exchanged. This information will be used to design life detection approaches that will be tested in well-characterized field locations as a first step towards the design of flight-capable life detection instruments for future Mars drilling missions.

1. WATER ON MARS: A SUMMARY OF CURRENT THINKING

The search for water has been identified as the principal objective and common thread of Mars research – its abundance and distribution having important implications for understanding the geologic, hydrologic, and climatic evolution of the planet; the potential origin and continued survival of life; and the accessibility of a critical in-situ resource for sustaining future human explorers. Of the planet's estimated 0.5 to 1 km global inventory of H_2O, ~90-95% is thought to reside in the subsurface (as ground ice and groundwater), with the remainder stored as ice within the polar layered deposits (PLD).

Evidence for a water-rich Mars is provided by the geomorphic interpretation of a long list of landforms (e.g., (Carr and Schaber, 1977; Lucchitta, 1981; Rossbacher and Judson, 1981; Kuzmin, 1983; Carr, 1986)(Squyres, 1989; Squyres et al., 1992). In particular, it is supported by the existence of the outflow channels, whose distribution, size, and range of ages, suggests that a significant body of groundwater was present on Mars throughout much of its geologic history and may still persist to the present day (Baker, 1982; Carr, 1986; Tanaka, 1986; Tanaka and Scott, 1986; Baker et al., 1992).

Based on a conservative estimate of the discharge required to erode the outflow channels, and the likely extent of their original source region, Carr (Carr, 1996) estimates that Mars may possess a planetary inventory of water equivalent to a global ocean 0.5 to 1 km deep. Of this estimated inventory, ~0.000001% is found in the atmosphere, while ~5-10% is thought to be stored as ice in the perennial polar ice caps and layered deposits. This leaves ~90-95% of this H_2O unaccounted for, the vast bulk of which is believed to reside, as ground ice and groundwater, within the planet's crust.

Expected Nature and Location of Primary Subsurface Reservoirs of H_2O.

The expected distribution and state of subsurface water on Mars, as well as plausible values of the large-scale physical, thermal, and hydraulic properties of its crust, have been discussed by Rossbacher (Rossbacher and Judson, 1981), Clifford (Clifford, 1984, 1993), Squyres (Squyres et al., 1992), Carr (Carr, 1996) and Clifford (Clifford and Parker, 2001). To a first order, subsurface conditions on Mars are expected to resemble those found in cold-climate regions on Earth, particularly the unglaciated, continuous permafrost regions of Antarctica, Siberia, and North America. This similarity is likely to extend to an equivalent level of geologic complexity and spatial variability in such characteristics as lithology, structure, stratigraphy, porosity, volumetric ice content, and mechanical and thermal properties.

Current mean annual surface temperatures on Mars range from ~154°K at the poles to ~218°K at the equator (±5°K), with radiogenic heating expected to result in increasingly warmer temperatures at depth. Consideration of the current best estimates of both the planet's mean geothermal heat flux (~15-45 mW m^{-2}) and the freezing temperature of the most geochemically plausible compositions of groundwater (~252-273°K) suggest that the present depth of frozen ground on Mars, a region also known as the cryosphere (Rossbacher and Judson, 1981; Clifford and Hillel, 1983; Kuzmin, 1983), should vary from ~2.5 – 5 km at the equator to ~6.5 – 13 km at the poles (Fig. 1). However, natural variations in crustal

heat flow, thermal conductivity, and the presence of potent freezing-point depressing salts, are expected to result in significant local departures from these predicted zonally-averaged values (Clifford and Parker, 2001).

At the Martian surface, the low relative humidity of the atmosphere means that ground ice is thermodynamically unstable at latitudes equatorward of ~40° (Leighton and Murray, 1966; Fanale et al., 1986) resulting in its loss by sublimation at a rate that is dependent on the mean annual surface temperature, as well as the local thermal and diffusive properties of the crust (Smoluchowski, 1968; Clifford and Hillel, 1983; Fanale et al., 1986). Depending on the nature of these properties, their variation with depth, and the potential for replenishment from any deeper reservoir of subpermafrost groundwater, these factors may result in local depths of desiccation at low-latitudes that range from centimeters to as much as a kilometer with the potential for significant and complex variations in saturation state beneath the sublimation front (Clifford, 1998). Such uncertainties preclude any reliable theoretical or geomorphic prediction of the present distribution of subsurface H_2O below the seasonal skin-depth.

If the Martian inventory of H_2O exceeds what can be stored as ice within the pore volume of the cryosphere, then the bulk of the excess will be present as a liquid, saturating the lowermost porous regions of the crust (Clifford, 1993). Given a large-scale crustal permeability comparable to that of the Earth, and the lack of any recent rainfall, the influence of gravity should result in a present-day groundwater system that is in effective hydrostatic equilibrium – except where it may be locally perturbed by tectonic, seismic or thermal processes. Because of the low porosity expected at depth, comparatively little water is required to produce a groundwater system of substantial extent. Thus, if a subpermafrost groundwater system is present on Mars, it is expected to underlie much of the planet's surface.

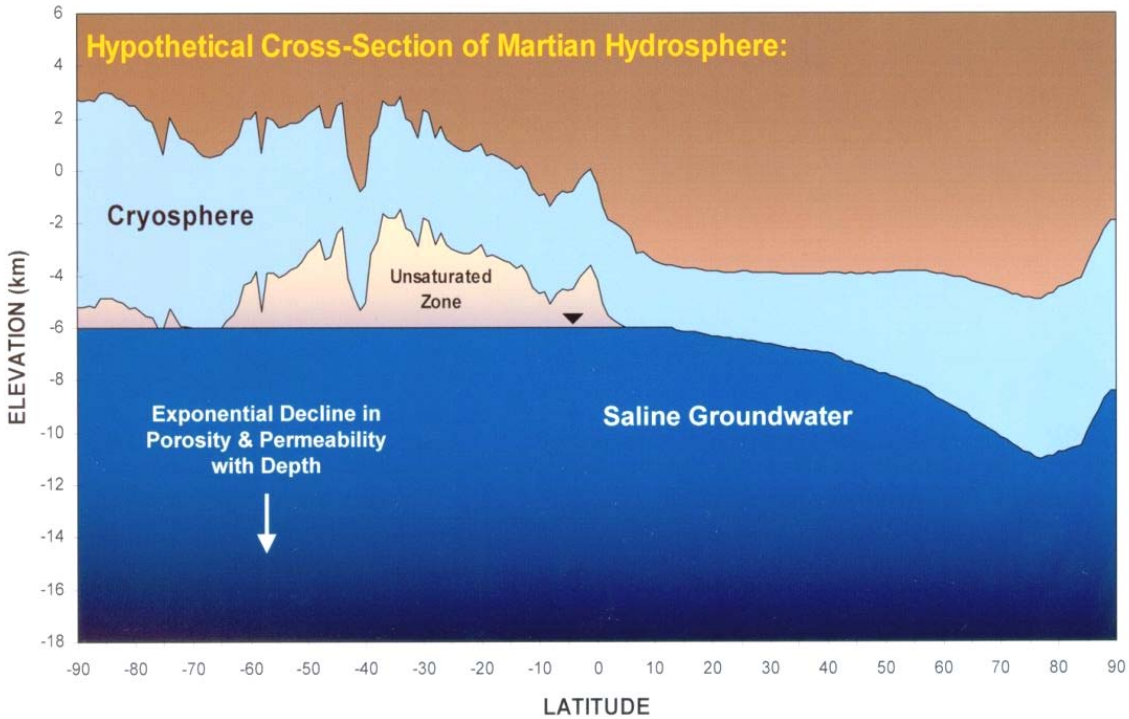


Fig. 1. Hypothetical, meridional, cross section of the Martian hydrosphere showing the distribution of ice, water and water vapor.

The distribution of ground ice is expected to follow the thermal structure of the crust, while the global groundwater table is expected to conform to a surface of constant geopotential. For this reason, the local vertical distance separating these subsurface reservoirs may vary considerably such that the intervening unsaturated zone is maximized in regions of high elevation and minimized (or absent) at lower elevations (Fig. 1). Within the unsaturated zone, the presence of a geothermal gradient is expected to give rise to a low-temperature hydrothermal convection system of rising vapor and descending liquid condensate. Such a system may have lead to the development of perched aquifers and the geochemical evolution of the underlying groundwater into a highly mineralized brine (Clifford, 1991, 1993). The liquid stability field of the brine encompasses much of the range in martian surface P-T conditions (Bodnar, 2001) and could be present just below a frozen duricrust.

Although there is evidence that Mars once possessed a sizable inventory of subpermafrost groundwater, it is also possible that such a inventory may no longer survive, outside of that which may be locally and transiently produced by the melting of ground ice from local geothermal activity. Such a state could well result from the progressive cold-trapping of a once large inventory of groundwater into the pore volume of the thickening cryosphere, as the planet's internal heat flow declined with time (Clifford and Parker, 2001).

The belief that any sizable body of liquid water on Mars must reside at depths of several kilometers or more (e.g., Figure 1) has recently been challenged by Malin and Edgett (Malin and Edgett, 2000), who have identified features in high-resolution Mars Orbiter Camera (MOC) images that they interpret as evidence of recent (and possibly contemporary) discharges of liquid water from near-surface aquifers (~100-500 m). However, given the enormous difficulty of reconciling the shallow aquifer hypothesis with both plausible environmental conditions and the need to explain the various enigmatic characteristics of the gullies (e.g., their restriction to mid- to high-latitudes and preferential occurrence on poleward-facing slopes), significant doubts have subsequently been raised about the uniqueness and plausibility of this interpretation.

Currently, there is only one explanation for the origin of the gullies that appears to satisfy the most serious environmental and observational constraints. The Martian obliquity is known to be chaotic on a time scale of ~10^7 years, varying from ~0° - 60° (Laskar and Robutel, 1993). For obliquities $\geq 45^o$, the peak insolation on poleward facing slopes at mid- to high-latitudes can yield summertime surface temperatures that easily exceed the melting point for continuous periods that range from hours to many months (Pathare and Paige, 1998; Costard et al., 2002; Paige, 2002). Under these conditions, large amounts of water ice are expected to sublime and melt from the summer polar ice cap – increasing the atmospheric vapor pressure of H_2O sufficiently to allow liquid water to flow readily across the surface. Under such conditions, formerly stable near-surface ice deposits could conceivably melt and produce sufficient run-off to form the gullies (Costard et al., 2002; Paige, 2002).

Potential Occurrence and Distribution of Gas Hydrates and Liquid CO_2.

In addition to the potential presence of such well-recognized volatile reservoirs as ground ice and groundwater, subsurface H_2O may also be present in the form of physical compounds of water ice and various gases, known as gas hydrates. Hydrates form when CO_2 and other gases (like CH_4 and H_2S), are concentrated under conditions of high pressure and low temperature in the presence of H_2O, where they can become stabilized by Van der Waals bonding within the cubic crystalline lattice of water ice molecules.

A number of investigators have argued that substantial amounts of CO_2 hydrate may be present in the Martian subsurface, most probably formed by the progressive cooling and freezing of CO_2-saturated groundwater (Miller and Smythe, 1970; Milton, 1974; Kargel et al., 2000; Komatsu et al., 2000). At 200^oK, CO_2 hydrate is stable at depths as shallow as ~5 m (corresponding to a confining pressure of ~50 kPa) and may remain so down to a maximum depth defined by the location of the 283^oK isotherm (Sloan, 1997). It is also possible that CH_4, has been produced on Mars by both biotic (Farmer, 1996; Fisk and

Giovannoni, 1999; Max and Clifford, 2000; Max and Clifford, 2001) and abiotic (Wallendahl and Treimann, 1999) processes resulting in local concentrations ranging from a dispersed contaminant, to massive deposits (Max and Clifford, 2000; Max and Clifford, 2001). The stability field of CH_4 hydrate is similar, but not identical, to that of its CO_2 counterpart extending from a depth as shallow as ~15 m (corresponding to a confining pressure of ~140 kPa) at 200°K, to a maximum depth of as much as much as a kilometer below the base of the cryosphere. As the internal heat flow of Mars has declined with time, the resulting downward propagation of the freezing-front at the base of the cryosphere may have incorporated both CO_2 and CH_4 hydrate in concentrations ranging from a dispersed contaminant, to massive deposits (Max and Clifford, 2000).

In addition to gas hydrates, several recent studies have argued that the pressure and temperature conditions expected in the Martian subsurface may also permit the stable existence of liquid CO_2 in amounts that may range from small inclusions to aquifer-like reservoirs that may have played a role in the genesis of the outflow channels (Hoffman, 2000). While it is possible to identify a P-T environment in the Martian subsurface where liquid CO_2 would be stable if emplaced after that environment had formed, it appears difficult to imagine an evolutionary scenario by which aquifers of liquid CO_2 could have initially formed and survived to the present day (Stewart and Nimmo, 2002). If, however, liquid CO_2 is currently present in the subsurface, it appears that it will most likely occur as inclusions and localized pockets.

In terms of subsurface martian ecosystems, therefore, three classes of environments exist; 1) the water ice and CO_2/CH_4 clathrate of the cryosphere with briny interstices; 2) a deep vadose zone dominated by either CO_2 or possibly CH_4 gas; and 3) a deep, saline, fractured aquifer potentially saturated with either CO_2 or CH_4 gas. These environments should be stable on the time frame of ~10^7 years, perturbed by changes in martian obliquity and the occasional meteorite impact. Our recent LExEn-supported research focuses on a terran subsurface ecosystem that is most analogous to the third class of martian environments.

2. BIOSUSTAINABLE DEEP SUBSURFACE TERRAN ECOSYSTEMS

For the past two decades an increasing number of microbiologists, molecular biologists, geochemists and mineralogists have been examining subsurface microbial ecosystems to understand their biodiversity, origins, impact on ground water chemistry and aquifer mineralogy and to remediate contaminated aquifers (Pedersen, 1993; Whitman et al., 1998). Only few of these studies have examined the microbial biomass and community structure at a depth greater than a few hundred meters primarily because of the lack of accessibility and the difficulties of contamination during drilling (Colwell et al., 1997). Most of these studies rely upon groundwater samples pumped from wells or a few cores (Phillips and Lappin-Scot, 1997; Onstott et al., 1998b).

Inferences about the subsurface microbial communities present in the ocean crust have been based in part on ODP sediment cores (Parkes et al., 1994), on observations and measurements of ocean ridge hydrothermal vents (Deming and Baross, 1993) and off axis vents and on fluids emanating from boreholes drilled into the ocean floor (Cowen et al., 2002). More recently, however, our group has been able to obtain saline water samples and rock samples from depths up to 3.3 kilometers below the surface in the deep mines in South Africa (Takai et al., 2001a; Takai et al., 2001b; Mormile et al., 2003). The attribute that sets this deep subsurface site apart from the other studies is that the saline water has been isolated for up to ~100 myrs. from the surface and are encased in highly impermeable, 2.9 Ga rock strata. Fluid flux in these environments are 10^4 times slower than those studied in the marine environment and any residual organic matter is confined to rare horizons and has been metamorphosed to lower greenschist faces. Yet, these isolated fluid-filled fractures yield intact cells with DNA suggesting that their survival has depended upon chemical energy sources and growth substrates tapped by a combination of gas/water/rock/microbial interactions. A number of recent studies have speculated that H_2 producing water-rock interactions may provide the energy for subsurface microbial metabolism deep in the earth's crust (Lilley et al., 2001) and may be possible terrestrial analogues for subsurface microbial ecosystems

under reducing conditions on other planets or moons (McCollum, 1999; Chapelle et al., 2002). But many questions still remain including:

- Would these communities, having survived for tens of millions of years, continue to survive *ad infinitum* barring a geological cataclysm? Barring the invention of time travel the only way to address this question is to begin studying the electron fluxes, genetic, lipid and protein composition of both the planktonic and sessile microbial communities trapped within these deep fractures.
- Are autotrophic and acetogenic microorganisms key marker species to these types of communities? Stevens and McKinley(Stevens and McKinley, 1995), reported the presences of these phenotypes at ~1 kmbls in the 14 Ma Columbia River Basalt aquifer. This observation combined with H_2 and $\delta^{13}C$ analyses led them to propose that oxidation of Fe-bearing minerals in the basalt generates H_2 that is consumed by methanogens and acetogens. The latter then provide an organic carbon substrate for heterotrophic microorganisms. Although this hypothesis, referred to as SLIME's, has been hotly debated (Anderson 1998), the fundamental assumptions still appear to be tenable (Chapelle et al., 2002) and the presence of methanogens and a gram-positive homoacetogen in a granitic aquifer at depths up to 420 m has been cited as evidence in support of this concept (Kotelnikova and Pedersen, 1997; Pedersen, 1997).
- Are these microorganisms still relying upon an organic nutrient source, tenuous though it may be, that was first formed photosynthetically in the Archean, deposited, metamorphosed and in some cases radiolytically altered? The extent to which heterotrophs in subsurface environments such as the Columbia River Basalt aquifer utilize acetate generated by homoacetogens or upon dissolved organic substrates exuded by the sediments inter-layered between the volcanic horizons was never established. With one notable exception, the heterotrophic members of subsurface microbial communities typically outnumber the autotrophs even within a Precambrian granite aquifer (Ekendahl and Pedersen, 1994). To answer this question will require detailed, compound specific ^{13}C analyses of the major organic species present and of the microorganisms themselves. One also has to look to the origins of other essential nutrients including N and P.
- If not, have these microorganisms developed mechanisms or a strategy for enhancing, manipulating and efficiently utilizing in situ chemical energy fluxes, sequestering key nutrients and energy polymers and efficiently repairing DNA, proteins and cell membranes so that they are truly independent of any surface biosphere? In terran marine and lake environments, photosynthetic autotrophic communities appear to mitigate the variations in C, N, and P such that they follow the Redfield ratios or the average C, N and P composition of the plankton. In subsurface ecosystems that have been isolated for geologic time intervals do similar ratios define covariation in C, N and P?
- Just how long can a single cell maintain its viability without dividing? Geochemical analyses combined with ^{14}C dating of inorganic carbon in groundwater and determination of the concentration of "viable" biomass from the Middendorf aquifer indicates cell turnover times on the order of thousands of years (Phelps et al., 1994). Based on the estimates for nM yr^{-1} methanogenic activity given below and on previous investigations by members of our team, it is clear that growth in the deep terrestrial subsurface is a very slow process with estimated doubling times for heterotrophic organisms in deep Atlantic coastal

plains sediments on the order of hundreds of years (Phelps et al., 1994). Estimates of respiration rates in ground water from this same environment range from 10 to 10^4 nM of CH_2O oxidized per year, depending on ground water age and electron acceptor (Murphy and Schramke, 1998). Geomicrobial investigations of a number of deep terrestrial environments with relatively low permeability and hydraulic conductivity suggest that they are inhabited by populations or descendants of populations that were present during the original depositional events millions to tens of millions ago (Fredrickson et al., 1995; Fredrickson et al., 1997). Thus, it is reasonable to hypothesize that most members of deep subsurface microbial communities are highly adapted to low nutrient conditions with very slow or infrequent cell division, and the utilization of energy sources for maintenance rather than cell division, are the norm. Although this finding is provocative, programmed cell death is not known for bacteria and hence no reason exists to preclude Methuselahian microorganisms. ^{14}C analyses of the microorganisms, lipids and dissolved organic matter will be required to directly tests this hypothesis, however.

The last question has been partially answered by the recent successful recovery and resuscitation of bacteria and Archaea trapped in salt crystals. The age of entrapment is ascertained from a combination of radiometric analyses and petrofabric relationships. The answer appears to be that at least in spore form, bacteria can survive trapped within crystals for 250 myr (Vreeland et al., 2000) and Archaea for at least 97 kyr (Mormile et al., 2003).

Long-term hydrological isolation

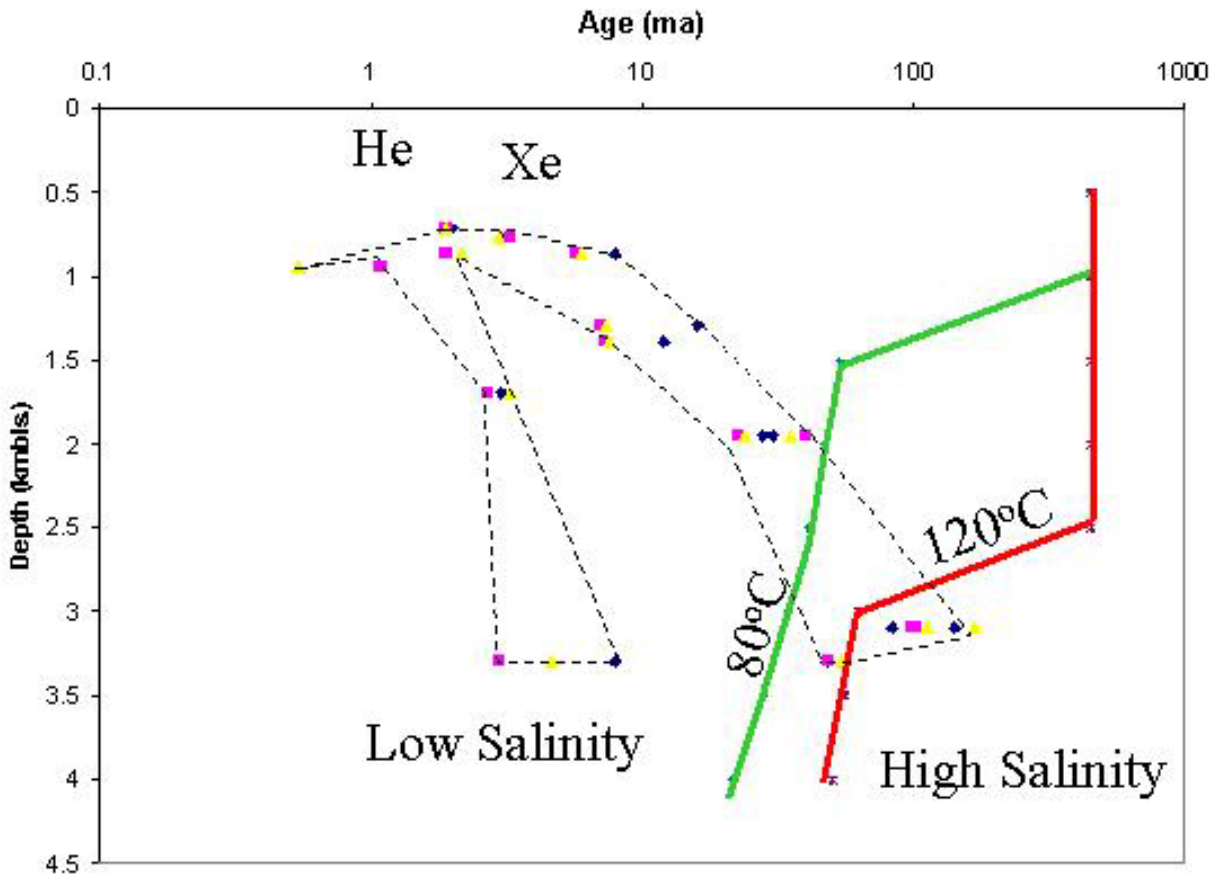
The highly saline fluid-filled fractures intersected in deep mines are arguably larger scale versions of mineral fluid inclusions, but more open to gas transport and storage, matrix diffusion and mineral surface reactions (Guha and Kanwar, 1987). Determining the age of entrapment, however, is far more difficult than in the case of mineral fluid inclusions and we have relied upon the following three approaches:

1. Fission track apatite dating-Because the partial annealing zone overlaps the maximum verifiable temperature of microorganisms (~<120°C) and the optimum growth temperature of many thermophiles (~>80°C) fission track apatite length distributions delineate the thermal history relevant to colonization of rock strata providing a *maximum* age for the indigenous community (Omar et al., 2003). As these age are younger at greater depths, the fission track apatite ages for rock strata at a few kilometers depth that have been the targets of microbial studies have ranged from 1.5 to 160 Ma (Tseng et al., 1995; Colwell et al., 1997). Much of the Precambrian/Paleozoic continental crust reflect the Mesozoic thermal overprinting or uplift associated with the breakup of Pangea with the exception of fission track apatite dates reported from the western Canadian Shield that range back to 650 Ma.

2. Noble gas isotope analyses-Because the saline fluids collected in South Africa yield $^{36}Cl/Cl$ values consistent with in situ nucleogenic production they are older than 1.5 myr. and their age beyond the reach of cosmogenic isotopes (Lippmann et al., 2003). Analyses of the $^{3}He/^{4}He$, $^{22}Ne/^{20}Ne$, $^{40}Ar/^{36}Ar$, $^{134}Xe/^{132}Xe$ and $^{136}Xe/^{132}Xe$ and the noble gas concentrations of the saline fluids and the pore waters in the rock strata and the chemical composition and porosity of the rocks can determine the flux of the radiogenic noble gases from their to the fluid filled fracture and the total subsurface residence time and unlike cosmogenic isotopes has no maximum age limit (Andrews, 1987; Andrews et al., 1989). The subsurface residence time represents a *minimum* age for the microbial communities since microorganisms strongly adhere to the mineral surfaces given the high ionic strength of the saline fluids and their migration through the crust should be strongly retarded with respect to that of the water.

3. $\delta^{18}O$ and $\delta^{2}H$ of H_2O and fracture minerals-The $\delta^{18}O$ and $\delta^{2}H$ of the water determines both its origin and its pathway, e.g. whether it has interacted with rock at low or high temperatures. In the case of low water/rock the $\delta^{18}O$ and $\delta^{2}H$ can determine how much fluid has been lost to the formation of hydrous minerals and how much mineral dissolution has occurred (Pearson, 1987). They provide valuable checks to the noble gas model ages in method 2. The $\delta^{18}O$ of the fracture minerals in conjunction with fluid inclusion analyses can determine the $\delta^{18}O$ of the fluid from which the mineral precipitated and the when tied to the fission track thermal history provides an additional constraint on the age and origin of the fluid and hence the origin of the microbial community.

In the case of the deep subsurface of South Africa the thermal history derived from fission track apatite length distributions (Omar et al., 2003) and noble gas isotopic analyses (Lippmann et al., 2003) constrain the maximum and minimum ages for microbial occupation and isolation as a function of depth

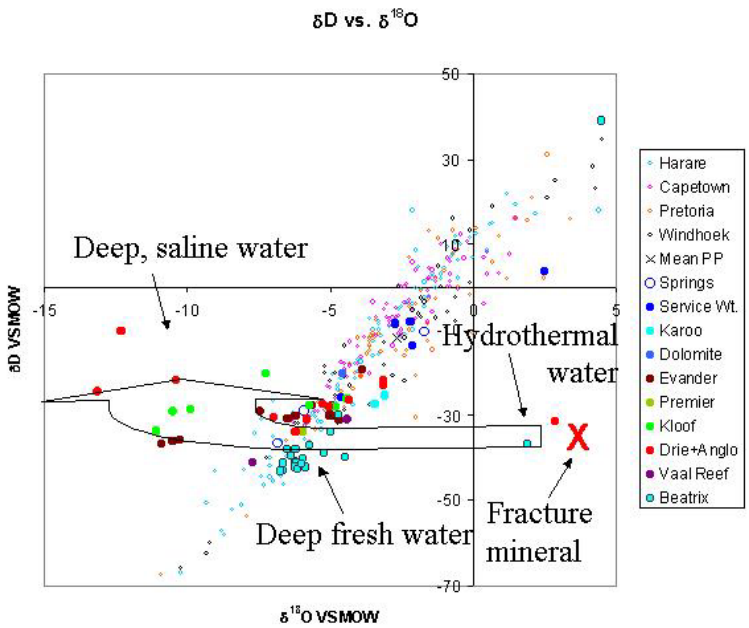


(Fig. 2).

Fig. 2. He and Xe ages for fissure water and 80°C and 120°C thermochrons as a function of depth for Witwatersrand basin, S. Africa. The thermochrons are based upon fission track length distribution data from a 3.7 kmbls apatite-bearing sample.

The maximum and minimum age estimates converge at a depth of ~3 kmbls. indicating that our deepest sites were quickly colonized following cooling below 120°C. Isotopic analyses of fracture coating calcite when compared to the $\delta^{18}O$ of the saline fluid indicates that the saline fluid must have changed its isotopic composition during this time either by flushing or by mineral/water exchange during slow cooling. Given that the present day fluid is distinctly non-meteoric (Fig. 3) the latter explanation is more likely, but requires a more detailed examination of the fracture minerals.

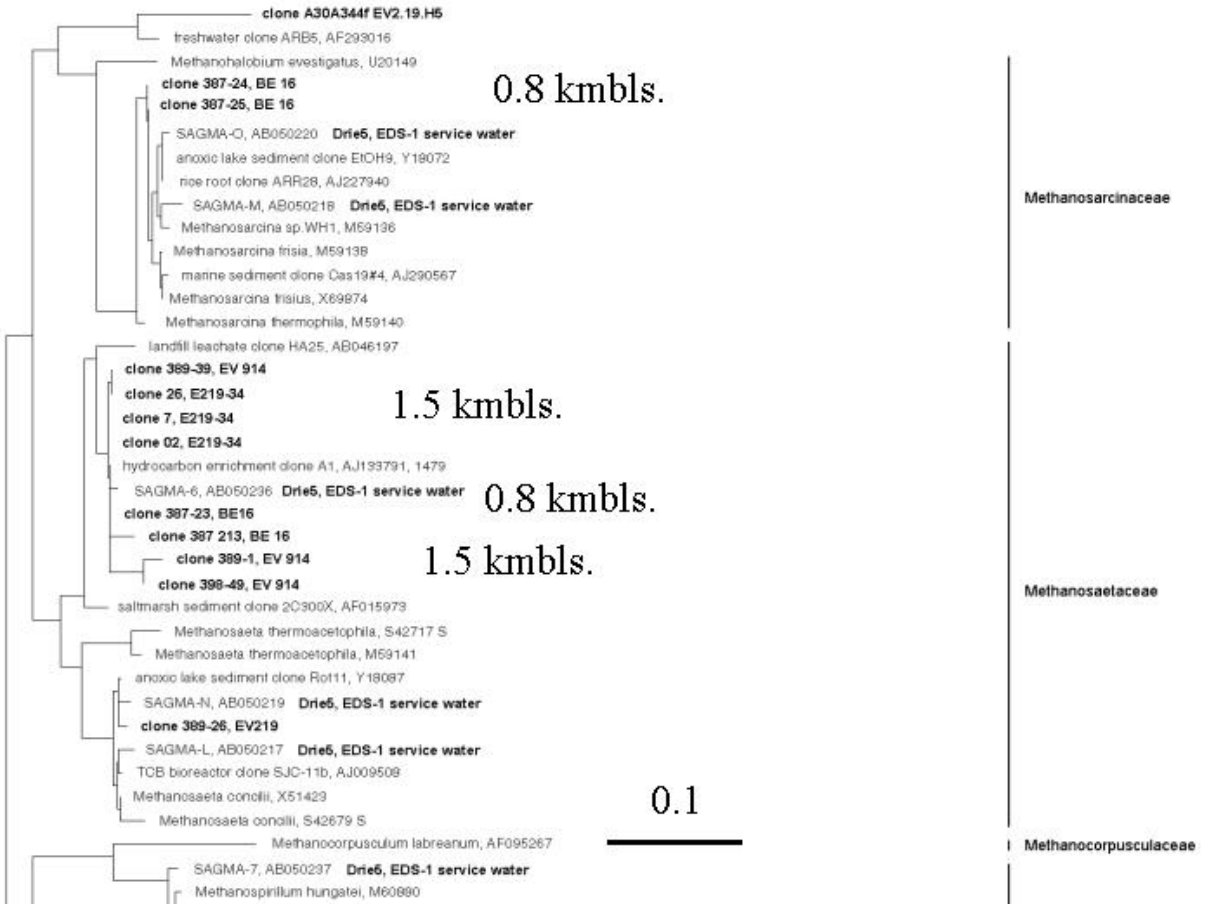
Fig. 3. $\delta^{2}H$ versus $\delta^{18}O$ of fissure water from various mines within the Witwatersrand Basin compared to precipitation, surface water and hot springs and carbonate crystals located along a fracture zone.



Autotrophs versus Heterotrophs-16S rDNA data

The identity of the microbial occupants on fluid-filled fractures has been determined by sequencing of the 16S rDNA gene of DNA extracted from filtered water samples and from thermophilic and mesophilic microbial enrichments (Takai et al., 2001a; Takai et al., 2001b; Moser et al., 2003; Onstott et al., 2003). Members of the bacterial and archaeal kingdoms are present and the similarity of some of the sequences to those in the RDP database for microorganisms with know physiology has permitted the identification of some of the potential metabolic processes in the deep subsurface. Methanogens have been detected in fracture fluids from depths up to 1.6 kmbls. and from the circulation water used for mining, service water, (Fig. 4), but appear to be absent from the deeper, hotter and more saline fracture fluids. A distinct cluster of Crenarchaeota have been detected in the service water and in water samples of the ~1 km thick, 2.5 Ga dolomitic aquifer that caps the Au-bearing formations. Sequences similar to those found within this clade have just been reported for Fe and Mn oxyhydroxide crusts in Lechuguilla cave (Northrum pers. comm. 2002). S and thiosulfate oxidizing autotrophs are also associated with the dolomite water, but are absent in the deeper saline environment. Of the 16S rDNA sequences recovered from the latter environments, the ones that are similar to the database match dissimilatory sulfate-reducing bacteria, SRB's, in the δ-Proteobacteria and gram positive (or Firmicutes) divisions (Fig. 5 and 6), members of the genus Thermus and Clostridia, potential thermophilic and hyperthermophilic Euryarchaeota (Fig. 7). Quantitative PCR indicates that the archaeal portion of the microbial community is minor compared to the bacterial portion. Some of the sequences associated with the genus Clostridia are suggestive of thermophilic homoacetogens (Fig. 8), but over 90% of the ~500 16S rDNA sequences recovered to date are novel and are too dissimilar from those in the database to unambiguously infer their environmental significance. In some cases, the metabolic significance of a novel 16S rDNA is inferred from the media used to enrich the

microorganism, as in the case of the gram positive DR504, which grew up in a Fe(III) reducing media



with only CH_4 compounds as electron donors (Fig. 8).

Fig. 4. Euryarcheota divisional tree showing positions of clades associated with Methanogens and clones related to hydrothermal vents rDNA sequences.

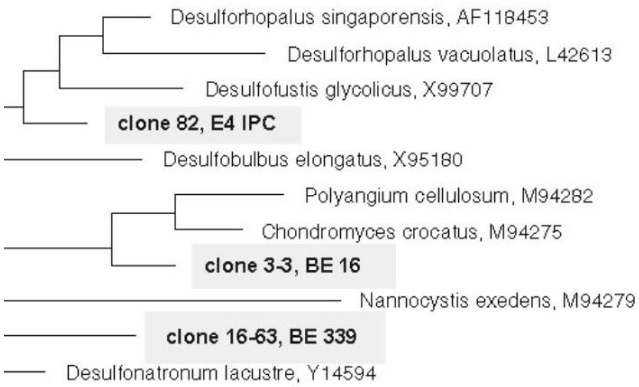


Fig. 5. δ Proteobacteria division, sulfate-reducing bacteria.

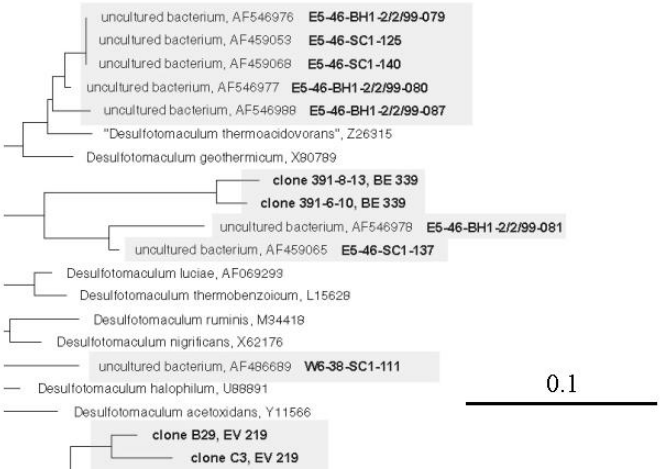


Fig. 6. Firmicutes or gram positive phyla.

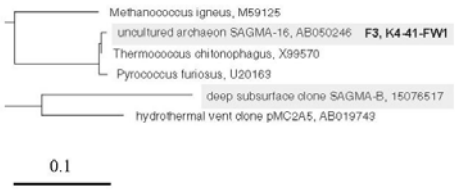


Fig. 7. Hyperthermophilic Euryarchaeota from 3.2 kmbls. (Takai et al., 2000)

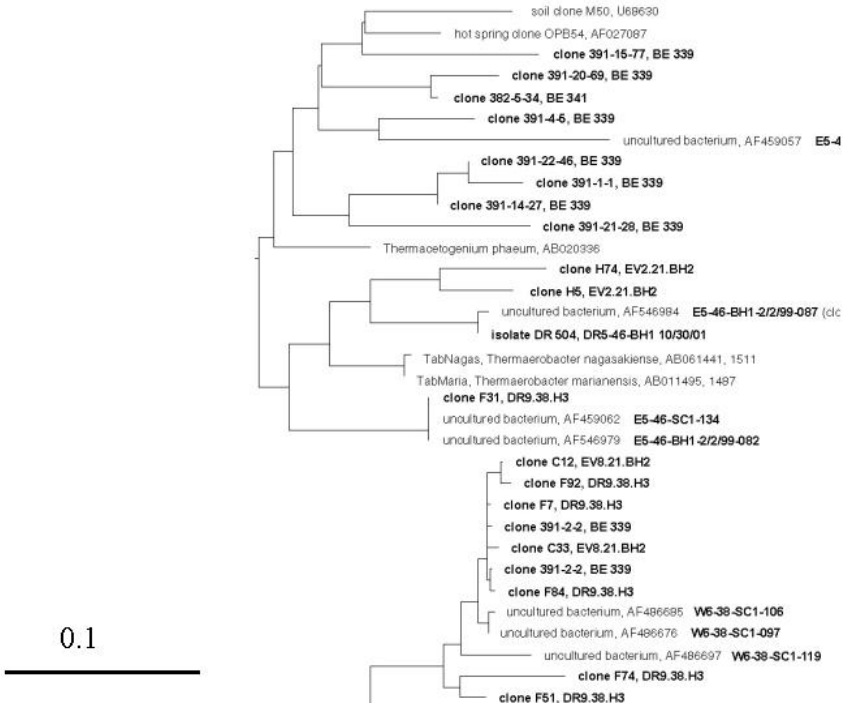


Fig. 8. Novel thermophilic? Firmicutes, including DR504 which was enriched at 50°C with CH_4 as the electron donor and Fe(III) oxide as the electron acceptor.

Autotrophs versus Heterotrophs-Stable Isotope Signatures

Because so many of the 16S rDNA sequences form unique clades, determination of whether or not autotrophs or heterotrophs dominate the communities relies upon stable isotope analyses of the dissolved gases and aqueous species. The $\delta^{13}C$ and $\delta^{2}H$ of the C_{1-4} compounds from the deeper, saline environments are similar to those reported for Canadian and Scandinavian shield brines and distinct from those of thermogenic hydrocarbons (Sherwood-Lollar et al., 1993; Sherwood Lollar et al., 2002). The Canadian Shield rock strata, a 2.7 Ga metamorphosed ultramafic/mafic hydrothermal complex, are distinct from the 3.0 Ga siliclastic and basaltic andesite strata of the Wits basin, yet the reaction mechanisms responsible for formation of the large quantities hydrocarbons and H_2 gas (up to 50% by volume with flow rates of 1-30 L gas/minute) appear similar.

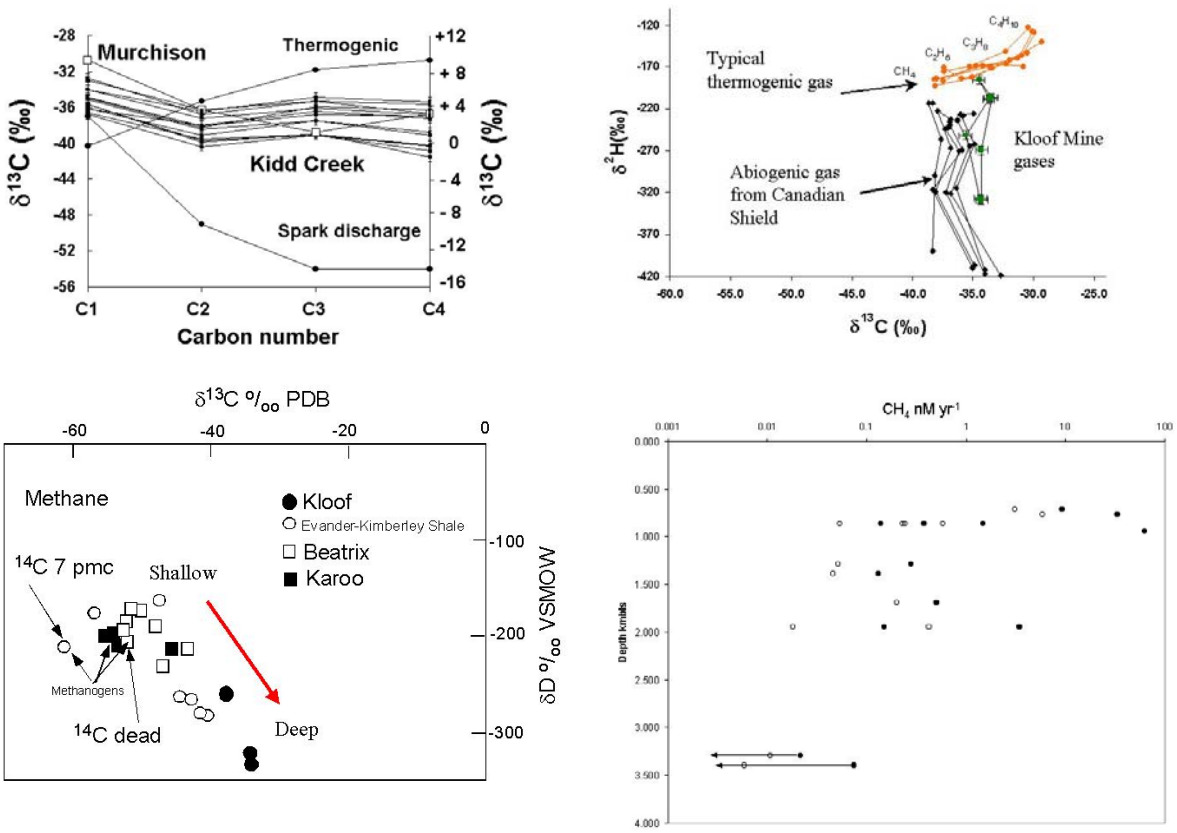


Fig. 9. From top left to lower right. a) The $\delta^{13}C$ for C_{1-4} compounds from Canadian Shield versus C number compared to those reported for the Murchison meteorite and hydrothermal experiments. b) δD and $\delta^{13}C$ for C_{1-4} compounds from Canadian Shield and South African brines. c) δD and $\delta^{13}C$ for CH_4 from South African fissure water. ^{14}C values reported for biogenic CH_4. d) The methane production rate as a function of depth in South Africa.

Sherwood Lollar et al. (Sherwood Lollar et al., 2002) demonstrated that the abiogenic reactions that may have generated prebiotic organic molecules in the early earth and on other planets may be the same reactions involved in the production of hydrocarbons in Precambrian Shield crystalline rocks. The most distinctive feature of these gases is the unusual pattern of ^{13}C values between the C_1-C_4 alkanes. Thermogenic hydrocarbons have been shown empirically and experimentally to have a characteristic

isotope distribution pattern whereby the C_{1-4} alkanes become more enriched in ^{13}C (less negative $\delta^{13}C$ values) with increasing molecular weight. This orderly isotopic distribution results from kinetic fractionation effects whereby alkyl groups separating from source organic matter cleave preferentially at weaker ^{12}C-^{12}C rather than ^{12}C-^{13}C bonds (Des Marais et al., 1981). In contrast, the Canadian and South Africa C_1-C_4 alkanes reveal a significant depletion in ^{13}C for C_2-C_4 with respect to C_1. No thermogenic or bacterial formation reaction or post-genetic alteration process is known to produce such a pattern (Fig. 9a).

Such a pattern can be generated, however, when hydrocarbons are produced from CH_4 in an abiogenic reaction. This was first demonstrated in a spark discharge experiment (Des Marais et al., 1981). The isotope pattern results during kinetically controlled synthesis of higher molecular weight homologues from lower ones due to the fact that $^{12}CH_4$ reacts faster than $^{13}CH_4$ to form chains, so that ^{12}C is more likely to be incorporated into larger hydrocarbon chains. The Canadian Shield brines were the first reported terrestrial samples to demonstrate this consistent depletion of C_2-C_4 with respect to C_1. Only values of $\delta^{13}C$ obtained for C_2-C_4 n-alkanes for the Murchison meteorite yield a similar pattern, also attributed to formation by abiotic polymerization reactions (Fig. 9a; Yuen et al., 1990). The absolute $\delta^{13}C$ values of the hydrocarbons from the Murchison are significantly more enriched in ^{13}C than the Canadian brines, reflecting their extraterrestrial origin. Nonetheless, the Murchison meteorite and the Canadian brines are remarkably similar in the isotopic pattern of the C_1-C_4 n-alkanes (Fig. 9a).

The δ^2H values for the C_1-C_4 gases provide additional support for an abiogenic origin. The distribution of $\delta^{13}C$ and δ^2H values is distinctly different than for thermogenic gas (Fig. 9b), and the inverse correlation of $\delta^{13}C$ and δ^2H values between C_1 and C_2 supports the participation of these compounds in an abiogenic polymerization reaction. (Des Marais et al., 1981) proposed that in a kinetically controlled synthesis of higher molecular weight homologues from lower ones, the lighter isotope (^{12}C) will react faster than the heavy isotope (^{13}C) to form a 2-carbon chain and the resulting C_2H_6 would be depleted in ^{13}C versus the CH_4 precursor. The reaction will also proceed more rapidly if the weaker ^{12}C-^{1}H bond versus the ^{12}C-^{2}H bond is cleaved, thus preferentially eliminating the lighter ^{1}H. The C_2H_6 should be isotopically enriched in ^{2}H and isotopically depleted in ^{13}C with respect to the CH_4 precursor as observed in Canadian and South African brine samples.

The $\delta^{13}C$ and δ^2H of the CH_4 (Fig. 9c) and the C_{2-4} compounds reveal that the CH_4 from the environments < 2.0 kmbls. does appear to have been produced by methanogens, many of which appear to be aceticlastic (Gelwicks et al., 1994), not CO_2 reducing autotrophs (Fuchs et al., 1979) on the basis of the 16SrDNA (Fig. 4). The biogenic CH_4 has no detectable ^{14}C, with one exception, suggesting that the source acetate is > 50,000 years old. The proportion of this biogenic CH_4 decreases with depth and based upon the Xe age of the fissure water (Fig. 2) the biogenic CH_4 production rated decrease exponentially with depth from 10 nM yr^{-1} at 0.6 kmbls to <0.01 nM yr^{-1} at 3.2 kmbls. Based upon estimated methanogenic biomass in the fissure water of these samples by flow cytometry and qPCR, these rates correspond to a cell turnover time a few decades at 0.6 kmbls. to hundreds of years at 2.0 kmbls.

The $\delta^{13}C$ of the dissolved inorganic carbon, DIC, doesn't follow the enrichment trend reported by (Stevens and McKinley, 1995) for the Columbia River Basalt aquifer and that expected for an autotrophic dominated environment, but instead yields isotopically light values more consistent with heterotrophic respiration of dissolved organic matter (Fig. 10). The S isotopes suggest that one of the principal electron acceptors to this respiration is sulfate. The difference in the $\delta^{34}S$ of sulfate and sulfide from the deep saline water is consistent with that produced by microbial fractionation by SRB's in lab cultures (Fig. 11)(Kemp and Thode, 1968). This fractionation is present in saline water with a sulfate concentration of 50 μM, lower than has yet to be reported for lab cultures and suggestive of very slow sulfate reduction rates

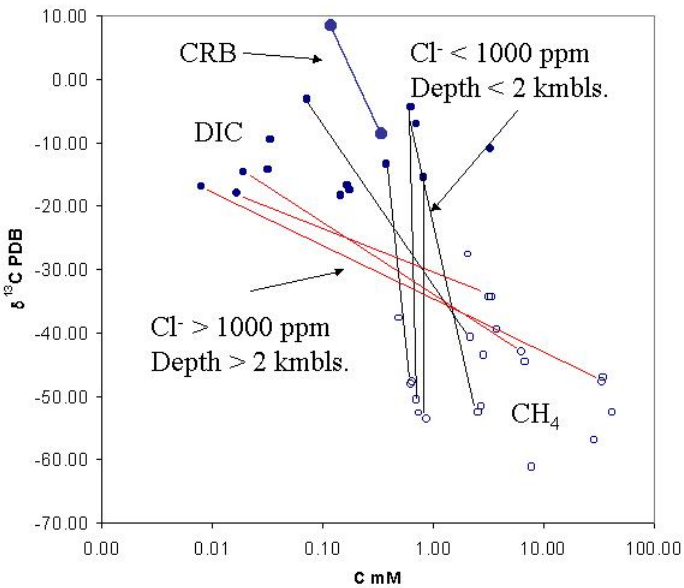


Fig. 10. $\delta^{13}C$ of CH_4 and DIC for South African fissure water and $\delta^{13}C$ of DIC Columbia River Basalt aquifer (Stevens and McKinley, 1995).

(Harrison and Thode, 1957). Dissimilatory sulfite reductase, DSR, genes recovered from two saline water samples yield some sequences that closely align with those recovered from the Columbia River Basalt aquifer and other sequences that are unique and deeply rooted in the DSR tree (Fig. 12; Baker et al., 2003). This may indicate that some SRB carry reductive enzymes that are uniquely adapted to deep subsurface environments, but until these enzymes are characterized this remains speculation. The acetate, sulfate and sulfide concentrations yield a free energy of ~28 kJ/mole sulfate, sufficient to support the formation of ATP (Schink, 1997).

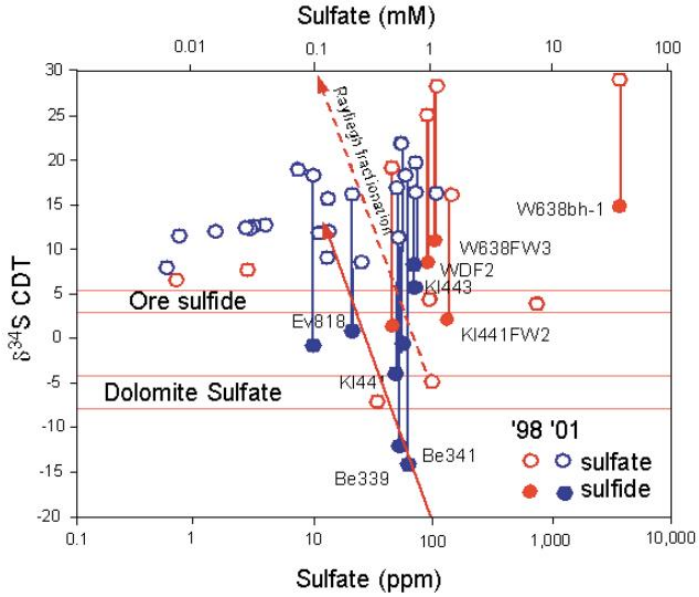


Fig. 11. $\delta^{34}S$ of sulfate and sulfide from fissure water in Witwatersrand Basin, S. Africa.

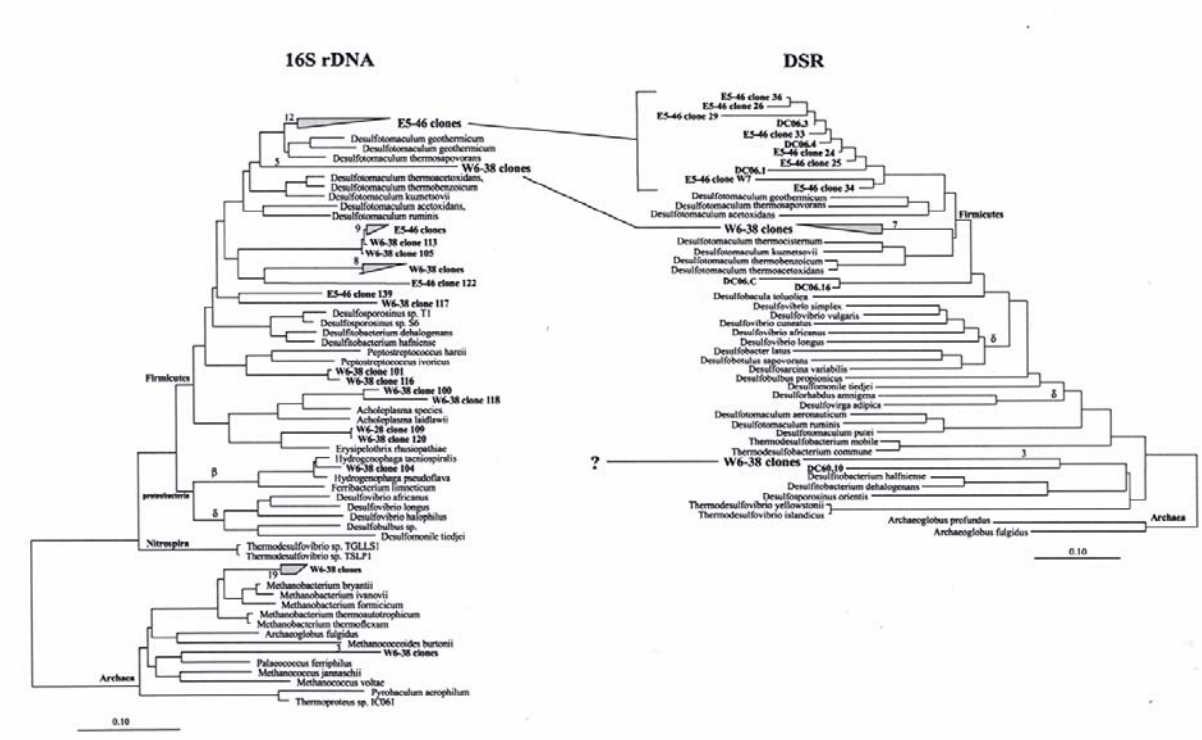
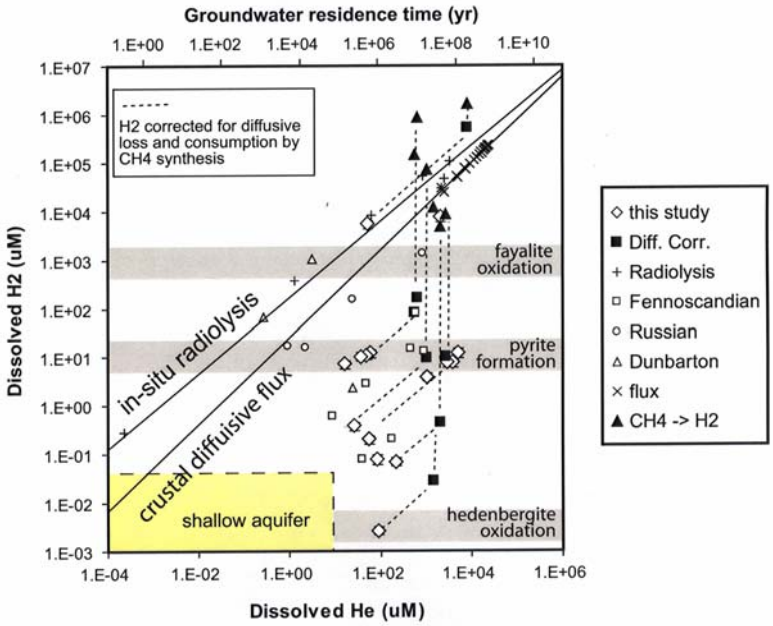


Fig. 12. 16SrDNA (left) and DSR (right) tree for two deep saline water samples in the Witwatersrand Basin, S. Africa.

The H_2, sulfate and sulfide concentrations yield a greater free energy, ~56 kJ/mole sulfate, suggesting that most of the electron flow is generated from H_2. The H_2 concentrations in the South African fissures span 6 orders of magnitude and are as high as 1 mM (Fig. 13)[1]. The lack of appropriate Fe-bearing



[1]Fig. 13. H_2 vs. He for fissure water from South Africa, Fennoscandian shield, Ukranian Shield and North American Triassic basin. Dashed line represents predicted radiolytic yield as a function of time and He concentration.

mineral phases and thermodynamic calculations indicate that this H_2 cannot result from water-rock interactions (horizontal bars in Fig. 13). Measurements of the TOC and carboxylic acid concentrations indicate that the Gibbs free energies of fermentation reactions are positive for all samples with the exception of one, so fermentation cannot explain the high H_2. Nor are ultramafic rock present in the lower crust underlying the Wits basin as can been seen in the crustal section exposed ~40 km south in the Vredefort impact structure. Given the concentrations of radiogenic elements in the strata, the porosity and the ^{4}He residence times (Fig. 2), the calculated H_2 concentration agrees with that of the highest measured values (dashed line in Fig. 13). The mostly likely source of the H_2, therefore, is radiolytic reactions (dashed line in Fig. 13; Lin et al., 2003b). Furthermore, if one assumes that the abiogenic CH_4 utilized radiolytic H_2 and correct for this loss, then many more of the corrected H_2 concentrations overlap the radiolytic production line (solid triangles in Fig. 13). Data from other Precambrian shields and from the Triassic basin in eastern North America have yielded similar H_2 versus He distributions. Unlike shallow aquifers where the H_2 concentration (lower rectangle in Fig. 13) has been used as a yardstick to infer the principal microbial electron receptor for respiration (Lovley and Goodwin, 1988; Jakobsen et al., 1998), the deep subsurface H_2 concentration appears not to be solely mitigated by microbial activity, but reflects a balance between abiotic production, abiotic hydrocarbon generation reactions and nutrient-limited microbial consumption. The microbial footprint appears more pronounced in the shallower depths, particularly < 2.0 kmbls. Similar behavior may be found for other metabolites and nutrients.

The elevated concentrations of H_2 also indicate that microbial respiration is not efficiently extracting all of the available chemical energy for growth. Other factors that might be limiting both microbial metabolism and growth are:

- Lack of electron acceptors-Onstott et al. (Onstott et al., 1998b) observed that the deep subsurface environment of a Triassic rift basin was electron donor rich compared to available electron acceptors. A similar discrepancy between electron equivalents of electron donor and electron acceptor compounds exists for South African deep subsurface samples. Most of this excess electron donor capacity is present in the form of CH_4. The only electron acceptor that could redress this imbalance is Fe(III) and if it was being utilized by or was available to anaerobic CH_4 oxidizers then the donor/acceptor balance might be restored.
- Lack of organic C. Unlike marine sediments, continental and ocean crustal rocks may not have sufficient organic carbon. This possibility seems less likely given the recent reports of long chain hydrocarbons from hydrothermal vent fluids and the abiogenically produced light hydrocarbons in Precambrian shield rocks. Hydrothermal redox catalyzed reactions may generate light-weight organics at deeper levels in the crust and they may diffuse upwards to nourish the subsurface biosphere as suggested by Tommy Gold (Gold, 1992). The 10^3 ml^{-1} biomass concentrations observed in deep saline water from South Africa are far less that would be expected from extrapolation of biomass concentrations from shallower, marine sediment environments (Fig. 15) and less than that expected given the 1 μM, minimum measured concentrations of carboxylic acids (Fig. 16).
- Lack of N. When dissolved organic carbon (DOC) and total inorganic N (NH_4^+ and NO_3^-) concentrations are compared to the C:N values for bacteria, some of the deep saline water exhibit a deficiency in N relative to DOC (Fig. 16). The minimum N concentrations of 0.1 nM correspond to a cellular concentration of 10^3 ml^{-1} and thus could be limiting. Given the surfeit of dissolved N_2 in these fissures, N_2 fixation would alleviate this deprivation, but only if sufficient energy was present to sustain this energetically costly enterprise.
- Lack of phosphate. The C:N:P values for the South African deep fissures indicate a relative paucity in P (Fig. 17a), which could account for the low levels of biomass. Dissolution of P bearing phases such as apatite could replenish this nutrient, but phosphate is very much oversaturated with respect hydroxyapatite (Fig. 17b) in these systems. The mechanism responsible for the apparently elevated phosphate concentrations has not been resolved.

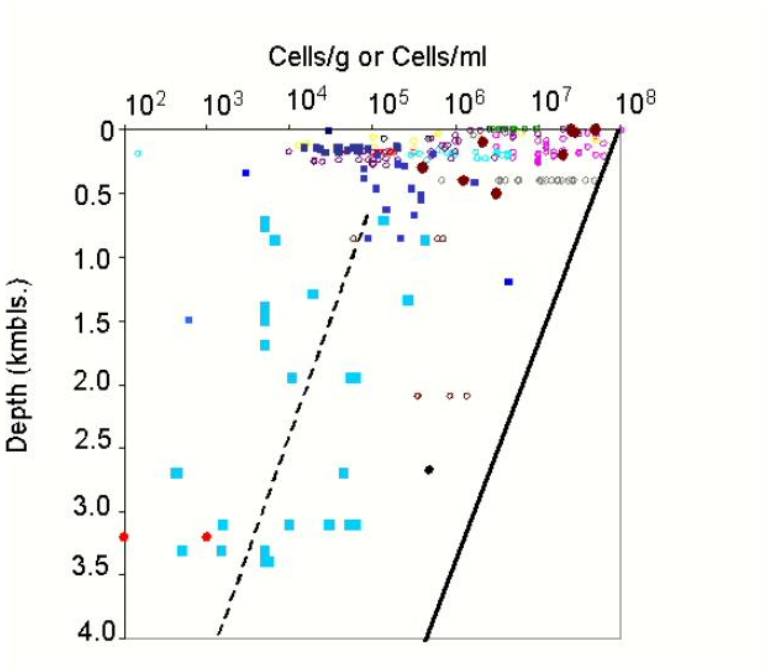


Fig. 15. Summary of biomass concentrations based upon concentrations of PLFA or direct cell counts per gram of sediment (open symbols) and per ml of groundwater (closed symbols). Data from (Onstott et al., 1998a), Hall (Pers. comm., 2003) and Pfiffner (Pers. comm. 2002). Red dots are biomass estimates for quartzite and organic-rich ore zone (Onstott et al., 2003). Solid line is based upon extrapolation of biomass values determined for deep-sea sediments (Parkes et al., 1994). Our results suggest three orders of magnitude lower subsurface biomass in continental crust.

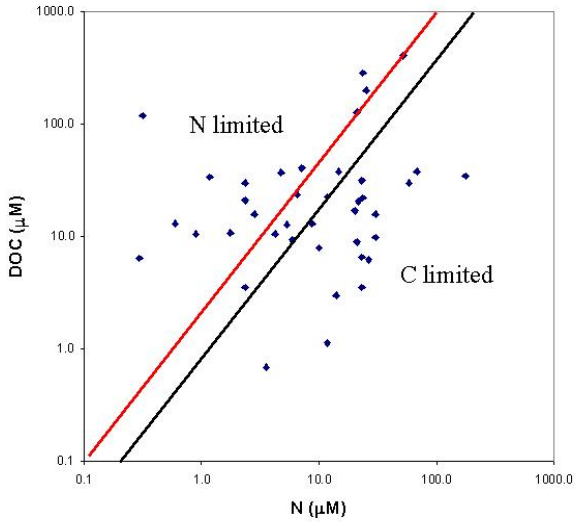


Fig. 16. Dissolved Organic Carbon (DOC) versus total inorganic N (NH_4^+ and NO_3^-) for South African deep groundwater. Red and black lines represent the C:N values measured on stationary and exponential phase bacteria, respectively, grown in lab (Vrede et al., 2002).

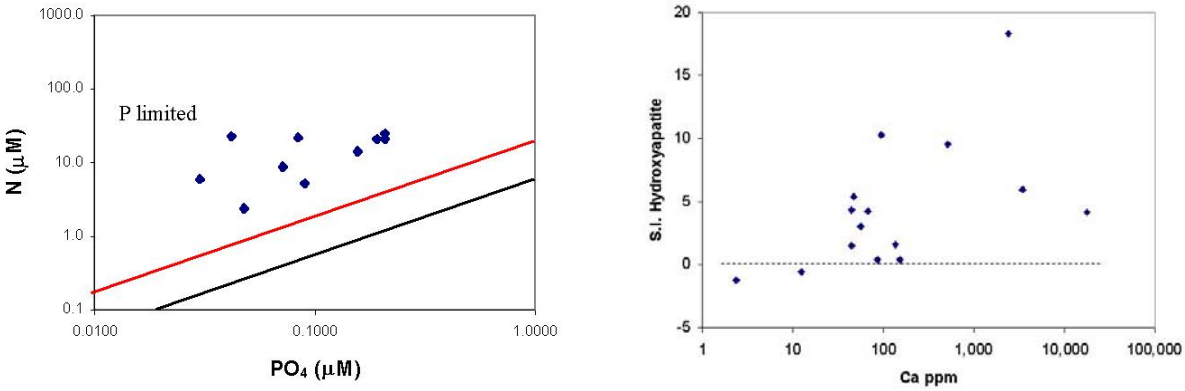


Fig. 17. Left-a) Total inorganic N versus PO_4^{2-}. Red and black lines represent the C:N values measured on stationary and exponential phase bacteria (Vrede et al., 2002). Right-b) Saturation Index of hydroxyapatite and calcite versus Ca concentration for South African deep groundwater.

- Lack of trace metals. In South Africa, fissure water <1.5 kmbls. tends to be bicarbonate rich, whereas, fissure water deeper than 1.5 kmbls. tend to be saline and sulfidic. Trace metals tend to have different valence states and form different anionic complexes for these to systems with many metals precipitating as sulfides. The lack of Fe, Ni and other transition metals may limit the total abundance of enzymes such as hydrogenase and CO dehydrogenase, which in turn may limit H_2 utilization and growth.

3. IMPLICATIONS FOR BIOSUSTAINABLE DEEP SUBSURFACE MARTIAN ECOSYSTEMS

Our research to date indicates that abiogenic hydrocarbons in Precambrian Shield geologic environments are a globally and volumetrically significant phenomenon, hitherto significantly underestimated. It has to been largely assumed that after the evolution of life on earth, biologically mediated reactions overprinted evidence of the pre-biotic abiogenic chemistry of the earth. This is the first indication that abiogenic reactions that contributed to the formation of primary organic molecules on the early earth continue to play a significant role in the isolated deep subsurface environments. Although our South African research indicates that some of these environments were inoculated with microorganisms, they do not appear to dominate the C or H cycle in the deepest, most saline fissure water. These isolated brines may host other abiogenic and even prebiotic reactions involving N, S and C compounds that have yet to be detected.

Based upon the radiogenic element concentration of SNC's, the hydrological model of Clifford (Clifford, 1993) and our results, substantial reservoirs of radiolytically derived H_2 and abiogenic CH_4 would likely exist in the martian subsurface, the former as trapped gas diffusing upwards through the cryosphere towards the surface and the latter as a clathrates within the cryosphere (Max and Clifford, 2000). The isotopic composition of the H_2 should be distinct from that of the martian atmospheric H_2. Light hydrocarbons may be trapped in residual saline brine as the cryosphere base deepens with temporal cooling of Mars and could provide potent electron donors and C substrate.

The abundance of S and Fe in martian crust estimated from the martian surface measurements (Clark et al., 1982; Reider et al., 1997) and from SNC's (Wanke and Dreibus, 1988) and especially of the bioavailable forms of sorbed sulfate, S in smectite and ferrihydrite in SNC fractures (Treiman et al., 1993)

suggest that sulfate and Fe(III) reduction could be two principal electron acceptor reactions. In this case, radiolysis would renew these resources from their biologically reduced mineral equivalents. The concentration of P is high compared to that of terrestrial rocks (Banin et al., 1992; Dreibus et al., 1999) suggesting that unlike terran deep subsurface systems, P limitation would not be a serious concern. Bioavailable N, however, is a serious dilemma given that N_2 abundance of the martian atmosphere is 5000 times less than that of earth and only trace amounts of nitrate have been reported in SNC's (Clark, 1998). Our results have detected microorganisms that are capable of N_2 fixation, but this function is energy-intensive. The high abundance of N_2 in some saline fissure water and its absence in others suggest that some mechanism is removing N_2 in these deep environments. Nitrate reducers have been found in the deep saline fissure water, but whether they are reducing nitrate in situ is difficult to assess given the absence of nitrate.

Our observations of a deep subsurface terran ecosystem that has been tectonically quiescent and isolated from the surface for ~10^8 years, therefore, carries implications about long term potential energy and nutrient flux in a martian subsurface environment that differ from previous assessments. Fisk and Giovannani (Fisk and Giovannoni, 1999) propose oxidants, such as dissolved O_2 from the martian atmosphere enter the subsurface through basal melting of the polar cap (see Fig. 1) and downward recharge to interact with H_2 produced by water dissociation from oxidation of fayalite. Assuming the polar ice traps dissolved O_2 at a concentration of 10-20 nM, a melt recharge rate of $10^{10\text{-}12}$ L yr^{-1} (Clifford, 1993) and a subsurface brine volume equivalent to 10 to 250 m global ocean, the O_2 recharge rate is 10^{-5} to 10^{-9} nM yr^{-1}, which is 8 to 12 orders of magnitude LESS than the oxidant production rate predicted by radiolysis (see Fig. 19 below).

PROPOSED RESEARCH

The mechanisms that are at work in deep biosustainable habitats in South Africa could clearly play critical roles in biosustainable environments beneath the surface of Mars. Our information doesn't however address the other two potential martian habitats mentioned above, the cryosphere and deep vadose zone. Nor do our results provide insight energy and nutrient cycling in saline aquifers where CO_2 is a dominant gas phase, but they do imply that over a period of at least ~10^8 years this CO_2 could be converted to CH_4 and light hydrocarbons.

Our proposed research program, therefore, has four major themes:

1. Abiotic Microcosm Experiments-Abiotic geochemical and radiolytic experiments designed to mimic the processes inferred to be taking place in the deep subsurface of South Africa and Canadian Shield, to quantify the resulting energy and nutrient fluxes, determine the stable isotopic fractionation and to develop simple aqueous geochemical and isotopic models that can be applied to martian hydrogeological conditions.
2. Biotic Microcosm Experiments-Biotic experiments similar to the abiotic experiments, but inoculated with selected subsurface microbial isolates that cycle C, N and S. These results will be used to develop models for martian biosustainable deep subsurface habitats. The chemical and stable isotopic composition of the metabolites will be compared to that of the abiotic experiments and from this comparison we will begin designing life detection tools that can be utilized in the field and potentially on mars missions.
3. Subsurface Simulator Stress Experiments-Both abiotic and biotic microcosm experiments will examine the effects of dessication to the limit of thin, saline films on mineral surfaces and the effects of CO_2 and CH_4 clathrate formation. These stresses are designed to examine the impact of these processes on energy and nutrient flux and on the chemical and isotopic composition of abiogenic species and biogenic metabolites. To examine the effect upon a subsurface microbial community field rock and water samples will be placed into large volume subsurface simulators.

This information will also be incorporated into models for martian subsurface habitats and in the design of potential life detection instruments.

4. In situ Activity and Substrate Utilization Experiments-Geochemical, isotopic and microbial analyses and in situ experimentation at a field site where a relatively shallow (< 1km), brine that has been isolated from the surface by permafrost for ~10^7 years is accessible through mining operations. Several candidate sites exist in northern Canada and the results from this field site will be compared to our observations from the deep subsurface of South Africa, which has experienced a much different surface climate history. The results of the experimental studies will be compared to the results from both field studies as an initial step towards scaling up biogeochemical modeling. Finally, the results from the northern Canada site will be compared to those of Artic permafrost deposits to determine if any evidence for biological or chemical transport between the two habitats exists.

1. ABIOTIC ENERGY AND NUTRIENT SOURCES

(Onstott, Myneni, Dismukes, Sherwood-Lollar, Pratt and Lehman)

Radiolysis

Background-Dissolved H_2 and He measurements indicate that radiolysis is the simplest explanation for the elevated H_2 concentrations reported for Precambrian crustal aquifers (Lin et al., 2003b). Radiolysis also generates strong oxidants, H_2O_2 and O_2 (Spinks and Woods, 1990) and the production rate of all three species depends upon the fluid-filled porosity and radiogenic element composition and concentration (Hoffman, 1992). The occurrence of H_2 and O_2 in fluid inclusions within quartz demonstrates the stability of these radiolytic products over the geological periods in the absence of variable valence elements (Dubessy et al., 1988). This has been confirmed by laboratory irradiation of complex brines at PNNL (Gray and Simonson, 1984). The relative production rates of H_2O_2 and O_2 depend upon the relative α, β and γ dosage and upon the salinity of the fluid since Cl inhibits H_2O_2 production (Lin et al., 2003a). H_2O_2 and O_2 will react with reduced metal oxides, organic matter and sulfides to generate potential electron acceptors, Fe(III), CO, CO_2, S^o, $S_2O_3^{2-}$, SO_4^{2-} and or with NH_4^+ to produce NO_2^- or NO_3.

Published estimates of radiolytic yields rely almost entirely upon irradiation of homogenous water solutions. The efficiency of radiolytic decomposition of water is increased in heterogenous water/mineral solutions since the ephemeral products, such OH, H, and e-, interact with the mineral surface before they have a chance to recombine into H_2O. The radiolytic of H_2 for thin films of water absorbed to oxides is ~15-50 times that of pure H_2O (Vovk, 1987a). In the presence of gas hydrates, the rate of radiolytic decomposition is 3 to 4 times that of pure H_2O (Vovk, 1987a). Laboratory irradiation of aerobic, organic-rich sediments has yielded both CH_4 and CO_2 with the production of H_2, the consumption of O_2 and surprisingly the consumption of N_2 (Vovk, 1987a).

Since most of the fluid filled porosity in deep crustal rock is associated with an effective porosity with sub-micron pore throats or fluid inclusions and not within the fractures (Nordstrom and Olsson, 1987), most of the radiolytic production takes place in the rock matrix. As a result, much of the Fe(III)-bearing oxyhydroxides and S^o precipitated during radiolytic reactions may not be bioavailable and the soluble electron acceptors, SO_4^{2-}, $S_2O_3^{2-}$, NO_2^- and NO_3^-, will diffuse from the matrix to the fracture zone along with the H_2. The oxidation reactions that take place within the matrix reduce the pH of the pore fluid thereby promoting the dissolution/precipitation of clays, feldspars and quartz and elevate the Eh thereby promoting the mobilization of other redox sensitive metal species, such as U. Pore water and fissure

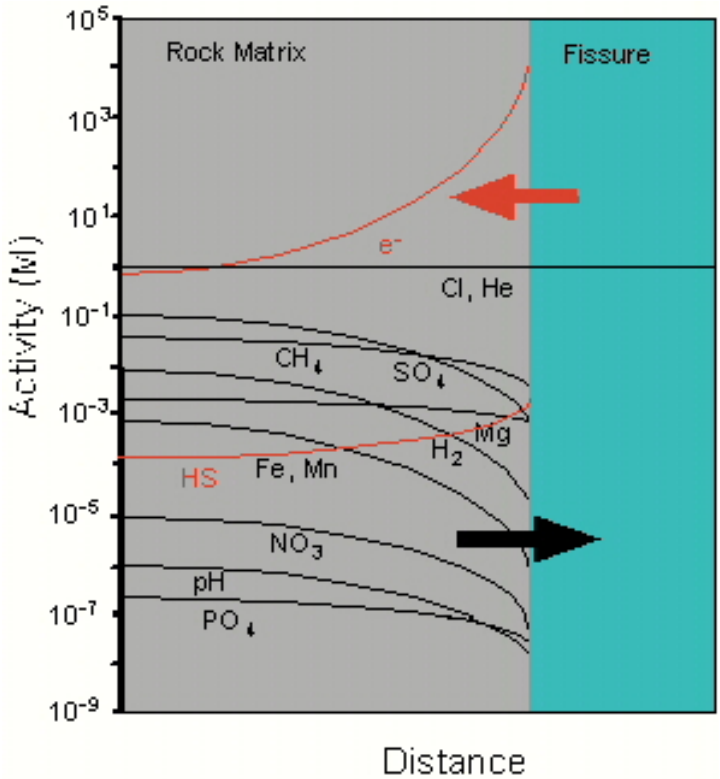


Fig. 19. Diagrammatic representation of observed concentrations gradients between rock pore water and sulfidic fissure water. Vertical scale is activity in M. Horizontal distance is diffusion controlled. Not included are mineral precipitating reactions on fracture face, such as sulfide or U oxide formation. Also not shown are coupled microbial redox reactions, e.g. reduction of nitrate that is diffusing outward by HS.

water geochemical results from South Africa and geochemical modeling have delineated some of these gradients (Fig. 19). SO_4^{2-} appears to be the principal electron acceptor diffusing into the fracture explaining the wide spread occurrence of SRB's. By reducing the SO_4^{2-} to HS^- the SRB's enhance the diffusive flux and their overall metabolic rate. The HS^- can either react with the metals diffusing from the matrix to form sulfides or act as an electron donor to other electron acceptors, such as NO_3^-. This redox couple can be utilized by microorganisms, such as *Thiobacillus denitrificans*, as an energy source, producing N_2 as a waste product (Fig. 19).

The potential chemical power made available to chemolithotropic microbial communities depends upon the relative volume of the fracture to matrix rock, the diffusivities of H_2 and the electron acceptors and the age of the fracture itself. A newly formed fracture in an ancient rock will immediately yield the maximum chemical power, which approaches steady state over a period of 10,000 to 100,000 years (see Fig. 20). Because of the tiny facture volume typical of deep crustal rocks (~0.001-0.01%; (Nordstrom and Olsson, 1987), the potential biosustainable chemical power production is greater than that present in Atlantic coastal plain aquifers that support a large diversity of microbial species.

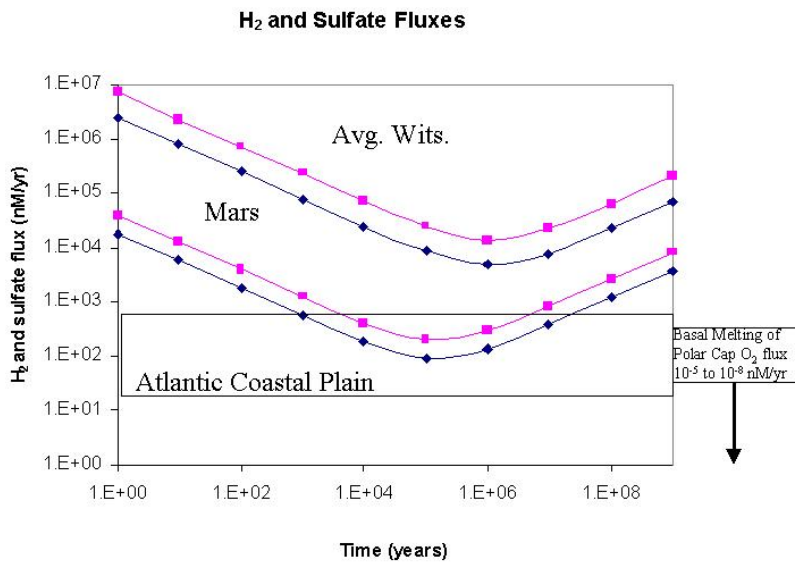


Fig. 20. Chemolithotrophically available power as determined by maximum production rates for H_2 and SO_4^{2-} in nM yr^{-1} based upon instantaneous and steady-state solution of diffusion equation for a semi-infinite medium, measured pore water concentrations and estimated radiolytic productions rates using experimentally determined yields for H_2 and assuming that all radiolytically produced O_2 and H_2O_2 oxidizes sulfide to SO_4^{2-}. The production rates for H_2 and SO_4^{2-} for Mars are based upon the radiogenic content of SNC meteorites. Shown for comparison is the chemical fluxes of H_2 and SO_4^{2-} for Atlantic Coastal Plain aquifers from (Lin et al., 2003b) and (Chapelle and McMahon, 1991) and also the redox power availiability for martian hydrosphere based upon basal melting of polar caps as the sole source of recharging oxidant (Fisk and Giovannoni, 1999).

Several questions need to be resolved, however, in order to determine whether this mechanism alone can sustain subsurface life.

- Does the presence of mineral surfaces enhance radiolytic yields and alter redox chemistry?

Several studies from the late 60's to early 80's reported these effects (Vovk, 1987a) but experiments utilizing modern surface spectroscopic techniques have not been performed. One would anticipate that different aqueous surface complexes whose type and abundance depend upon the pH and mineral would influence both the radiolytic yield and the surface reaction. The surface reaction would entail both dissolution/precipitation and oxidation/reduction reactions.

- How do dissolved organic and inorganic C and N species behave in this system?

Only a few studies have reported the effect of radiation on organic matter and of those most focus on kerogen and bitumen associated with U ore deposits where the calculated α dosage is ~10^{13}-10^{14} rads (Leventhal and Threlkeld, 1978; Leventhal et al., 1987). Aromatic carbon complexes that are stable to γ irradiation and depleted in H and O relative to immature organic matter typify these deposits (Zumberge et al., 1978) or sometimes enriched in O (Dubessy et al., 1988). The reported loss of N_2 during sediment irradiation experiments (Vovk, 1987a) the susceptibility of NH_3 to UV photolysis and the reported correlations between N_2 and He in natural gas fields (Ballentine and Sherwood-Lollar, 2002) are all very suggestive, but nothing is known about radiolysis and N cycling.

- What S species are formed by radiolysis of sulfide/saline anaerobic water?

Radiolytic production rates for SO_4^{2-} by γ and β irradiation of S^o in water ice (nonsaline and microaerophilic) were recently reported by (Carlson et al., 2002). Under these conditions SO_4^{2-} was the principal S species, but further experimentation is required as oxidation of sulfide by H_2O_2 typically

yields a coating of microcrystalline S^o and pore water analyses of U-bearing ore zones in South Africa also detected the presence of $S_2O_3^{2-}$. Because the $S_2O_3^{2-}$ can be simultaneously oxidized to SO_4^{2-} and reduced to S^{2-} its radiolytic production and subsequent destruction could potentially cause large values in the $\Delta^{34}S$ of SO_4^{2-} and S^{2-} (Jorgensen, 1990).

- Are there any isotopic or molecular signatures that are indicative of radiolytic processes?

The $\delta^{13}C$ of the solid phase organic matter in U ores has ranged from –17 to –44$^o/_{oo}$PDB (Leventhal and Threlkeld, 1978; Zumberge et al., 1978; Leventhal et al., 1987) and, in one case, that of occluded CH_4 and CO_2 was –44 and –35$^o/_{oo}$ PDB, respectively (Leventhal et al., 1987). The light isotopic values and petrographic observations have fueled speculation that some of the organic matter formed by radiation-induced condensation of CH_4 and or CO_2 (Robb et al., 1994). To date, no compound specific stable isotopic analyses have been performed. Although the δ^2H of radiolytically produced H_2 is isotopically light, its signature will equilibrate with that of water too quickly to be preserved (Lin et al., 2003a). The same argument may apply to the $\delta^{18}O$ of H_2O_2 and O_2 formed by radiolysis and preserved in the SO_4^{2-} formed by oxidation sulfide bearing rock strata, but no measurements have been performed to date to test this.

Energy Flux for Thin Films on Minerals

Background-It is well known that heterogeneous reactions at the mineral-water interfaces modify the energetics of several reactions on the surface of the Earth (Brown et al., 1999). The energetics of redox reactions mediated by mineral surfaces are vastly different from that of homogeneous aqueous phase reactions. Our recent vibrational spectroscopy studies indicate that water sorbed on mineral surfaces (thin films of water on surfaces) interacts with mineral surface hydroxyls and modify their H-bonding environment significantly (Myneni, unpublished). In addition, these H-bonding interactions vary as a function of water film thickness. This suggests that the interfacial reactions involving minerals do not entirely depend on the type of metal coordination of surface hydroxyls but on the H-bonding environment of solvated water at the interfaces. In unsaturated subsurface environments including the deep martian vadose environment, most of the water exists as thin films on mineral surfaces, and it is expected that the thickness of water films, the composition of the vapor phase, e.g. CH_4 and CO_2 and with N_2, mineral substrate composition and interfacial chemistry would play a critical role in various geochemical reactions, including the radiolysis of water. Total radiolytic yield of H_2 should diminish with decreasing abundance of H_2O, but the yield of O_2 and H_2 O_2 could be quite different. Very little information is currently available, however, on redox reactions and water radiolysis in thin film conditions.

We propose to investigate the role of mineral surfaces on the radiolytic yields of different chemical species. Since the H-bonding environment at mineral-water interfaces changes significantly as a function of pH (modifies the point of zero-charge and thus the interaction of different ions at interfaces), water film thickness (modifies the coordination of water and the strength of H-bonding environment at interfaces), solution composition (ionic strength and composition of fluid phase modifies the overall distribution of charged and uncharged species at interfaces) and substrate composition, we would like to explore the influence of these important variables on the radiolysis of water. Using the vibrational and the soft X-ray spectroscopy accessories that our group has built in the past few years, we would like to explore the chemical state of water on mineral surfaces under different conditions stated above, and probe the radiolytic breakdown of water and its reaction products. The OH stretching and bending vibrations of water and the 1s electronic transitions of O in water are sensitive to the H-bonding environment of water molecules in aqueous solutions and at interfaces (Myneni et al., 2002).

Hydrocarbon Yielding Water/Mineral Reactions

To date we have been able to identify C and H isotopic fractionation patterns consistent with abiogenic versus thermogenic and bacterial processes for C1-C4 hydrocarbons, but we have not been able to distinguish between different potential abiotic pathways and reactions. An additional goal of the proposed research is elucidate the specific reaction mechanisms responsible for the production and fate of

hydrogen and hydrocarbon gases in this geological setting, taking advantage of the enormous potential that isotopic signatures have as diagnostic indicators of reaction mechanisms. Several reactions have been suggested in the literature as potential mechanisms for production of abiogenic gases including:

1. CH_4 from dissolved bicarbonate (HCO_3^-) under hydrothermal conditions with metal catalysts (Horita and Berndt, 1999).
2. CH_4 and higher hydrocarbons via the Fischer-Tropsch synthesis (Yuen et al., 1990; Charlou and Donval, 1993; Hu et al., 1998; Lilley et al., 2001).
3. CH_4 and higher hydrocarbons via serpentinization of ultramafic rocks (Charlou and Donval, 1993; Berndt et al., 1996; Kelley, 1996).

While some work has been done to establish C isotope fractionation effects associated with CH_4 production, a paucity of information exists on formation of the higher hydrocarbons and on H isotope effects. The objective of the proposed research is to undertake a focused experimental program to determine both the C and H isotope fractionation associated with these important abiogenic reactions and to extend the information base we have developed to incorporate higher molecular weight hydrocarbons in order to address the following questions:

- To what extent do the different potential abiogenic gas formation reactions have distinct isotopic fractionation associated with them?
- If distinct isotopic differences do exist between these reactions are they observed in field samples?
- If they are observed in the field to what extent can the inferred reaction be related to the geologic setting and geochemical history of the locale as provided by fluid inclusion analyses?
- If these gases occur in ore bearing rocks does this association reflect a casual relationship whereby the dispersed metals act as catalysts to facilitate the conversion of inorganic carbon to hydrocarbons?

It will be particularly important to distinguish whether the gases are primarily controlled by geological/geochemical features of the host rock or are controlled by structural features, implying migration from the point of origin. If the former, then the differences in gases observed in different geological settings will yield important information on potential reaction mechanisms and the production of gases via water-rock interaction.

Experimental Approach

Objectives-The focus of research into radiolysis will be to perform laboratory irradiation experiments on homogenous fluid and heterogeneous fluid/mineral systems to obtain radiolytic production rates, to identify the reactive species forming on the mineral surfaces and to quantify any isotopic fractionation effect associated with the radiolytic products. Both homogenous and heterogenous fluid experiments will utilize anaerobic, Na-Ca-Cl solutions as these best represent the bulk composition of deep subsurface brine. Heterogeneous experiments will also use pyrite, magnetite, olivine, pyroxene and quartz. The initial pH of the solutions will vary from 3 to 10 in order to examine the effects of surface species and at 4, 25, 45 and 80°C. To examine the effects of mineral surface reactions, the experiments will be performed without mineral substrates, with an equal volume of mineral and saline solution and just with minerals whose surface is saturated with a thin film of saline solution. Total dosage from the ^{60}Co γ source used at Columbia University will vary from 0 to 10^4 Gy. The surfaces of the minerals will be examined with XAFS to identify surface adsorbed aqueous complexes. Finally, the heterogeneous experiments will be performed without irradiation, but with low levels of H_2O_2 and O_2 to see if the

kinetics and products of these reactions can approximate that of the irradiation experiments. If so, this will greatly facilitate incorporation of radiolytic effects into standard geochemical modeling codes.

Tasks-The experiments will focus on H, C, N and S cycles.

1. H cycle-The production rates of H_2, H_2O_2 and O_2 will be determined as a function of temperature, pH, water saturation and Fe concentration in the mineral. The non-irradiated control experiments will be used to further quantify the effect of H_2 production via oxidation of Fe in mineral phases.
2. S cycle-For irradiation experiments utilizing FeS_2, the aqueous S species will be measured by ICMS and the surface absorbed S species by XAFS. The production rates and species distribution will be determined as a function of temperature, pH and water saturation. The S isotope composition of the various products will be determined.
3. N cycle-Anaerobic saline water with NH_4^+ and Ar and with N_2 will be irradiated to determine the stability of both phases during irradiation and whether oxidized N species, NO_2^- and NO_3^-, are produced. The aqueous N species will be measured by ICMS and the surface absorbed species by XAFS. The N and O isotopic composition of the different species will also be determined. The homogenous experiments will be compared with the heterogeneous experiments to determine the effects of temperature, pH, water saturation and Fe concentration in the mineral.
4. C cycle- Anaerobic saline water with CH_4 and CO_2 and with N_2 will be irradiated to determine the stability of the C phases during irradiation and whether oxidized or reduced C species, such as low molecular weight hydrocarbons or carboxylic acids, are produced. The aqueous C species will be measured by ICMS, HPLC and the surface absorbed C species by XAFS. The C and H isotope composition of the different species will also be determined. The homogenous experiments will be compared with the heterogeneous experiments to determine the effects of temperature, pH, water saturation and Fe concentration in the mineral.

New Gas Detection Methods

In the past decade, Cavity Ring Down Spectroscopy (CRDS) has become a widely used optical spectroscopic technique for detection of very weak absorption, including by trace species(Busch and Busch, 1999). In this method, absorption of a gaseous sample is detected by a change in the decay rate of an optical cavity former by very high reflectivity mirrors when the sample concentration between the mirrors is changed. Lehmann (1996)(Lehmann, 1996) and (Romanini et al., 1995; Romanini et al., 1997) have pioneered the application of a continuous wave laser to excite the optical cavity. Using such lasers, optical absorptions as small as one part per billion per pass of the cell can be detected. The methods developed at Lehman's laboratory at Princeton University have led to introduction of the first commercial trace gas analyzer based the CRDS method (Dudek et al., 2003).

This instrument can detect concentrations of moisture in a wide range of gases at a concentration of 100 ppt by volume. The laboratory prototype had a noise equivalent concentration ~10x more sensitive. With minor modifications, this instrument can detect other of simple hydride species, including HCl, HF, CH_4, NH_3, and C_2H_2 with detection sensitivities within one order of magnitude of the moisture sensitivity. The detection is capable of isotopic analyses and is also quite fast, limited primarily by the time required to exchange the sample in the cell. The method is most compatible with a slow, continuous flow of sample through the detection cell.

In order to apply this technique to problems relevant to Astrobiology, we propose several developments for the instrument. By minimizing volumes of the cell and transfer line, we should be able to reduce the size of the sample to be analyzed to about one standard cc of gas. With a detection sensitivity of 100 ppt, this would correspond to a detection limit of about 100 femtogram of analyte or ~0.006 nM of CH_4 as an example. This is about 100 to 1000x more sensitive than analytical techniques

employing GC-IRMS but less sensitive than those utilizing ^{14}C. The second direction is to combine this detection technology with chemical transformation methods to selectively convert other chemical species into ones we can detect. For example, both CO and CO_2 can be quantitatively converted with a methanizer into CH_4, which can then be analyzed. We will also explore methods to pre concentrate larger samples to allow similar absolute quantity sensitivity. Finally, we will determine the concentrations required for obtaining C, H and N stable isotope precisions of ~1$^o/_{oo}$. The distinct advantage of this approach is that it conceivably can be adapted to fly on Mars drilling missions.

2. BIOTIC ENERGY AND NUTRIENT CYCLING

(Onstott, Myneni, Dismukes, Lehman, Pratt, Sherwood-Lollar, Hazen, Moser)

Introduction

The research program will focus on two main, long-term objectives:

1. To determine the distribution between abiogenic and microbial C, H, N and S metabolites and the geological, structural, geochemical and microbiological controls on the transition between these two regimes in deep subsurface brines.
2. To determine whether the abiogenically derived C, H, N and S compounds act as substrate(s) supporting the microbial communities.

Although these two objectives may appear contraindicative, i.e. if microbial communities are utilizing abiogenically derived C, H, N and S compounds wouldn't they be transformed into microbial metabolites and erasing their former abiogenic signature? To successfully distinguish between abiogenic source and microbially produced metabolites we will rely heavily upon compound specific stable isotope analyses and isotopically labeled substrates. These methodological approaches in concert with laboratory microbial microcosm and macrocosm experiments, with a field site that offers a range in environments with more or less microbial activity and with in situ experiments performed in boreholes will enable us to document the processes that operate to sustain subsurface microbial communities for 10^7 to 10^8 of years.

Evolution of the C Cycle From Abiotic to Biotic Processes

Background-The $\delta^{13}C$ and δ^2H analysis of CH_4 clearly detected biogenic methane production in our deep subsurface systems in South Africa (Fig. 9a) and in the Canadian Shield (Doig, 1994). The absence of ^{14}C in some of this CH_4 suggests it is the product of indigenous, deep subsurface methanogens. The 16SrDNA data indicate that aceticlastic methanogens are responsible for most of the biogenic CH_4. The challenge now is to characterize the interface between these two major gas-producing geochemical/microbial systems in the complex and dynamic field settings encountered the deep biosustainable geological environments.

- What is the degree of chemical/biogeochemical interaction between the abiogenic hydrocarbon gases and the deep subsurface microbial community?
- To what extent do indigenous anaerobic microbial communities utilize these compounds as substrates?
- To what extent do indigenous anaerobic microbial communities utilize these compounds as electron donors for energy and convert them back into CO_2 and cycling the C compounds between the abiotic and biotic systems?
- If C_{1-4} utilizing, anaerobic microorganisms exist, then how do they alter the C and H composition of the C_{1-4} compounds?

Field sites will be sampled extensively to determine the nature and spatial distribution of gases within the mine. Sampling will be targeted to cover different depths, geologic formations and structural features. A key feature of this research is close coordination with the mine geologists and exploration drilling programs to sample both from newly completed boreholes and to re-sample these boreholes over time to

determine temporal as well as spatial variability in measured parameters. This has never been systematically accomplished in the mines in Canada, South Africa or Finland and will allow us to resolve whether the microbial hydrocarbons are stored over long times periods in the host rock (as are the abiogenic gases) or are a phenomenon that appears post-borehole completion. If the latter, it will be essential to determine if this is due to mixing with *in situ* native microorganisms or due to colonization of boreholes with surface microorganisms associated with mining contamination. The goal will be to establish both the contribution of microbial processes to the groundwater and gases, and also the substrates (DIC, DOC, H_2, CH_4, CO) that the *in situ* microorganisms are utilizing as a basis for this deep subsurface community.

Samples will be collected for compositional and isotopic analysis of CHN-bearing gases and dissolved organic compounds as well as associated saline groundwater and brines. Concentration data, as well as stable C and H isotope signatures, have traditionally been used to identify microbial gases mixing with non-microbial end-members (Bernard et al., 1977; Schoell, 1988). Sherwood-Lollar et al.(Sherwood Lollar et al., 2002) have now established a model for isotopic fractionation patterns in abiogenically-produced gases that can be used to identify this novel end-member and provide constraints to determine the relative contribution of the end-members to each sample. Once the distribution pattern of microbial and abiogenic hydrocarbons is established for these systems, radiocarbon analysis (^{14}C) of the hydrocarbon gases from selected systems will be undertaken to determine if the microbially-produced CH_4 is young (and potentially related to mining contamination of the deep subsurface), or geologically old, representative of an *in situ* microbial community.

Prior to this, determination of substrate utilization will be done by collecting microbial samples, isolating them in laboratories by conventional or Gel Microdroplet techniques and culturing them under the same suite of substrates with natural isotopic abundances and optimal and in situ growth conditions. The isotopic fractionation associated with substrate utilization will be determined from these experiments. While valuable, extrapolating this type of result back to the field is uncertain. While such tracer experiments to evaluate substrate utilization have not to our knowledge been attempted systematically before in the challenging environment of the deep subsurface, the results will provide critical data for resolving the question of substrate utilization in these unique geological and microbiological systems.

Evolution of the N cycle from abiotic to biotic?

Background-In conjunction with our research on biogeochemical cycling of C in deep subsurface biosustainable systems, the role of N cycling must be addressed. Our work in the Canadian Shield and South Africa has demonstrated that N_2 is often the second major component (ranging in concentration from 5% to in some cases as high as 60 vol.%), but the origin of this important component has yet to be resolved. The South African mine geochemical and dissolved gas samples have revealed some high N_2 gas partial pressures (P_i ~72 bars) and NH_3 concentrations as high as 200 μM and undetectable NO_3^- concentrations (<1 μM). Non-atmospheric N in geologic systems is predominantly derived from four sources: 1) the thermal deamination of organic material within sediments; 2) N held as NH_4^+ in the lattice structure of silicate minerals within metasediments; 3) N associated with recent magmatism, or of an igneous/mantle origin; and, 4) hydrothermal reduction of N_2 to NH_3 (Brandes et al., 1998). The absence of ^3He in both the Canadian Shield (Sherwood-Lollar et al., 1993) and South African (Lippmann et al., 2003) gas occurrences rules out a mantle origin. Nor is "recent" magmatism a potential candidiate. In the 3.0 Ga siliclastic/volcanic rocks of the Witswatersrand basin clays might provide a source of NH_4^+ although its bioavailability has not been tested. Only one thin, marine, shale unit, the Kimberly Shale, might represent a potential source of bioavailable NH_3. The N_2 (0.1 – 1 M), NH_4^+ (30 to 90 mM) and NO_3^- (up to 50 mM) present in fluid inclusions of hydrothermal veins cutting the Witswatersrand rock strata (Drennan et al., 1999); Frimmel et al., 1999) and the NO_3^- in the leachates of crushed ore-bearing rock (Onstott et al., 2003) suggests that fluid inclusion may provide a source for all three N species.

Since the variation in N_2 isotopic signatures for these different sources is overlapping, N_2 isotope systematics alone cannot conclusively resolve all possible origins of N_2, but stable isotope studies do have

the potential to distinguish between atmospheric-derived N and nitrogen-derived form crustal or mantle processes. Unfortunately, compared to the subsurface C cycle, isotopic studies of the subsurface N Cycle are in their infancy. Sherwood-Lollar et al. (Sherwood-Lollar et al., 1993) have observed $\delta^{15}N$ values for N_2 that increase toward a heavy signature with increasing concentration, consistent with the type of signatures expected for N_2 derived during devolatilization reactions associated with metamorphism (Haendel et al., 1986; Bebout and Fogel, 1992)). Ballentine and Sherwood-Lollar (Ballentine and Sherwood-Lollar, 2002) find the N_2 and ^4He concentrations are correlated suggesting a crustal source. No information on the $\delta^{15}N$ values of NH_4^+, N_2, NO_3^-, NH_3 or NO_2^- in the aqueous or solid phases have been reported, however, from either of these deep subsurface environments.

In the case of our South African research, nitrifying and dissimilatory denitrifying bacteria have been detected by 16s rDNA analyses and thermophilic, nitrate reducing bacteria have been isolated (Kieft et al., 1999), but which of these are mining contaminants or indigenous subsurface habitants is currently being resolved.

Objectives-Like the C cycle objective 1, N cycle research will focus on characterizing abiogenic and biogenic components of N in the deep subsurface of South Africa and Canada. This will involve the N isotopic analyses, N species analyses by Ion chromatography mass spectrometry, DNA extraction, amplification and sequencing of the 16S rDNA gene, and possibly functional genes, analyses of deep groundwater. The N:C and the $\delta^{15}N$ and $\delta^{13}C$ of the microbial biomass will be analyzed. Finally the N:C concentration of $\delta^{15}N$ and $\delta^{13}C$ of the Kimberly shales and of the ore-associated kerogen and bitumen and the $\delta^{15}N$ of the siliclastic and volcanic units enveloping the fissure water will be analyzed.

The research will examine the source of the various components of the N cycle, including the excess N_2 gas. Probable pathways for N_2 formation will be addressed by looking at the typical isotope signatures observed for such pathways and comparing that with the field data, by looking for certain functional genes for the processes of nitrification and denitrification within our environmental microbial population, and by examining the fluid inclusions to determine the chemical composition, isotopic composition, and the source of the mineralizing fluid. In addition to the field component, some (high-temperature, high-pressure) laboratory experiments to explore certain abiotic possibilities for sources and sinks of N, such as mineral-catalysed reduction of NO_3^-, NO_2^-, and N_2 to NH_3. The fundamental database derived will be used to compare N isotopic results to that of other Archean sediments to address the question of correlating the modern terrestrial N cycle to that of the Archean. The research will address four major questions:

- What are the $\delta^{15}N$ values for the solid phase NH_4^+ and the N_2 gas in the rock strata? What is the $\delta^{15}N$ value of the organic-rich Kimberly shales? What are the $\delta^{15}N$ and $\delta^{18}O$ values for the NO_3^- and the $\delta^{15}N$ for the NH_4^+ of the pore water and the fluid inclusions? What are the $\delta^{15}N$ and $\delta^{18}O$ values for the NO_3^- and the $\delta^{15}N$ for the NH_4^+ of the mining water contaminated with explosive residues? Do these isotopic values correlate with other geochemical, isotopic, microbial or geological parameters? Is there any suggestion of an abiotic source for the N_2?
- Do we see evidence in the microbial community of nitrifiers, N_2 fixers and denitrifiers by the presence of 16S rDNA or certain functional genes?
- What are the $\delta^{15}N$ and $\delta^{13}C$ values of the microbial biomass? Do these values reflect the source of N? Are the indigenous microorganisms deriving their N from an abiogenic source?

Nitrate and ammonium isotope analyses-The isotope measurements on NO_3^- will be made using the 'denitrifier method', in which the NO_3^- sample is quantitatively converted to N_2O, which is then measured by gas chromatography-isotope ratio mass spectrometry [Sigman et al., 2001]. This method offers several improvements relative to previous methods that are important to this study. It is extremely sensitive (down to 10 nmol N and 0.5 μM NO_3^-), reducing the constraints and burdens associated with sample concentration and volume. It is completely free of cross-contamination by dissolved organic N or NH_4^+.

Finally, it measures both N and O isotope ratios of NO_3^- in a much more sensitive and time-efficient way than previously available methods [Casciotti et al., 2002].

The NH_4^+ isotope measurements will be made using an adaptation of the 'passive NH_4^+ diffusion' that is optimized for natural abundance isotope work [Sigman et al., 1997]. This method is not as sensitive as the denitrifier method but is more sensitive than other NH_4^+ methods, and the abundance of NH_3/NH_4^+ in many of the systems being studied makes this less of an issue.

Both the NO_3^- and NH_4^+ isotope measurements will be made in Sigman's lab at the Department of Geosciences at Princeton University. The N_2 isotopic analyses will be performed in the Sherwood-Lollar's lab at the University of Toronto.

Evolution of the S Cycle From Abiotic to Biotic Geochemistry

Background-Sedimentary sulfide minerals are typically depleted in ^{34}S due to their formation from sulfide (H_2S or HS^-) produced during bacterial sulfate reduction. The extent of ^{34}S-depletion recorded by the sulfide is dependent primarily on the rate of sulfate advection or diffusion to the site of sulfate reduction but factors such as the concentration of sulfate, pH, temperature, substrate availability, bacterial species, and general growth conditions are important secondary controls. Laboratory studies have shown that the kinetic isotope effect associated with dissimilatory bacterial sulfate reduction produces sulfide that is depleted in ^{34}S by 5 to 46‰ relative to the source sulfate (e.g.,(Kaplan and Rittenberg, 1964; Habicht and Canfield, 1996). Measured ^{34}S values of marine sulfides may show values as low as -50‰ (70‰ depletion relative to a 20‰ sulfate, e.g., (Canfield and Teske, 1996). The additional depletion in sulfide is likely due to isotopic fractionation produced during bacterial disproportionation of S^0, SO_3^{2-} (sulfite), and $S_2O_3^{2-}$ (thiosulfate) (e.g., Habicht et al., 1998)). Intermediate sulfur species are produced without isotope fractionation during biotic and abiotic re-oxidation of sulfide that is not sequestered as metal sulfide minerals and is, therefore, available for reactions in sediment pore water (Habicht et al., 1998). Both biotic and abiotic pathways readily transform these intermediate compounds. Consequently, study of sulfur isotope signatures is crucial for distinguishing between chemical gradients that sustain microbial life and chemical gradients that are abiogenic. At temperatures in the range of -5 to 110°C, biotic isotope fractionation of sulfur allows for rapid establishment of substantial ^{34}S depletion in sulfides while abiotic isotope exchange is minimal due to slow reaction rates. If abiotic equilibrium were reached in this temperature range, however, the isotopic partitioning between sulfate and sulfide would be 70 to 80‰.

In any type of hydrothermal environment, circulating geothermal waters may react with, and dissolve, sedimentary sulfides with strongly negative ^{34}S values. If the waters are strongly oxidizing, sulfate with anomalously low ^{34}S values may result. If the waters are reducing (but still sufficiently acidic to dissolve sulfide minerals), sulfide with low ^{34}S values will result. If the water is characterized by a fO_2 value near the sulfate/sulfide boundary then an equilibrium fractionation between aqueous sulfide and sulfate species may result ($\Delta^{SO_4}_{H_2S}$ values ~15 to 25‰ at temperatures between 450 and 250°C). Sulfide ^{34}S values more negative than those of the sedimentary sulfide source may coexist with ^{34}S-depleted sulfate under these conditions. A similar scenario would apply to leaching of virtually any type of sulfide (mantle or sedimentary) with a ^{34}S value less than ~ 20‰. Depending on the fO_2 conditions and produced SO_4^{2-}/H_2S ratio, plus the degree of isotopic re-equilibration during cooling of the hydrothermal fluid, ^{34}S values of sulfide could range from strongly negative to highly positive.

A third method for producing sulfides with low or variable ^{34}S values is through low-temperature mineral-fluid interaction that leads to the production of intermediate S species such as SO_3^{2-} and $S_2O_3^{2-}$. Rapid, but abiotic, disproportionation to sulfate and sulfide has been proposed by (Warren, 1972) to explain ^{34}S-depleted pyrite in the ore-zone of roll-front-type sandstone-hosted uranium deposits. Laboratory reactions have not confirmed abiotic disproportionation reactions involving intermediate S species produced as oxygenated groundwater interacted with disseminated sedimentary pyrite in the reduced, down flow, portions of the sandstone. In light of recent discoveries, it is likely that microbial

communities are associated with these ore-producing chemical gradients and that microbial reduction and disproportionation are responsible for ^{34}S-depleted pyrite in these types of U deposits.

The above model for sulfide oxidation and the production of unstable sulfur intermediaries depends on the availability of large volumes of oxygenated water. If such water is absent from the geologic environment in question then the proposed reactions cannot proceed. (Vovk, 1987b) has proposed that in the absence of oxygenated groundwater, radiolysis of H_2O by α, β, or γ rays may produce oxygen-equivalent oxidants (primarily H_2O_2 and OH). In sandstone-type U deposit the interaction between a radiation source and oxygen-poor interstitial water may produce radiolytically generated oxidants that would be similar in abundance to those derived from an atmospheric source. Radiation oxidation of sulfides in low-O_2 water results in the formation of both SO_3^{2-} and $S_2O_3^{2-}$ (DellaGuardia and Johnston, 1980). Vovk ((Vovk, 1987b)) suggests that the large spread in the ^{34}S values of pyrite and groundwater sulfate in roll-front-type U deposits are produced through a radiolytic process of oxidation followed by disproportionation of the intermediate S species. As noted previously, microbial processes may influence pyrite associated with uraninite because laboratory experiments have not demonstrated low-temperature S isotope fractionation during abiotic disproportionation of SO_3^{2-} and $S_2O_3^{2-}$.

Objectives-We propose to further evaluate the possible effectiveness of radiolytic sulfide oxidation in the production of $\delta^{34}S$ values that resemble those produced by bacterially mediated sulfide oxidation and disproportionation reactions. Initial experiments will involve ultra-pure water, freshly cleaved pyrite, H_2O_2,, and H_2 sealed in quartz tubes. ^{34}S values will be determined for residual pyrite and aqueous S species, and monitored with respect to the progress of oxidation reactions. Follow-up experiments will involve a low-level radiation source (e.g., Polonium-210 foil), ultra-pure water, and pyrite. Results will be used to evaluate the possible role of disseminated uraninite in the radiolytic oxidation of sulfide minerals and the sulfur isotopic systematics that accompany this process. Results of these experiments have far-reaching consequences for understanding how biosustaining chemical gradients can be established in the subsurface.

Sealed tube experiments will be performed to evaluate the S and O isotopic effects associated with the oxidation of FeS_2 by potential products of the radiolysis of H_2O. Initial experiments will track the changes in FeS_2 ^{34}S values, the sulfur isotopic composition of the aqueous phase, and isotopic variations in headspace an N_2:H_2. Pyrite in the experiments will be from hydrothermal veins in Park City, Utah.

Finely ground FeS_2 (< 20 μm) will be placed into silica glass reaction tubes and 5 ml of 1 and 10 mM H_2O_2 solution will be added. The water and H_2O_2 will be frozen into the tubes using a liquid N_2 cold trap and the sample tubes will then be evacuated. The mixture will then be thawed and the process repeated to ensure that all O_2 has been eliminated from the H_2O_2 solution. Although oxidation of FeS_2 by H_2O_2 does not normally yield H_2, an amount of H_2 equal to $1/100^{th}$ the molar concentration of H_2O_2 will be added to the tubes to evaluate H_2 isotopic exchange during the reaction. The reaction tube will then be sealed and placed into a horizontal resistance furnace controlled to 1°C. Experiments will be conducted at 60, 80, 100, and 110°C, with run durations ranging from 100 to 700 hours.

We anticipate that at the temperatures and run durations of these experiments only partial isotopic exchange will be achieved. We will monitor the extent of FeS_2 oxidation by determining the SO_4^{2-} and $S_2O_3^{2}$ concentrations in the aqueous phase. S species produced will be determined via spectrophotometric methods. The S isotopic compositions of residual FeS_2 and aqueous species (converted to barite) will be measured using elemental analyzer-continuous flow isotope ratio monitoring mass spectrometry. S isotopic fractionation between the residual FeS_2 and aqueous S species will be evaluated as functions of time, temperature, and extent of oxidation. The H_2 isotopic exchange and fractionation will be evaluated by measuring δ^2H on initial H_2O, H_2O_2, and H_2 followed by measuring δ^2H on final H_2O and H_2 gas. Similarly, O isotopic exchange and fractionation will be evaluated by measuring $\delta^{18}O$ on initial H_2O and H_2O_2 followed by measuring $\delta^{18}O$ on final H_2O and SO_4^{2-} and $S_2O_3^{2}$. If equilibrium is not attained in the longer duration runs we will be able to accurately estimate these values from the partial exchange results. Dissolution and precipitation of new minerals on the FeS_2 surfaces will be monitored using soft X-ray

spectroscopy and SIMS (Riciputi at ORNL). For experiments where the degree of S isotopic exchange has been small, it will be necessary to measure $\delta^{34}S$ values at grain margins on a 20 to 30:m scale. Ion microprobe $\delta^{34}S$ analyses will be required for these samples. Ion microprobe data will allow us to better model S isotopic exchange that may occur prior to the conversion of FeS_2 to aqueous S species and will allow detection of grain-to-grain isotope variability in experiments where sulfide oxidation is rapid.

Using the experience gained from the sealed-tube experiments, we will design an experimental reactor utilizing only ultra-pure water, FeS_2, and α radiation from a Polonium-210 foil. The configuration of the experimental reactor will allow for continuous monitoring of the chemical gradients across a 1-2 mm aqueous layer between the α source and the FeS_2 target. We will use the microelectrode chemical array (pH, oxygen, sulfide) currently in use at Indiana University for studies of sub-millimeter chemical gradients in algal/bacterial mats. All S, O and H species described in the sealed-tube experiments will be isotopically monitored for exchange and fractionation. After doing sterile radiolysis experiments, we will inoculate the reactor with sulfate reducing bacteria with and without growth nutrients and monitor the chemical gradient between source and target.

The common association of uraninite and pyrite in upper Archean and lower Proterozoic sedimentary rocks from Australia, South Africa and Canada (see references in Kimberley (1978)) suggests the intriguing possibility that biosustaining radiolysis facilitated the transition from anaerobic to aerobic ecosystems by allowing microbes in some sedimentary basins to evolve oxygen tolerance prior to oxygenation of the atmosphere.

Experimental Approach

Objectives-The focus of this research will be to perform biotic versions of the abiotic laboratory experiments, with and without irradiation, on heterogeneous fluid/mineral systems to obtain radiolytic production rates and biological utilization rates. The nonirradiated experiments will provide some indications as to whether certain microorganism can modify their environments to enhance microbial activity. The microorganisms selected will come either from our current set of subsurface isolates or from the ATCC culture collection yielding an isolate with the appropriate physiology and 16SrDNA most similar to our environmental 16S rDNA results. The physiologies to be included will be: an autotrophic methanogen, acetogen, aceticlastic methanogen, an H_2- and acetate-utilizing sulfate-reducing bacteria, a hydrocarbon-utilizing sulfate-reducing bacteria, an H_2-utilizing Fe(III)-reducing bacteria, an N_2 fixer, a nitrifier and denitrifier. Electron donor and acceptor concentrations will be measured by IC-MS and growth by flow cyotmetry. Depending upon the success of the Polonium-210 experiment described above, this approach will be used to establish a micrometer scale redox gradient that should prevent radiation damage of the selected microbial species. The reactive species forming on the mineral surfaces and to quantify any isotopic fractionation effect associated with the radiolytic products. These heterogenous fluid experiments will utilize anaerobic, Na-Ca-Cl solutions used in the abiotic experiments. Heterogeneous experiments will also use pyrite, magnetite, olivine, pyroxene and quartz. The pH and T of the solutions will be set at the optimal growth of the selected microorganisms and will be set at the pH and T of the fissure water if these two do not coincide (as they often do not). A mixture of inorganic salts will be used to buffer the solution. To examine the effects of dessication on microbial activity, the experiments will be performed with an equal volume of mineral and saline solution and then allowed to evaporate into an anaerobic glove bag over the course of several weeks until the mineral surface and Polonium-210 foil is saturated with just a thin film of saline solution. The surfaces of the minerals will be examined with XAFS to identify surface adsorbed aqueous complexes and by SR-FTIR to record changes in the metabolic state of the microorganisms. Finally, the heterogeneous experiments will be performed without irradiation, but with low levels of H_2O_2 and O_2 to see if the kinetics and products of these reactions can approximate that of the irradiation experiments. If so, this will greatly facilitate incorporation of radiolytic effects into standard biogeochemical modeling codes.

Tasks-The experiments will focus on H, C, N and S cycles.

1. H cycle-The production rates of H_2, H_2O_2 and O_2 will be determined as a function of temperature, pH, water saturation and Fe concentration in the mineral. The non-irradiated control experiments will be used to further quantify the effect of H_2 production via oxidation of Fe in mineral phases.
2. S cycle-see experimental description above.
3. N cycle-Anaerobic saline water with NH_4^+ and Ar and with N_2 will be irradiated to determine the stability of both phases during irradiation and whether oxidized N species, NO_2^- and NO_3^-, are produced. The aqueous N species will be measured by ICMS and the surface absorbed species by XAFS. The N and O isotopic composition of the different species will also be determined.
4. C cycle-Anaerobic saline water with CH_4 and CO_2 and with N_2 will be irradiated to determine the stability of the C phases during irradiation and whether oxidized or reduced C species, such as low molecular weight hydrocarbons or carboxylic acids, are produced. The aqueous C species will be measured by ICMS, HPLC and the surface absorbed C species by XAFS. The C and H isotope composition of the different species will also be determined. The homogenous experiments will be compared with the heterogeneous experiments to determine the effects of temperature, pH, water saturation and Fe concentration in the mineral.

Synchrotron FTIR Direct Analysis of Stress Changes in Living Cells.

The ability to image and characterize microbial responses to stresses in complex and extremely heterogeneous and transient natural environments has been one of the most challenging areas. Recent work from the Holman group (Holman et al., 1999; Holman et al., 2000; Holman et al., 2002) suggests that one can meet this challenge by using synchrotron-radiation-based Fourier transform infrared (SR-FTIR) spectromicroscopy, especially by combining SR-FTIR results with information from other bioanalytical and imaging techniques. Here, we propose to use this as a real-time technique to characterize microbial resistance to stress.

Synchrotron radiation is utilized because the infrared photons extracted from the radiation source can be focused to a spot less than 10 μm (in diameter). The LBNL Advanced Light Source, the brightest radiation source in the world, routinely obtains high infrared intensities with a signal-to-noise ratio that is at least 100 times better than any infrared thermal source at a spatial resolution of 3 to 10 μm (Lumppio et al., 2001). This unique property allows very sensitive discrimination of IR spectra, even on geological materials that inherently have very low infrared reflective surfaces and at the same time often have a spatial variation of typically 3 to 10 μm. More recently, Holman's group has proved experimentally that although the infrared light from ALS's sychrotron radiation source is very intense, it neither heats the sample nor affects the physiology of a living biological system (Holman et al., in press). Unlike other surface chemistry microprobe techniques (e.g., UV or x-ray microprobe), the SR-FTIR spectromicroprobe is truly a nondestructive and noninvasive technique that provides for direct interrogation of the same location of geological materials with microorganisms. This provides researchers with an extremely beneficial opportunity to quickly track and image the fine-scale (3–10 μm) chemical changes in different compartments of a living biofilm on surfaces of geological materials without the need for staining procedures or chemical tags (see Fig. 21).

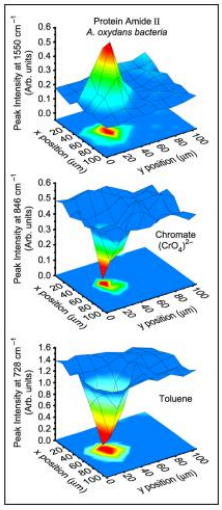


Fig. 21. SR-FTIR mapping showing *Arthrobacter oxydans* bioremediating mixtures of Cr^{6+} and toluene on a mineral surface (Krupa, 1999).

The microcosm experiments, such as microbial response to dessication and formation of thin H_2O films can be observed directly with SR-FTIR. The DSPS and in situ experiments (discussed below) will provide polished rock chips from the sampling site can be inserted into and removed from the chambers and reactors at different points to monitor biofilms on the surface using the SR-FTIR technique. This way, the resistance to stress in these systems can be measured, IR signature changes can be documented, and population changes can be imaged in the biofilm on the chip, all in real-time. This alternate method for measuring stress changes in the cell can also cross-verify the expression analysis studies for the stress regulation pathways in the environmental simulators and chemostats.

Thus although geological materials are inherently heterogeneous and have low reflectivity surfaces, the high brightness of the synchrotron beam enables one to measure, identify, and locate key compounds undergoing changes, as well as microbes involved in the changes. The technique has been successfully used to identify a new pathway of chromate reduction on surfaces of geological materials (Holman et al., 1999) and to provide direct insight into the relationship between localization of chromate reduction, toluene transformation, and *Arthrobacter oxydans* bacteria distribution on surfaces of mixed-valence iron oxides (Fig. 21).

3. LABORATORY SIMULATION OF SUBSURFACE MICROBIAL COMMUNITIES

(***Phelps, Pfiffner, Pratt, Sherwood-Lollar and Hazen)***

SPS Operations Overview of Seafloor- and Deep Subsurface- Process Simulators (SPS and DSPS)

Background-Recent technological developments enable examination of biogeochemical interactions at temperatures and pressures of native deep biosphere environments. Temperature controlled high pressure test facilities at ORNL are available through this research to perform experiments examining the biogeochemical interactions between life processes and the physical environment typical of those several km below land surface or beneath seafloors at temperatures >100°C and pressures greater than 300 bars.

Laboratory experiments typically accomplished with small test tube-sized vessels differ fundamentally from field experiments because of scaling, poor visualization, insufficient monitoring, lack of detailed sampling and wall effects. These limitations make it difficult to extrapolate from small-scale experiments to natural systems. Scaling of dimensions, appropriate for detailed scrutiny with precisely controlled meso-scale experiments, can be accomplished with the ORNL Seafloor Process Simulator (SPS; Fig. 22) a large (72-L usable volume, maximum linear dimension 0.9 m) corrosion-resistant Hastelloy pressure vessel located in an explosion proof cold room, as described in Phelps et al. (2001)).

Characteristics of the SPS include operating ranges of -2 to 20°C (with the addition of a heating element can go to >50°C) at pressures of 0.1 to 20 MPa that covers the relevant regions of the seafloor hydrate phase diagrams, most near surface systems including regions of supercritical CO_2. The SPS also features the ability to extensively sample without significantly altering pressures or volumes. Sampling will be accomplished through a series of high-pressure ball valves connected to 1/4 -3/8 high-pressure stainless steel tubing that is again sealed with high-pressure ball valves and a vent needle valve. Accordingly, by sequentially operating the ball valves 1-50 ml (g) aliquots can be transferred to and from the vessel. Six sapphire-viewing windows (3/4 to 2 in diameter) facilitate visualizations and video or digital photography. A Saphwire-windowed boroscope provides a 10 μm size resolution. Laser-Raman spectroscopy is also available through the boroscope or through sapphire windows. Injections into the SPS include gaseous, liquid injections into the liquid, and headspace or sediment phases through.

Capable of even higher temperatures and pressures is the newly established Deep Subsurface Process Simulator (DSPS; Fig. 23), a Monel-alloy high-pressure apparatus capable of >325 bar at >100°C. This system was designed for evaluating deep terrestrial carbon sequestration and is capable of evaluating various fluids including; aqueous, brines, hydrocarbons and super critical carbon dioxide in native subsurface solid matrices. In this system the subsurface materials are placed into 10-50m length columns of a nominal 3/8" internal diameter. Fluids and or gases are then injected into any of a dozen Monel pressure chambers (50-500 ml volumes) where they are equilibrated at pressure and temperature prior to being pumped into and or through the pre-packed sediment columns. Effluents from the test apparatus automatically pass into an autosampler that can aliquot samples into fraction collectors, subsamplers, a GC and or an HPLC. Similar to the SPS the system is computer operated and data logged by LabView.

Both temperature-controlled, high pressure systems represent unique experimental facilities, ideally suited to the determination of biological, biogeochemical, kinetic, thermophysical, and mechanical properties of the deep subsurface environment and martian hydrosphere while permitting direct observations of processes, and investigation of how these processes are influenced by of partial pressures, temperature, salinity, gas or liquid phases, or biogeochemical transformations. Through the use of natural rock fragments in our experiments, we can investigate the response of microbial communities to high gas/liquid ratios where the saline fluid will "wet" the surfaces and internal pores spaces of the rock fragments.

Objectives-The overall objective of the proposed task is to gain a basic understanding of complex biogeochemical effects of perturbations such as changes in temperature, pressure, nutrient bioavailability and their impacts on the diversity, abundance, activities and metabolomics of subsurface derived microbial trophic groups.

1. Quantify key biogeochemical consequences of perturbations;
2. Evaluate the changes in microbial community composition and assess the functional gene diversity and metabolomics of the microbial community; and
3. Identify potential molecular markers from the microbial community that can be used for the detection and assessment of biogeochemical perturbations and associated consequences.

Experiments- Experiments will rely on rock fragments from cores of the 2.7 Ga Ventersdorp volcanic formation and 2.9 Ga ore-bearing Witwatersrand quartzite/conglomerate collected in South Africa and rock fragments collected from cores obtained from the new field site in northern Canada, complemented by sediment gathered from Ocean Drilling Program (ODP) Legs 204 and 201. Phelps was affiliated with Leg 204 ("Hydrate Ridge") that collected and preserved >30 meters of methane hydrate-rich sediments off Oregon in Summer 2002 archived under in situ CH_4 partial pressures at the ODP TAMU site, College Station, TX.

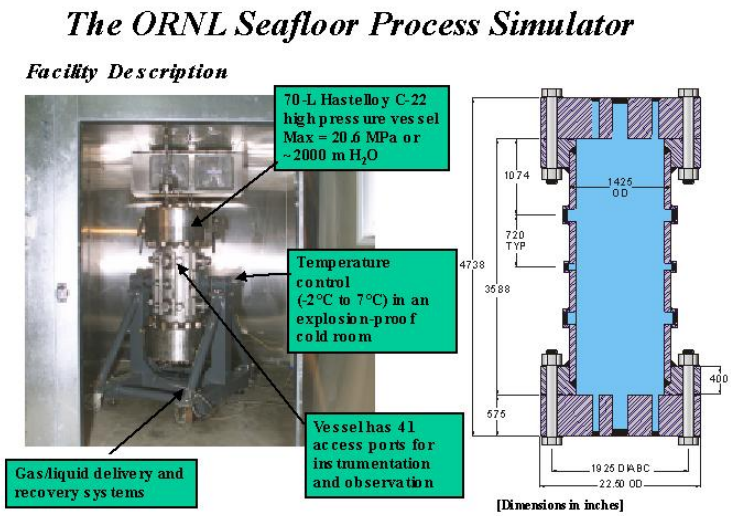


Fig. 22. SPS-Seafloor Process Simulator is constructed of corrosion resistant C-22 Hastoloy with a 72 L internal volume. The 32 cm I.D. x 90 cm length is capable of operation at any angle. 41 access ports with ¾" to 2" NPT fittings. Six sapphire windows. Influent and effluent flow meters. Flow rates can be accurately metered from 0.1 ml/min. to 10 ml/min. Simulator resides in an explosion proof cold room maintained from –2°C to 20°C (type K thermocouples +/- 0.2°C). Fluid pH monitored by 6 high-pressure probes. Visual records obtained with digital camera and boroscope. Internal pressures are measured using a series of piezoelectric transducers with a working range of 0 to 200 bars. Temperatures within the vessel are measured using a series of Hastelloy-sheathed type K (Riestenberg et al., 2003) thermocouples - which are calibrated to an accuracy of +/- 0.2°C. Six pH probes are also currently adopted for internal use. Data are collected in time sequences of 10 - 60 seconds using a data acquisition system consisting of National Instruments Field-point modules connected to a PC using LabView v5.1.

Early experiments will be used to develop the baseline operation of all injectors and sample withdrawal equipment. Liters of sediment will be added to the SPS (or <kgm volumes to the DSPS) vessel in the upright position and rotated ~120 degrees to the horizontal position to spread the sediments uniformly across the curved bottom of the SPS. If necessary, an additional 3 to 5 liters of rock fragments may also be added or a window unit removed to facilitate the spreading of rock fragments via raking to provide adequate coverage. Six liters of rock fragments should provide ~5 cm depth at the deepest location throughout the 0.9 m length of the vessel. It is estimated that 12 liters of sediment would provide a maximum depth of nearly 8-cm, the length of the SPS. The vessel will then be nearly filled with injected, artificial aqueous solution of fresh or sea water (previously sterilized and cooled), such that the headspace is less than 1 liter (eliminating headspace gas is unlikely in horizontal SPS operations) and equilibrated overnight at ~5°C and at pressures ~70% of those experimentally desired. For this project, we anticipate experimental temperatures of ~5°C and pressures of ~100 bar, which it the anticipated formation T and P at 1000-m depth in northernmost Canada. Previous experiments demonstrated that after 12hr of equilibration the vessel contents varied less than 1°C and after 16 hr vessel contents vary less than 0.2°C, which is near the accuracy of thermocouples and thermistors.

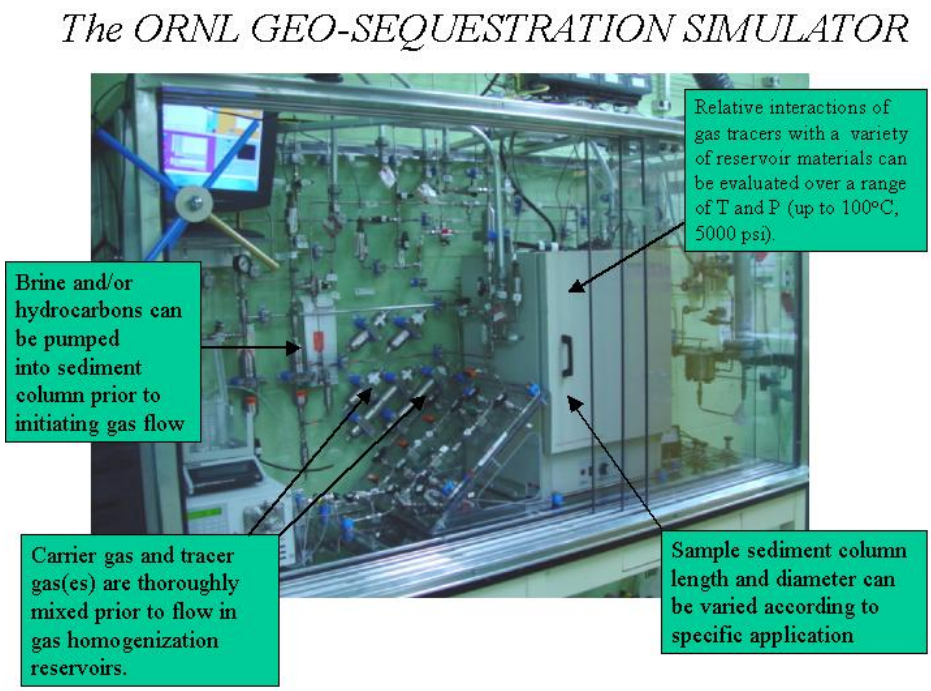


Fig. 23. Constructed of corrosion resistant Monel (brine injection areas) and 2507 stainless steel with Swagelock valves and tubing. Working pressure range up to 344 bars. System can operate with CO_2 as a gas, liquid, or supercritical fluid. Working temperature up to 100°C. Temperature measurements by type T thermocouples. Pressure measurements using piezoelectric transducers (0-6000psi). Data continuously measured using Labview System can be remotely operated via internet connection. Metered flow rates from 0.01ml/min to 10ml/min. Multiple gas homogenization reservoirs (75ml, 225ml, 50ml) allow for continuous sampling. Valco sample valve inject gaseous effluent directly into HP gas chromatograph with an electron capture detector. Conservative tracers include stable isotopes, noble gases, nonreactive salts, and perfluorocarbonsCapable of measuring fate and transport of biogeochemical parameters including varied gas, aqueous, and solid phases under reactive subsurface media

Upon equilibration (SPS or DSPS), the final pressures will be adjusted through additions of subsurface saline water or brine, N_2, CO_2 or liquid CO_2 or CH_4 and allowed to equilibrate for approximately 4 hrs. Upon secondary equilibration completion, samples (time =0) will be withdrawn and analyzed, as described later below. All sediment and aqueous subsamples will be retrieved using the high-pressure transfer chambers. These post depressurization subsamples will be microbiologically, geochemically, and mineralogically characterized during time course perturbations. Throughout the course of each experiment, temperature, pressure, and pH measurements will be monitored from three or more locations. Once quasi-steady state has been established (~6-9 days into an experiment), the vessel will undergo one or more perturbations (liquid or gas) via injection into the aqueous or porous rock phase. Experiments planned for the first year will pursue end member analyses of highly perturbed levels of pCO_2, pCH_4, pH_2, pH, redox, and nutrient amendments, on microbial metabolism and biogeochemistry. Such highly perturbed end member experiments will allow us to fine-tune the boundary conditions for SPS/DSPS operations. Second and third year experiments will focus more on biogeochemical interactions over time. The experiments will be iterative in that lessons learned will be incorporated and factored into designs. Time course characterization will include assessments of changes in the microbial community composition over time, assessment of the changes in metabolic diversity in response to altered nutrient bioavailability, and perturbations and environmental biogeochemistry alterations with time.

Analyses-Total C, N, and S will be determined on rock samples using a Leco dry combustion furnace. Rock fragment surfaces from time-course sampling will also be mineralogically characterized SEM with

energy dispersive X-ray. A JSM-35CF scanning electron microscope (SEM), utilizing both secondary electron imaging and backscattered electron imaging in conjunction with energy dispersive and wavelength x-ray spectrometer, will be used for the analysis of morphology, particle size, and elemental distributions on rock fragment surfaces.

Analysis of the pore fluids and overlying water phase will be important to constrain mass balances and subtle chemical changes in the rock system that may not be measurable by solid phase techniques. In addition to measuring the chemistry of these aqueous phases (e.g., Ca) by ICP-AES and ICP-MS, major anions will be analyzed by IC. $Fe^{2+/3+}$ and HS^- will be measured colorimetrically. Methane, inorganic carbon, and volatile fatty acids will be determined by GC (TCD/FID). H_2 will be measured as a headspace gas using GC-thermal conductivity detection (TCD). Reduced metal precipitates will be assayed mineralogically as described below. After dramatic shifts in bioprocessing are noted, ^{13}C-substrates will be added to select experiments with the distribution of the stable carbon isotopes followed over time into microbial biomass, lipids, and end products such as acetate, methane and VFAs determined by GC-MS. When radioactive ^{14}C or ^{3}H are used in time course metabolomic studies the radioactive end products will be determined by GC-gas proportional counting (GC-GPC) as described previously or by newer procedures being demonstrated by the PI's ((Cochran, 2001)). Microbial lipids and metabolic functions will be analyzed by personnel at UTK under the direction of White/Pfiffner/Peacock (see section 4 below).

DNA extraction, amplification and sequencing will follow the protocols of Qui et al. ((Qiu et al., 2001)). The sequences obtained will be compared with reference sequences from GeneBank and aligned with ClustalW and compared with reference sequences ((Thompson et al., 1994)). Phylogenetic and molecular evolutionary analyses will be conducted using MEGA version 2.1 ((Kumar et al., 2001)), and phylogenetic trees constructed with distance matrices and the neighbor-joining method.

The functional gene diversity has been assessed for a variety of environments and has been shown to serve as an indicator of biochemical capacity and stability. In conjunction with metabolimic and phylogenetic analyses, gene microarrays will be used to assess the response of the community activity to the perturbations during time course experiments. These microarrays have been fabricated for the DOE-GTL program with thousands of gene probes on a small surface area so that genes from an entire community can be monitored. Accordingly, no new microarrays will be developed for this work, rather accessing existing microarrays developed by DOE-funded research to provide additional insights into functional diversity of extreme environments. In addition to high throughput and parallel analysis, microarrays offer a number of other advantages over conventional molecular methods including rapid detection, high sensitivity, multi-color detection for differential display, lower cost, automation, and high signal to background detection (Shalon, 1996). Both DNA and oligonucleotide microarrays have been successfully used for monitoring gene expression and detecting differentially expressed genes in bacteria (Tao, 1999; Peterson, 2000; Ye et al., 2000). These studies demonstrate that microarrays allow researchers to switch from the very focused single gene view to a global view of entire genomes and communities. In addition, the applicability of this method has been tested for studying microbial community mRNA and DNA isolated from soils and marine sediments (Wu et al., 2001). The functional gene array (FGA) will contain many genes of interest as well as the following controls: 16S rRNA genes as positive controls and five yeast genes as negative controls. DNA samples will be arrayed with a single pin (ChipMaker 3) using a PixSys 5500 robotic printer (Cartesian Technologies, Inc.) on silane-modified slides (Cel Associates). The slides will be post-processed as previously described (Wu et al., 2001). We will also use denaturing gradient gel electrophoresis (DGGE) to monitor changes in the microbial composition as previously described (Ferris et al., 1996).

4. FIELD GEOCHEMICAL, ISOTOPIC AND MICROBIAL ANALYSES AND IN SITU EXPERIMENTS

(Onstott, Moser, Sherwood-Lollar, Brockman, Fredrickson, Pratt, Pfiffner, Phelps, van Heerden and Hazen)

Field Sites for Biosustainable Subsurface Environments

South Africa-As a result of our previous characterization studies in South Africa, we have at our disposal five flowing boreholes distributed in depth from 0.95 kmbls. to 3.2 kmbls. and ranging in salinity from 0.01 M to 0.5M and temperatures up to 60°C. The most saline of these boreholes has a δ^2H and δ^{18}O values that lie off the meteoric water line (Fig. 3) and He-Xe ages of 40-80 Myr (Fig. 2). The lipid and 16SrDNA analyses of these boreholes are complete and reveal a diverse population of Archaea and bacteria, isotopic evidence of sulfate reduction and abiogenic C_{1-4} formation. This large diameter borehole inclines downward at 60° and intersects a couple of fractures at depths >2.0 kmbls. As such, it is ideal for using a multi-level sampler designed by our team for determining substrate incorporation and quantifying in situ respiration.

Northern Canada-The brines encountered by Con Au mine near Yellowknife, NWT have been the subject of numerous previous publications (see, for instance, Frape and Fritz, 1987). The mine contains numerous boreholes emanating brine at various depths. The origin of the brine has been somewhat controversial, but recent ^{129}I analyses yield a minimum residence time of 80 Myr (Bottomley et al., 2002). This is considered by the authors to be a minimum subsurface age as their preferred interpretation for the brines origin is that it represents ancient seawater, trapped in these metamorphosed mafic rocks during the Middle Devonian at which time the Canadian Shield was covered by seawater (Bottomley et al., 1999). Bein and Arad (1991) suggest that the brines could also be created by freezing of seawater and infiltration beneath the continental ice sheet during the Pleistocene at ~ 1 Myr. Isotopic data are most consistent with the former interpretation and indicate these subsurface brines have been beneath a frozen permafrost cap for ~10^7 years until quite recently. In this respect, their origin and hydrogeological history is very distinct from that of the saline groundwater in South Africa, which originated as meteoric water infiltrating from the surface, as this part of South Africa has not been covered by seawater since 2500 Myr. Given the different origins of these brines and the lower formation temperatures at Con Au mine, it will be of interest to know whether the subsurface microbial communities exhibit similar dominant species and activities. The isotopic signatures of CH_4 from Con Au mine is similar to that of Kidd Creek suggesting an abiogenic origin. The antiquity of this brine qualifies it as a biosustainable subsurface environment, if we find evidence of indigenous microorganisms. The chief geologist at Con Au mine has offered their assistance in providing access to these boreholes (see letter of collaboration).

Nearby, the Ekati diamond mine has started developing an underground mine, which should be available to our team within a year or two. The 50 million year old kimberlite cuts quartz-feldspathic basement and brine has already been encountered at 0.5 kbmls. in an exploration borehole that can be sampled by our team. As fresh brine intersections will be encountered during the lifetime of this mine, it represents an opportunity to obtain both core samples and to monitor drilling contamination during these intersections. Ekati mine is accessed from Yellowknife and like Con Au mine is logistically the most convenient and least costly.

The disadvantage of Ekati and Con mines is that they no longer have permafrost caps. Lupin Au mine and Nanisivik Pb-Zn mine in Baffin Island do have permafrost caps and underground works that would potentially provide an opportunity to examine the relationships of subsurface brine communities and the freezing front. Contacts with mine geologists have been made and access is being discussed (see email correspondence).

Finally, our team has contacted Kidd Creek Au mine in Timmins, Ontario and the Pt mine in Sudbury as potential brine sampling sites given their isotopic and geochemical similarity to Con Au mine, but distinctly different rock types and depths.

Regardless of which mine becomes the host of the proposed research, reconnaissance sampling of brine emanating boreholes will be performed in year 1. Like our South African site, a suite of isotopic and geochemical water and gas samples will be collected along with filters of the water for microbial analyses by 16SrDNA and microbial enrichments. For Con Au mine brines, because so much work has already been completed on the isotopic signature of the water, its age and its major anions and cations and because Clark's research group at Univ. of Ottawa is already performing noble gas isotope analyses, our team will focus be able to focus on the redox metal chemistry, the microbial and DNA analyses, the $\delta^{34}S$ of the sulfate and sulfide and the $\delta^{2}H$ and $\delta^{13}C$ of the C_{1-4} compounds. The $\delta^{33}S$ and of $\delta^{36}S$ sulfate will also be measured for the mines in Archean rock since their sulfide should preserve an anomalous signature of mass independent fractionation (Farquhar et al., 2000). The presence of mass independent signatures in the sulfate would support local derivation by subsurface oxidation and not transport from overlying post-Archean formations.

The fissure water sample collected from mine boreholes in South Africa typically have temperatures ranging 50 to 60°C, which discourages the growth of mesophilic contaminants. For the Canadian mines with their lower formation temperatures, however, much greater care will be required to avoid mining contaminants. Field data such as salinity, sulfide and O_2 concentrations will be used to target brine samples and minimize the probability of contamination. Associated core samples, if available, will be frozen and processed back at the lab, paying particular attention to segments corresponding to water-bearing features. With this information and by working closely with mine geologists, it should be possible to collect water from fresh intersections within hours or days of a water strike and before contaminating microbes have had a chance to bloom within the flowing borehole. Flowing boreholes will be sealed and valved using a well-tested telescopic packer and sampling manifold design (Moser *et. al* 2002) and with stainless steel cartridge filters with pressure relief valves and check valves, which allow for the aseptic and anaerobic sampling of boreholes.

Based upon these results obtained on these samples we will select a mine in year 2 for follow up study that focuses on Gel microdroplet (GMD) enrichments and in situ experiments.

HomeStake Au mine-A new National Underground Science Laboratory that will house higher sensitivity neutrino detectors at 2.5 kmbls. at Homestake Au Mine in Lead, South Dakota. As part of this intiative, a new multimillion dollar NSF program called EarthLab is being submitted to NSF next year to establish an underground laboratory for geomicrobiological investigations. The facility will undertake coring down to 6 kmbls. reaching formation temperatures of >100°C to search for subsurface hyperthermophiles in uncontaminated rock formations. Fracture networks will be instrumented with boreholes, geophysically imaged and used for in situ experiments and microbial transport experiments. Hydrofracturing experiments will investigate the effects of rock fracturing on nutrient supply to subsurface microbial communities. A deep recharge experiment will instrument one fracture zone from 0.1 kmbls. to 2.7 kmbls. to examine interactions of photosphere microbial communities and metabolites with deep subsurface communities. This underground lab will provide opportunities that will not be attainable at a working mine. The time frame for the construction of this laboratory will overlap with the 5 year duration of this proposal and conceivably will present new opportunites and capabilities for investigating deep subsurface biosustainable habitats.

Gel Microdroplet Enrichment

Background-Traditional procedures for cultivation of microorganisms from environmental samples rely on the use of solid, semisolid, or liquid medium containing relatively high concentrations of nutrients to encourage relatively rapid growth or microorganisms to high cell densities. The success of such techniques is often equated to the formation of visible colonies on plates or extensive increases in turbidity of the medium. Yet, the concentrations of cells required to detect a change in turbidity by spectroscopy is ~10^6 cells mL^{-1}, much higher than that found in our deep subsurface fractures (Fig. 15). Visible colonies on plates requires a microcolony of at least 10^5 cells and strongly selects for microbes that are fast-growing, resistant to high concentrations of nutrients, and able to grow in isolation. We

argue that these strategies are counter to the normal growth habit of most deep subsurface microorganisms and a major reason why most microorganisms in subsurface environments appear to be uncultivable. The 16S rDNA gene sequencing results from the South African samples record a considerable prokaryotic phylogenetic diversity including members of the *Archaea* and novel lineages within the *Euryarchaeota* (figs. 4-8; (Takai et al., 2001a), but only a few living and archived isolates have been obtained to date. This is despite the fact that our team utilized filter-sterilized fissure water for enrichment media preparation in order to exactly replicate the major element chemistry. Our continued lack of success in attempts to transfer multiple times microorganisms from positive enrichments of fissure water into fresh media comprised of artificial fissure water is attributed our requirement to obtain ~10^6 cells mL^{-1}. Another complicating factor with dilution and transfer approaches is that it requires the target physiology to grow in isolation. In the fracture environment, however, the planktonic species are probably associated with a much larger microbial community that has colonized the fracture surfaces. Because of the difficulty of obtaining fresh, uncontaminated samples of these fracture surfaces our only strategy to sample this part of the population is to immerse into the borehole or pass the emanating fissure water through cartridges containing sand or crushed formation rock in the hopes that the sessile species will colonize these surfaces. The enrichment strategy then employs a mixture of this sand or rock and its pore water where multiple species can grow in proximity to each other.

Objectives-The objective of this task is to apply a novel technology for the cultivation of previously uncultivable microbes with an aim towards their subsequent characterization by genomic and physiologic methods and eventual use in laboratory microcosm experiments. This technology, developed by and to be conducted by Diversa Corporation, is based on encapsulation of single (or a small number) of cells within individual gel microdroplets (GMD's) followed by reconstitution of the starting microbial community by loading the GMD's into a column. The community is grown in the column under very low nutrient flux conditions using filter-sterilized water from the sampling site. Over time, each cell capable of growth under the conditions in the column forms a microcolony within the its GMD. A high-speed flow cytometer and cell sorter is then able to identify GMD's containing microcolonies of >100 cells using intrinsic forward and side scatter and sort each positive GMD into a well of a microtiter plate. The individual cultures can then be: grown further by adding effluent collected from the growth column and performing metabolic analyses, studied by FISH with various gene probes or used for partial genomic sequencing in combination with rolling circle amplification.

Although the use of dilution culture has been successfully applied to the cultivation of "oligobacteria" from natural aquatic assemblages (Button et al., 1993) it has not, to our knowledge, been applied to the cultivation of deep terrestrial microorganisms. The Diversa approach extends Button's oligotrophic medium-limiting dilution approach by replacing the glass tubes with microscopic "polymer cages" (i.e., the GMD). This confers several advantages for cultivating microbes. First, the GMD's allow the microbial community to be grown together versus individually in glass tubes, yet allows each "caged microcolony" to be later separated and analyzed. Second, microbes are grown in a flowing open system that simulates natural environments, where microbes are exposed continually to a very low concentration of nutrients. This is in contrast to a closed batch system where microbes receive a high concentration of nutrients at one point in time and metabolic byproducts can build up to unnaturally high and inhibitory concentrations. The low concentration of nutrients also minimizes overgrowth of fast-growing organisms, thereby allowing propagation of microbes with extremely slow growth rates and/or that only grow to low cell densities. Third, the ability to reconstitute the community within the GMD's of the column allows for diffusive cross-feeding of metabolites and other molecules (e.g. regulatory molecules) between members of the community. This feature also simulates the natural environment, and thus preserves some of the community interactions and other specific requirements that may be needed for successful cultivation.

The Diversa approach has been shown to enable cultivation and isolation of previously uncultivated microbes ((Zengler et al., 2002)). Methanogens have been successfully encapsulated, grown, sorted by flow cytometry, and grown post-sorting, demonstrating the technology can be combined with standard

anaerobic methods to grow strict anaerobes. In addition, recent data has shown fluorescent nucleic acid probes will hybridize to microcolonies within gel microdroplets, enabling flow cytometric screening and sorting based on nucleic acid probes or antibodies. In summary, we believe this novel culturing approach will result in the ability to culture and study a much greater number of organisms from the ultra-deep South Africa subsurface environment.

Cell concentration-Because of the low cellular concentration in the South African fissure water several cell concentration approaches have been developed and will be used in parallel to assure the availability of undamaged, cells of a suitable density for encapsulation. Cells will be concentrated *in situ* behind 0.1 μm MediaKap hollow fiber filtration units, such that particulates from hundreds or thousands of liters will be concentrated into a small volume (ca. 150 ml). These filters will be back-flushed in the field into sterile, Ar-filled serum vials and transported to surface on ice. An alternative method will involve tangential flow filtration (TFF), which has been used in preparation for gel encapsulation of planktonic microbes (Zengler et al., 2002) and a field-ready technique for the strictly anaerobic concentration of cells by TFF has already been successfully demonstrated in previous sampling in the mines. Another approach will be to use high surface area cartridge filters in stainless steel containers. Like the Mediakap hollow fiber filters they will be directly attached to the borehole and will filter tens of thousands of liters, but can handle greater flow rates and pressures. They can then be sealed and removed from the borehole without exposure to air and the canister with filter and ~1 L of filtered water and several sealed canisters of the filtered water will be shipped to the U.S.

Encapsulation-As few as 10^4 cells in 0.1 ml can be encapsulated at ~ 10% efficiency, and efficiencies improve to >90% at >10^6 cells per 0.1 ml. Cells concentrated from fissure water will be encapsulated directly, while those from rock samples will be first concentrated by nycodenz gradient centrifugation (Zengler et al., 2002) prior to encapsulation. Singly occupied GMD's, or multiply-occupied if multiple cells are attached to one another) of approximately 50 microns in diameter will be generated using a microdrop maker licensed to Diversa. Only a very small percentage of the total GMD's contain a cell. The encapsulation procedure will be carried out in an anaerobic glove bag.

Growth columns-To minimize the need for collecting and shipping large quantities of groundwater, the cultivation system will be a closed, recirculating loop (Fig. 24). Since the media is pumped through the column at a low rate (10 mL/hr), we propose to use 3 L of media for each column assembly. At this rate, the water reservoir will have a turn over time of 9.6 days. Each assembly will consist of a growth column and an upstream cartridge containing crushed rock chips, sourced from as near the fissure zone water source as possible. These chips will provide fracture surfaces, which may generate soluble weathering products potentially required by the microbes for energy or nutrition. It may also be possible to seed the growth column with milled and cleaned 0.1 to 1 mm-diameter rock from host rock from the location fissure water was sampled. In this case rock particles would be carefully removed by anaerobic centrifugation prior to sorting of GMD's. The GMD's (10 million per column) will be dispensed into sterile chromatography columns equipped with a 0.1 um membrane filter at the inlet and a 8 um filter at the outlet. A second 0.1 um filter will be placed immediately upstream of the media reservoir return. This prevents cells from contaminating the media reservoir and retains GMD's in the column while allowing any free-living cells to be washed out.

Preliminary work at Diversa indicates the GMD formulation can be modified to generate microdroplets that are stable at elevated temperatures. Diversa's cultivation system allows for different medium conditions in different growth columns. Individual columns are snapped into place on a rack and connected to media reservoirs via quick-connects. Individual columns can also be temperature controlled. Incubation of growth columns will be in an anaerobic glove bag.

Growth media-As in the past, we will use on-site filter-sterilized mine water as a basal medium rather than synthetic basal medium or simulated mine water and does seem to work better than using strictly artificial media (Rappe et al., 2002; Zengler et al., 2002).

Deep fissure waters contain high concentrations of various gases. While readily available, for safety-related reasons, we do not feel that the collection and transport of mine gas in quantity is advisable. Nor will it be possible to infuse the cultivation system with gases at the 10's of mPa pressures expected in the deep subsurface. Rather, the entire cultivation system will be infused with a custom gas mix that approximates the composition noted in the mines and maintained at ca. 2 bar (Fig. 24). Infusion with reduced gases may be important, since it is possible that they serve as respiratory electron donors for certain microbes in this environment. A typical brine gas mix might contain, in decreasing abundance, CH_4/ N_2/He/H_2/C_2H_6/C_3H_8/CO. Previous N and P analysis have shown many brine samples with NH_4^+ at 0.1 to <0.0001 μM and PO_4^{3-} at 0.5 to <0.05 μM. Geochemical analyses of these trace elements and trace metals will be used to determine if any amendments are required to ensure adequate availability of these compounds at <0.1 μM levels.

Our general philosophy follows that of others (Bruns et al., 2002; Janssen et al., 2002) who simulated key chemical aspects of an environment to improve the cultivation of microbes from that environment. Cells will be grown with filter-sterile and gas-charged groundwater under 4 different anaerobic electron donor-acceptor combinations at or near the *in situ* temperatures of the samples (Table 1). The addition of electron donors and acceptors simulate the known geochemistry and biogeochemical processes of the waters and are used in different combinations to stimulate growth of different physiologies in the growth columns. One assembly will be maintained without supplementation other than the headspace gas mix. In three other assemblies, alternative energy substrates will be tested. This may vary as the work progresses, but initially we propose to test alternative electron acceptors coupled with, in addition to the dissolved gases, supplemental organic carbon sources. In case the fissure water contains adequate sulfate to support sulfate reduction (Takai et al., 2001a; Moser et al., 2003), one column assembly will be supplemented with only organic carbon sources. The acetate may also stimulate acetoclastic methanogenesis. In some cases, saline fissure water already contains as much as 100 μM of acetate or formate. In this case an alternative organic acid, e.g. benzoate, will be used to select for fermenters or aromatic oxidizers. To stimulate microbial Fe(III) reduction, another will be supplemented with organic substrates and Fe(III)NTA. Both of the column assemblies with organic substrates added may also support lactate fermentation and propionate-oxidizing syntrophs. Finally, one column assembly will be established with an N_2/CO_2 headspace and supplemented with thiosulfate ($S_2O_3^{-2}$). In this case, a small amount of MnO_2 will be injected into the media reservoir to react with the sulfide and regenerate thiosulfate to enhance favorable thermodynamic conditions for thiosulfate disproportionation.

The flow cytometer is able to analyze 5,000 GMDs per second, which is important because the vast majority of GMD's do not receive a cell during the encapsulation procedure. The flow cytometer can easily distinguish between the light scattering properties of unencapsulated single cells, empty or singly occupied GMD's, and GMD's containing a microcolony (Zengler et al., 2002). The cell sorter function of the instrument will distribute microcolony-containing GMD's into separate wells of 96-well microtiter plates.

Analysis of microcolonies-Microcolony-containing GMD's will be grown in microtiter wells by adding effluent (to a total of 0.5 ml) collected from the parent growth column, and/or by addition of simulated fissure water derived from geochemical analyses of the sampled water. Prolonged growth of the 0.5 ml cultures typically leads to rupture of the GMD (Zengler et al., 2002) and provides biomass sufficient for multiple types of analyses. Cultures will undergo metabolite and 16S rDNA sequence analysis, and also be archived. For metabolite analysis, culture supernatant will be flash frozen for subsequent metabolite analysis by GC and HPLC. An aliquot of each culture will be archived for potential future growth studies by adding glycerol and flash freezing with liquid N_2.

As noted above, DNA will be extracted from the cartridge filter produced during purification of the fissure water used in cultivations. This DNA will be amplified with Archaeal and Bacterial 16S rDNA-targeted primers, followed by the generation and sequencing of clone libraries. This will establish a baseline microbial community structure that will be compared to the community cultivated in the GMD growth columns.

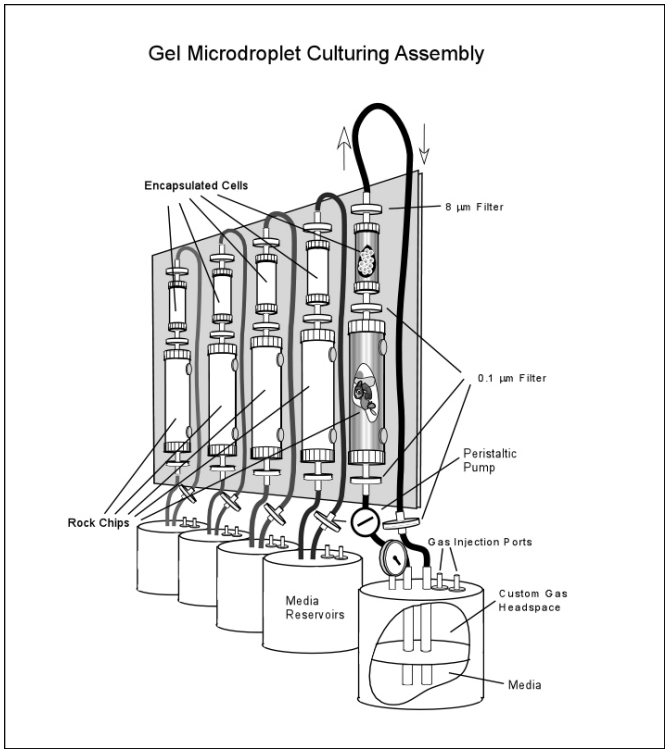


Figure 24. Cell cultivation assembly for GMD's.

Table 1. Supplements to be added to filtered fissure water in column experiments.

Target physiology	Gas mix	Organic electron donor	Electron acceptor
Multiple physiologies[a]	yes[b]	no	no
Sulfate reduction; acetoclastic methanogenesis	yes[b]	yes[c]	no[d]
Iron reduction [500 µM Fe(III)NTA]	yes[b]	yes[c]	yes[e]
Thiosulfate disproportionation (500 µM $S_2O_3^{-2}$)	yes[f]	no	no

[a] Includes hydrogenotrophic methanogenesis. [b] $N_2/CH_4/H_2/CO_2$/Propane. [c] Formate/acetate/lactate/methanol/benzoate, 20 µM each. Benzoate is a model for aromatic compounds detected in fissure water.

[d] Fissure waters typically contain natural sulfate. [e] Fe(III)NTA at 1 mM. [f] N_2/CO_2, 80:20

Sorting- The GMD's will be sorted after 4 to 8 weeks of growth. Anaerobic sorting of GMD's is performed by connecting the growth column (residing in the anaerobic grove bag) to a flow cytometer/cell sorter pressurized with N_2, and using degassed liquid containing O_2-scavenging chemicals.

The diversity and phylogeny of cultures from each growth column will be sequentially screened at the level of the 96-well plate. An aliquot of cells will be removed from each well, all 96 aliquots pooled, DNA extracted and amplified with universal 16S rDNA *Bacteria* and *Archaea* primers, and a clone library constructed from each PCR. Randomly-chosen clones (n=96) will be sequenced and analyzed by blast to determine their phylogeny. Because a GMD may have multiple cell types, rarefaction curves will be constructed to estimate diversity in the clone library from each plate. If diversity is high, additional clones may be sequenced. Plates with clones representing highly novel and/or previously uncultured

groups of microbes will be sub-divided into groups of 8 cultures, and screened again to determine the region of the plate containing the microbe(s) of interest. Single wells from that section of the plate will then be individually amplified, cloned, and sequenced to determine the wells containing the microbe(s) of interest. Alternatively, it may be possible to design a highly specific gene probe for the microbe(s) of interest and use fluorescently labeled nucleic acid probes to identify the wells containing the microbe(s) of interest. Fluorescent nucleic acid probes could also be mixed with GMDs before flow cytometry and sorting carried out to collect GMDs with hybridized microcolonies; however, this approach would be used to analyze only a portion of a growth column, as GMD microcolonies would be killed after exposure to the chemicals used for fluorescence in situ hybridization.

Sequencing of pathways from isolated microbes that appear unique or deeply rooted based upon their 16S rDNA sequences will be performed by Hazen's group at LBNL as part of their Genes-to-Life program. The ability to grow such microbes in GMD's will allow us to partially sequence their genome. Because the genome is a biomolecular record of ancient life (addresses goal 4 of NAI roadmap), these ancient genomes will provide insight into molecular, genetic, and biochemical mechanisms that control and limit evolution, metabolic diversity and acclimatization of life (goal 5 of NAI roadmap). The genomic content of GMD microcolonies can be studied using multiple-primed rolling circle amplification (MP-RCA). This DNA polymerase from bacteriophage phi-29 performs in vitro DNA amplification from linear and circular single- and double-stranded DNA templates (Dean et al., 2001). The method employs random hexamers for priming DNA synthesis and results in exponential amplification of the original target sequence. This method has been used by the Department of Energy sequencing center to directly amplify genomic DNA from as few as approximately 1000 cells of the microbe *Xylella fastidiosa* (Hawkins et al., 2002). Current research at DOE, Diversa, and other private companies is examining post-sequencing genome coverage when genomic DNA is amplified from low numbers of cells. Currently, genome coverage in shotgun libraries (3-4 kb inserts) produced from MP-RCA with an input of 100-1000 cells appears to be equivalent to that obtained by 2x to 3x sequencing of shotgun libraries produced from 10^{10} cells (unpublished data). This will no doubt improve significantly over the next few years. Thus, a whole genome shotgun library can be produced from the cells in a single (or few) GMDs using the MP-RCA technology, and sequencing of the library will capture a substantial portion of the genome. If ancient microbes are identified in the deep subsurface biosustainable environments, GMD's linked to MP-RCA and genome sequencing has the potential to make large contributions to the evolution of life on Earth.

In Situ Experiments

Introduction-A major challenge in determining experimentally in situ microbial respiration and incorporation rates is obtain meaningful information without disruption to the natural setting of the organisms. Traditionally determination of substrate utilization was done by bringing samples to surface laboratories and culturing them under a variety of potential substrates and growth conditions. While valuable, extrapolating this type of result back to the field is always uncertain. We propose instead to perform in situ tracer experiments to evaluate substrate utilization. Such experiments have not to our knowledge been attempted systematically in the challenging environment of the deep subsurface, the results will provide critical data for resolving substrate utilization in biosustainable environments and for identifying biogenic versus abiogenic metabolites.

Experimental Design-Based on these results of the previous investigations, we will piggyback on 1-3 exploration boreholes to be drilled at a Canadian mine site using a recirculating fluid spiked with rhodamine and fluorescent microspheres, two standards approaches for determining the extent of drilling fluid penetration of the fluids and fractures, and will be used for in situ field experiments. Immediately after borehole completion, the holes will be sealed off at the head to minimize any further contamination but allowed to flow slowly through a check valve. Geochemical, microbial and isotopic samples will be collected over time and analyzed until the borehole parameters stabilized and all traces of the drilling fluids have been flushed out of the system.

Compound specific stable isotope analysis and the use of stable isotope tracers (^{13}C, ^{15}N, ^{18}O and $^{34,36}S$ and ^{81}Br) will be used to address the issue of what substrates, provided by the fluids, gases and or rocks themselves, are being utilized by the communities in the deep subsurface.

To introduce these tracers we will utilize (in-situ) microcosms of solid phase "bio- traps" with BioSep® beads in flow-through Delran sampling tubes arranged along a supporting flexible spine. Each sampling tube is connected with gas impermeable tubing to the head of the borehole ((Mislowack et al., 2002)) and will also contain either crushed rock fragments of the country rock or specific rock or mineral substrates. A prototype was successfully deployed for 1 month in a South African mine. Each microcosm will contain a different labeled substrate and one microcosm without substrate is used as a control. The system has a modular design for autoclaving prior to assembly and insertion into the borehole and resealing of the borehole. The outlet of the gas impermeable tubing for each microcosm drips (~1 drop/minute) directly into an evacuated 140 mL serum vial sitting on dry ice. The serum vials are changed on a daily basis depending upon the drip rate.

1. In order to be able to detect the nanomolar levels of activity anticipated, in situ experiments will be conducted using ^{13}C-labelled substrates of a) DIC; b) CH_4 and C_2-C_4; and c) selected organic acids such as acetate, propanoate, butanoate, benzoate and formate identified in the *in situ* waters, ^{15}N-labelled substrates of NH_4^+ and NO_3^- and ^{36}S labelled SO_4^{2-}, $S_2O_3^{2}$ and sulfide. The substrates selected for the experiments will be based in part upon the 16SrDNA analyses of the borehole community and the results of GMD isolation.
2. Over a period of two months, samples will be taken to determine rate of conversion of various substrates and potential incorporation of ^{13}C-label into microbial biomass. Isotopic analyses of inorganic and organic C pools and biomass will be done to develop a C mass balance for these systems, and used to identify the substrate basis for the borehole microbial community.
3. After two months if the results on the effluent samples indicate microbial activity is occurring in some of the microcosms, the multi-level sampler is removed and the tubes disconnected, stored and returned to the lab. In the lab, the contents can be removed and the lipids and DNA of the microbial biomass adhering to the rock chips and the BioSep® beads can be extracted for analyses. Assessment of the extant in-situ microbial communities in subsurface environments will be most effectively accomplished if aliquots of solid phases in the samplers can be readily colonized by the resident microbiota and those systems be subsampled and analyzed over time.

BioSep® beads-BioSep® beads accurately capture viable biomass diversity and community composition and serve as excellent enrichment devices. We have recently employed Bio-Sep® beads (Sublette et al., 1996), 2-3 mm spherical beads consisting of 25% (w/w) aramid polymer (Nomex) and 75% (w/w) powdered activated carbon (PAC). The bulk density is about 0.16 g/cm^3 with a porosity of 74%, and an adsorptive surface is greater than 600 m^2/g. The beads are surrounded by an ultrafiltration-like membrane with a median pore diameter of 1.9 microns and with some large macropores > 20 microns. Beads can be purged of organic carbon by incubation at 350°C for at least 5 hours prior to deployment representing an organic-free inert adsorptive matrix ideal for microbial colonization in extreme environments. Bacteria are immobilized inside Bio-Sep® beads by culturing the bacteria in the presence of the beads in a natural environment. These beads were recently successfully deployed for several days at ~1800 mbls. in Evander Au mine in S. Africa. As in other installations, solid phase samplers with these beads have been shown to enhance the formation of biofilms in drinking water. For example, when deployed in groundwater Bio-Sep® beads generated a viable biomass (as measured by PLFA) that was more than 4-10 times greater than that formed on a plastic surface or glass wool (White et al, 2003, Peacock et al, 2003, Gouffon et al, 2002). Recovery of both lipid and DNA from these bio-traps proved much easier than from sediments and membrane filters. Enrichment of subsurface populations with solid-phase BioSep beads was demonstrated after infusion of 1-3 mM acetate induced a greater than

3-fold increase in viable biomass, with a 7 to10-fold increase in the specific PLFA characteristic of Geobacter, low-G+C Gram-positive Clostridium, and dissimilatory Fe(III) reducers in a metal-reducing groundwater system. Experiments performed in collaboration with the UFZ in Leipzig, Germany, used BioSep® beads containing ^{13}C-benzene in a polluted aquifer. GC/IRMS analysis of PLFA extracted from the beads showed ^{13}C incorporation into a small specific subset of the total PLFA indicating a specific set of organisms in the biofilm could utilize the ^{13}C-benzene. These experiments revealed the power of ^{13}C as a biomarker and allowing scientists to follow the metabolism of labeled compounds through extreme environments, including the synthesis of lipids in organisms.

Analyses

Lipid- Lipid analyses will be performed using previously reported important precautions to achieve quantitative results (White and Ringelberg, 1998) and published analytical procedures (Lytle et al, 2000a,b).

DNA analyses-Nucleic acids will be analyzed using the procedures discussed in section 3.

Stable Isotope Analyses-Stable isotopes (^{2}H, ^{13}C) combined with PLFA have recently been used to link degradation/metabolic activity with specific microbial populations (Boschker et all 1998; Nold et al, 1999; Hanson et al, 1999, Alexandrino et al, 2001; Pombo et al, 2002; Boschker and Middelburg, 2002). The problem with ^{2}H of lipids is that they reflect a combination of organic substrate and water and are fractionated and in that regard have yet to be proven diagnostic of a particular source substrate. Carbon isotope compositions of the FAME will be determined by GC-IRMS by Pratt research group in Indiana University. The measured isotopic ratios of methylated fatty acids are corrected for the methyl moiety (Abrajano et al. 1994) using:

$$\delta\, ^{13}C_{FA}\ (‰) = [(C_n + 1) \times \delta\, ^{13}C_{FAME} - \delta\, ^{13}C_{MeOH}]/C_n$$

where $\delta\, ^{13}C_{FA}$ is the $\delta\, ^{13}C$ of the fatty acid, C_n is the number of C atoms in the fatty acid, $\delta\, ^{13}C_{FAME}$ is the $\delta\, ^{13}C$ of the methylated fatty acid, and $\delta\, ^{13}C_{MeOH}$ is the $\delta\, ^{13}C$ of the methanol used for the methylation reaction. Precision of an internal fatty acid standard (19:0) is ±1.01‰ (n = 5) for the FAME. Carbon isotope ratios of total biomass and CO_2 will also be determined and all C isotope values reported against the PeeDee belemnite standard.

The $\delta^{15}N$ of NO_3^-, NH_4^+ and the whole cells extracts from the BioSep® beads will be made in Sigman's lab at the Department of Geosciences at Princeton University. The $\delta^{2}H$ and $\delta^{13}C$ of the C_{1-4} compounds and the $\delta^{15}N$ of N_2 isotopic analyses will be performed in the Sherwood-Lollar's lab at the University of Toronto. The $\delta^{36}S$ and $\delta^{18}O$ of SO_4^{2-} and $S_2O_3^{2}$ and the $\delta^{36}S$ sulfide will be measured at Indiana University.

FISH-Using the results of 16SrDNA analyses of the borehole samples and serum vial samples, probes will be designed to target the 16S rDNA gene. These probes will be applied to biofilms that have colonized rock chips from the samplers for which two opposing faces have been previously polished. Microscopic observations will be performed at Princeton University using an Olympus BX60 microscope operating at 2000x with epifluorescence illumination. The microscope is equipped with a digital, color CCD camera, an image analysis software package, an internet connection and UV cubes and filter sets appropriate for DNA and protein stains.

Geochemical analyses-Cations and trace metals will be measured by ICP-MS and anions by IC-MS (Princeton University). Gas compositions will be measured at the University of Toronto.

Data analysis-It will be necessary for this multi-dimensional data to be processed so that microbial ecologists can achieve understanding of the relationships among bacterial presence, function, geochemical characteristics and other environmental factors. Non-linear analysis, i.e., artificial neural network analysis, will be used to analyze relationships between PLFA or gene frequency data and site geochemistry (Pfiffner et al, 1999, Almeida et al, 1998, Noble et al, 2000).

4. STRESS RESPONSE PATHWAYS IN DEEP SUBSURFACE BIOSUSTAINABLE ENVIRONMENTS

(Hazen, Phelps, Onstott, Brockman, Pfiffner, Moser)

In July 2002 the Department of Energy funded a major new Genomes to Life Program (GTL) project lead by Lawrence Berkeley National Laboratory. This 5 year, multi-institutional project is focused on the rapid deduction of stress response pathways in metal and radionuclide reducing bacteria. This project was used to start the Virtual Institute for Microbial Stress and Survival (http://vimss.lbl.gov) whose mission is research on stress and survival mechanisms in microorganisms in a variety of environments. This institute's principal research goal is to determine stress and survival pathways and networks from the molecular level to the ecosystem level. The stress response pathways being studied by this DOE funded project, e.g. O_2, pH, T, N, and P, are some of the same parameters that impact the survival and sustainability of microorganisms in deep subsurface and extraterrestrial environments.

The Focus of the VIMSS GTL Project.

The GTL Project addresses DOE cleanup and collateral research needs. Our focus is on experimentally elucidating and computationally modeling the stress response pathways of three target metal and radionuclide reducing organisms: *Desulfovibrio vulgaris, Shewanella oneidensis* MR-1, and *Geobacter metallireducens*. Microbial metal reduction plays an important role in biogeochemical cycling of C and N, as well as in the bioremediation of metals, radionuclides, and organic contaminants. A number of bacteria have demonstrated the capability to reduce radionuclides and other metals. Numerous microorganisms capable of coupling the oxidation of organic compounds to the reduction of metals have been isolated and studied from the standpoints of physiology, ecology, and phylogeny.

The overarching goal of our DOE research is to develop criteria for monitoring the integrity (health) and altering the trajectory of an environmental biological system (process control). To achieve this requires a more complete understanding of how the biological "units" comprising the system are organized, regulated, and linked in time and space (genes, genomes, cells, populations, communities, and ultimately, ecosystems). Key to these objectives is a more complete understanding of stress response systems and their environmental context.

These studies will directly support the proposed NASA research by enabling a comparison of stress response pathways in deep subsurface microbial environments. Indeed, a critical factor that enables biosustainability in deep subsurface and even extraterrestrial environments are stress response pathways. By comparing the stress regulatory pathways that we are currently studying to those we will examine in deep subsurface microbial communities, we will be able to discriminate between those stress regulatory pathways and conserved components that are shared across species and habitat boundaries from those that maybe unique and critical to the survival of life in deep subsurface environments.

Overview of the VIMSS GTL Approach.

The goals for our DOE project fall into two categories: applied and pure. The pure goals, which are particularly relevant to the proposed NASA NAI project, are to understand, from a physical-chemical, control-theoretical, and evolutionary point of view, the structure, function, and dynamics of the pathways involved in the biogeochemistry of soil microbes under a wide variety of conditions.

First, we measure in physical detail the time-dependent activity of as many pathway components as possible under a variety of conditions, stresses, and other perturbations. From both perturbation-response data and direct measurements of molecular interaction, we will then deduce pathways involved in the stress response of target organisms during the task of metal reduction. The same perturbation-response data are also a necessary precursor for understanding how changing soil conditions and the applications of external stimulatory agents to these organisms will change and control their behaviors.

In the case of this proposal, our team will analyze time-dependent activity for 1) experiments performed in the Deep Subsurface- Process Simulator using deep mine fissure water and crushed rock and 2) for the in situ borehole experiments.

Why Many Pathways from Multiple Organisms in Great Detail?

By creating detailed causal/physical models of the stress response pathways, we will learn what the principles of control in these pathways are at a molecular level. In order to do this we must have extensive measurements of the time-dependent changes in activity for all molecular players and their interactions including for the base line, pre-stimulation or pre-perturbation state. It does not suffice to have only the cis-regulatory structure that comprises the immediate control of gene expression, nor only the protein-protein interactions. Ideally, all the state-dependent interactions among the cellular components should be traced. This is because bacterial regulatory networks are less stratified than it might seem at first. For example, the sporulation initiation pathway in *Bacillus subtilis* (a cryptobiotic pathway; Fig. 25) demonstrates that all types of interactions can play a major role in cellular decision-making. That is, control of gene expression, signal transduction, secretion of small peptides and proteins, and metabolite consumption are all coupled to arrive at the final decision to sporulate. The network in Figure 25 was experimentally discovered over a 25-year period and contains a large amount of genetics and biochemistry. Even so, the exact control of sporulation has still not been solved. To match and exceed this level of detail in our target organisms will require a concerted effort to measure as many cellular species as possible, as well as their interactions and their behaviors, under a wide variety of external conditions and mutant states. The functional genomics bioanalytic pipeline described below is designed to address this need.

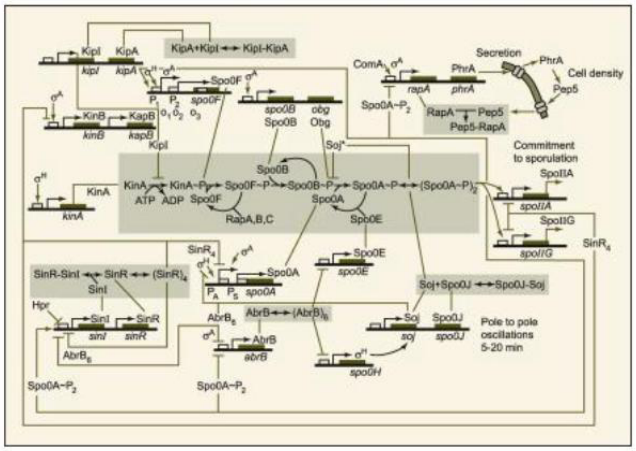


Fig. 25. The sporulation initiation pathway in *Bacillus subtilis.*

The sporulation initiation pathway diagram in Figure 25 is artificially truncated. Many other molecular species are integrated into the function of this pathway. In addition, many of these other species, for example ComA, are involved in other pathways. Figure 26 shows how the seemingly innocuous notation for ComA in Figure 25 (middle, upper right of that figure) can lead into more complex networks. One of the central challenges of network biology will be development of methods that discover modular structure in these pathways, if such exists. Certainly the pathways can at least be conceptually broken up by overall function. For example, the stress response pathways in *Bacillus subtilis* interact with each other in such a way that a perturbed population of cells splinters into a number of different cellular behaviors, each cell implementing one or more stress responses (Figure 27). These stress responses modulate each other so that incompatible behaviors such as sporulation and chemotaxis are not expressed simultaneously (or contemporaneously). This sort of interaction among pathways is more than simple cross-talk. Cross-talk, at least in engineering, implies parasitic signals that move

uncontrolled among closely spaced systems. Yet in this discipline, cross-talk has strong biological implications. This strong coupling and the resultant population heterogeneity necessitate the study of more than one stress response and the development of experimental methods to follow which cells choose which responses. Indeed, a central goal of the theoretical work in our group is to derive formal methods for determining modularity in these networks so that subsystems can be tested and modeled without complete information about the "cross-talked" systems.

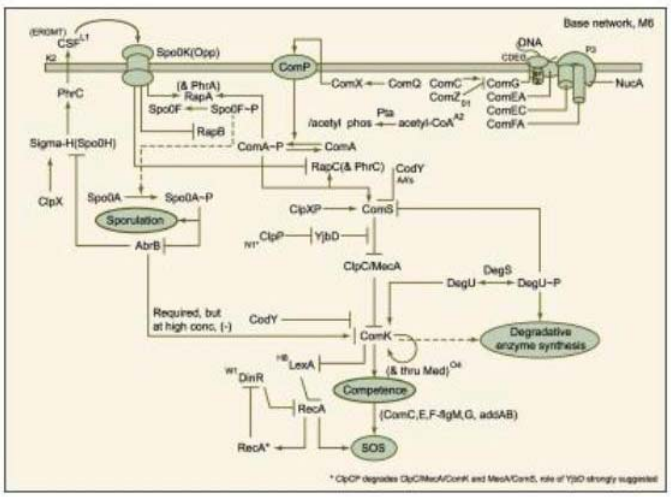


Fig. 26. Coupling of sporulation to other pathways.

Figure 27 shows the interaction among stress response pathways within a particular organism (in this case *B. subtilis*). Although other organisms may have homologous pathways to *B. subtilis*, the regulation among and within these pathways is likely to be different. As an example, in a recent comparative study of the chemotactic pathways in *Escherichia coli* and *B. subtilis*, it was found that, although the major complement of proteins was the same between the two organisms, there were both component differences and important differences in regulation. The study concludes that the systems respond very differently to perturbation in their machinery. Two classes of question arise in the face of this information. First, how do the differences in perturbation response behavior lead to differences in the endogenous ability to track chemical signals, and what do these dissimilarities imply about the differences in control that may be achieved by external (unnatural) signals? Second, are these differences traceable to the particular environments in which these two organisms live? *E. coli*, a gram-negative microbe, is an enteric organism that needs to live in animal hosts as well as in external environments, whereas *B. subtilis,* a gram-positive, spends most of its life in soil. Are there evolutionary roots to the disparate regulatory structures, or are they merely artifacts of evolutionary drift?

Although the three key organisms studied in our DOE will provide core models of the stress response pathways, obtaining information on the regulatory strategies of other organisms and their relative overall responses to different conditions will provide further confidence in the estimation of regulatory structure differences across and between niches and of how these regulatory differences lead to alteration of a variety of biogeochemical processes. For the NASA NAI proposal we will examine the diversity of regulatory strategies, both by genomically characterizing the microbial community in the deep subsurface, biosustainable environments and by comparing the response of this community to stress or stimulus with that of our three key organisms.

PNNL-Diversa team, homologous to the pathways identified in our key microbes, will lead to identification of both conserved and divergent sets of proteins and DNA cis-regulatory elements. Deducing which pieces of a given pathway are central to a given function can be done by observing which factors are conserved across niches. Within-niche variation of regulation should give insight both into the plausible flexibility of the regulatory network in achieving similar goals and the differential

regulation of organisms competing for resources within the same niche. Conserved differences between niches should yield insight into niche-specific regulatory strategies. Combining this information with experimental measurements on the population dynamics of the community under different stress conditions will yield a mapping between regulatory strategy and behavioral phenotype. Models of the different strategies, modified from the highly tested models of the target organisms, will yield hypotheses for the role of each regulatory difference in the survival of the microbe and the fabric of microbial community interaction with the environment that controls biogeochemical processes.

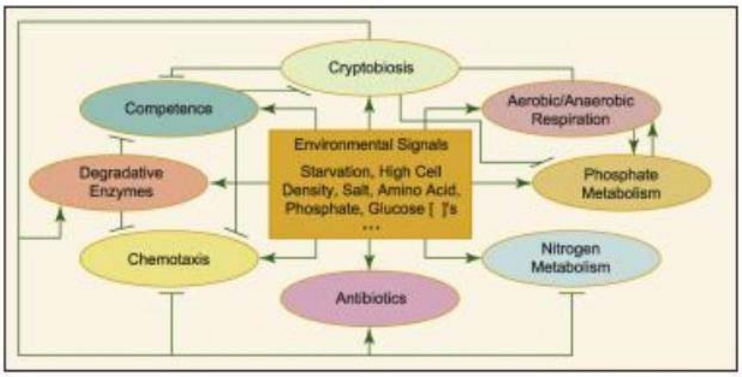


Fig. 27. Different stress responses caused by splintering of perturbed population of cells after interaction of stress response pathways in *Bacillus subtilis.*

Environmental Considerations: An Ecological Framework for Defining Stress

Extensive application of direct molecular measures of microbial community structure in different environments/habitats (primarily based on comparative 16S rDNA sequencing) has revealed some general themes that are directly relevant to the goals of the proposed research. First, population diversity is present in the deep-seated brines and the greater part of environmental diversity has yet to be recovered in pure culture.

Second, a natural organization to microbial diversity within this habitat appears to exist with heterotrophic sulfate reducers being the most obvious dominant respirer and aceticlastic methanogens dominating less saline and more carbonate rich fissure water. Generally, populations (as identified by the 16S rRNA sequence type) are affiliated with well-resolved phylogenetic groups (clades or "phylotypes") (Britschgi and Giovannoni, 1991; Liu and Stahl, 2001). This is also true of the Columbia River Basalt aquifer in which metal or sulfate reduction is a major process have revealed a high diversity of related populations (e.g., members of Geobacteraceae and Desulfovibrionaceae) (Fry et al., 1997). Thus, at higher taxonomic ranks there is a less complex natural order—high species diversity is captured by a limited number of major phylotypes. This suggests that the natural assemblages share a similar ecological function (e.g., metal reduction or sulfate reduction).

Little understanding of the ecological factors contributing to, or sustaining, high diversity within a phylotype (e.g., within the families Geobacteraceae or Desulfovibrionaceae) exists for the deep subsurface. We suggest that high species diversity among populations comprising a single phylotype contribute to functional stability within an environmental system. Since environments are not static but are constantly changing with respect to key physical-chemical variables (e.g., substrate concentration, temperature, pH, salinity, osmolarity, light, redox potential, etc.), it follows that not all populations in a system are simultaneously experiencing optimal growth. Those populations growing under suboptimal conditions, or entering resistant (e.g., spores) or moribund states, are experiencing stress even under the slowly evolving conditions of deep subsurface brine systems. Thus, we suggest that it is not sufficient to monitor stress. It is essential to understand the environmental context of stress response for different

populations. We hypothesize that within any well-adapted and dynamic system, some fraction of the community will be experiencing stress. The key question is, what levels of stress response signal a system that is in distress or that is not optimally adapted or sustainable over geological time intervals?

Experimental Methods for Determining Stress Response Pathways in Biosustainable Deep Subsurface Environments.

The two major experimental approaches to examine the stress response in deep subsurface environments were outlined above. Using the deep subsurface stimulator, the inoculate microbial population and constituent water and crushed rock will be exposed to good stress, nutrients amendments, and bad stress, CO_2 injection and dehydration with water ice and clathrates formation. Using a brine emanating borehole and down hole multi-level sampler array the microbial community, hypothetically in a overall state of stress, will be exposed to growth or energy supplying substrates. Both experiments will be performed over a time scale of months. Both experiments will be preceded with microcosm experiments using single species isolated from the environments by either conventional or GDM techniques.

Identification and Recovery of Stress-Responsive Populations from Complex Communities

Deep subsurface environments change at a much slower rate that the shallow environments being studied by DOE, yet at the same time, microbial turnover rates are also orders of magnitude slower in deep subsurface environments than shallow aquifers. Consequently, it still follows that all populations in a natural system are not simultaneously growing optimally. Those populations growing under suboptimal conditions, or entering resistant (e.g., spores) or moribund states, are experiencing stress and this component of the community may be greater in deep subsurface environments than shallow aquifer environments. Thus, we suggest that it is not sufficient to monitor an extrinsic parameter known to be associated with stress—it is essential to understand the environmental context of stress for individual populations. We hypothesize that within any well-adapted and dynamic system, some fraction of the community will be experiencing stress. This component of the community will be identified using a general activity measure (rRNA synthesis) to identify populations that respond to changing chemical or physical environments by entering a cryptic or low growth state. Cells comprising specific stress-responsive populations will then be physically recovered using a combination of a high-speed cell sorter and fluorescent in situ hybridization (FISH), using fluorescent probes designed to target specific rRNA sequence types. In this way, we will achieve full integration of system elements, linking specific stress-regulated genes to specific populations shown to respond differentially to specific stresses.

These studies will be directly integrated and in support of the expression analysis and modeling studies of our DOE GTL project. For example, these systems will later be used to monitor whole-system stress response using microarrays developed from the collection of stress responsive genes and related to model predictions, etc. The same microarrays will also be used to measure the stress responsive genes in the biosustainable deep subsurface environments being studied in the current NASA proposal.

Radiomicroarray Analysis.

Change in growth status will be evaluated using a radiomicroarray format to measure changes in the rRNA synthesis of individual populations resident in the DSPS and the in situ borehole experiments described above. The microarray will build upon our research program developing a platform for comprehensive monitoring of microbial populations in complex communities. Unlike the more standard glass-slide array, the gel-based format immobilizes DNA probes in small acrylamide pads ($100 \times 100 \times 20\mu m$) arrayed on a glass surface. This provides for much higher probe density (and target capture capacity) than possible by direct immobilization on the glass surface. Probes immobilized on individual pads are designed to complement specific rRNA sequence types, either unique to individual species or encompassing larger phylogenetic groups. Our studies have demonstrated the utility of this format to directly detect environmental rRNAs using an optimized fragmentation and fluorescent dye labeling

protocol (Guschin et al., 1997). This microarray format has been well characterized with respect to specificity and reproducibility.

We have recently combined the gel-based array with radiolabeling. In this format the microbial community is labeled by adding $^{33}PO_4$ (or ^{3}H uridine) to the growth medium immediately following perturbation/stress (e.g., altered pH, metal addition). The $^{33}PO_4$ is incorporated only into the nucleic acids of actively growing populations. Following recovery of total rRNA and hybridization to the microarray, the array is coated with a Ag halide emulsion. The emulsion is developed following an appropriate period of incubation, and radioactivity of the rRNA bound to each individual gel pad is quantified by observing precipitated silver grains with a diameter of approximately 0.2 µm using microscopy. Thus, nongrowing populations are labeled only with fluorescent dye, whereas active populations are identified by generating both fluorescent dye and Ag grain deposition signals. We recognize that not all cells of a specific population will be of comparable physiological status, for example, if cells are attached to rock chips versus being planktonic. Unfortunately we have no feasible approach for examining changes in attached cells during either the DSPS or in situ borehole experiment. Microcosms studies on the other hand that are examined with nondestructive SR-FTIR approaches may provide some insights into changes occurring on the rock surfaces during environmental changes.

Recovery of Stress Responsive Populations via Flow Cytometry

Flow cytometry is a well-established method of measuring physical characteristics of individual cells, including light scatter (size and shape) and fluorescence emission at wavelengths of interest. This technique was initially used in biological oceanography to quantify and sort naturally fluorescent marine picoplankton. Our earlier work documented the feasibility of using flow cytometry in combination with FISH to quantify specific microbial populations using probe-conferred fluorescence (Amann et al., 1990) and this method has now received general use in environmental microbiology (Fuchs et al., 1998).

Our more recent studies have shown that high-speed sorting can be used to physically recover specific populations, using FISH as the sorting parameter. Figure 29 shows the results of a successful sort in which a specific population of cells was recovered based on probe-conferred fluorescence. The pooled cells then serve for general and specific genetic characterization. For example, a few thousand sorted cells were sufficient to recover 16S rRNA genes using PCR amplification.

Previous attempts to view cells from South African fissure water using a flow cytometer and FISH were unsuccessful due to the low signal (small number of ribosomes). In the in situ borehole experiment, however, where isotopically labeled nutrients are being added, if growth does occur, then sufficient numbers of cells will likely be recovered for use in other sequence-based analyses, including hybridization and large-fragment cloning for the directed recovery of homologous stress response genes. This will require the use of evacuated serum vials partially filled with ethanol in order to preserve the cellular integrity and rDNA for FISH hybridization.

The proposed combination of methods provides a technical framework to link a physiological response of a specific population within a community setting (radiomicroarray) and to associate that population with a specific genetic system of stress response.

Flow Cytometric Sorting of FISH-labeled Populations

Flow cytometry has been productively used to provide general information of single-cell abundance and certain aspects of their activities (e.g., cell size, DNA and RNA content, membrane potential). Most past applications have been of relatively coarse resolution because analyses were restricted to populations having unique intrinsic properties such as autofluorescence (Button and Robertson, 2001).

The use of fluorescent dye-labeled probes to label single cells via fluorescence in situ hybridization (FISH) now provides the basis to selectively confer fluorescence on any population using genetic criteria. Our group contributed to both the early development of the basic format and the early documentation of the combined use of FISH and flow cytometry (Amann et al., 1990). A key goal of our proposed research

will be to use fluorescent probes complementary to the 16S rDNA's to selectively recover specific environmental, DSPS and in situ borehole experimental populations via flow cytometric sorting, providing a mechanism to associate genes recovered from the bulk community with specific members of the community. Probes will be developed to target specific populations highlighted by the radiomicroarray studies. The success of sorting a population from field-site samples will in part depend on our ability to isolate a relatively clean cellular fraction from the environmental substratum and correlate its forward and

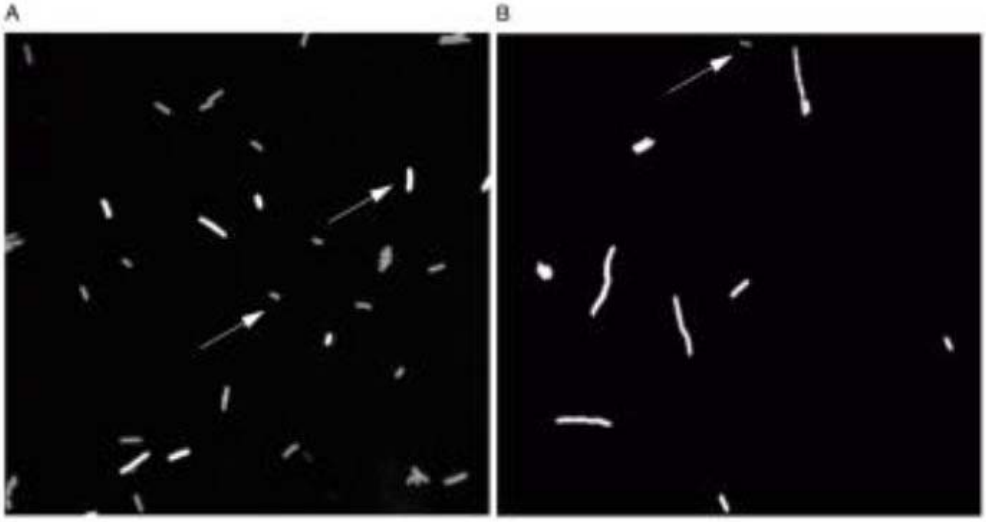


Fig. 29. Fluorescence photomicrograph of bacterial cells fixed with paraformaldehyde and labeled by hybridization with fluorescently labeled, group-specific 16S rRNA oligonucleotide probes. Panel A shows a mixture of *Escherichia coli* and *Pseudomonas aeruginosa* (noted with arrows) cells before flow cytometric sorting. Panel B shows the same population of cells after sorting. The arrow denotes a single cell not properly excluded during the sort. Courtesy of A. Schramm.

side scatter properties with a specific 16S rDNA sequence. We will also evaluate the use of enrichment cultures developed from environmental samples, eliminating or reducing the need for fractionation of cells from a solid substratum. Sorted populations will be further characterized using PCR and hybridization to link them with specific genes implicated in stress response pathways.

Direct Stress and Community Comparison with PLFA and Metabolite Analyses.

As discussed above PLFA has been used extensively for characterizing and monitoring microorganisms in all types of environments. The normal detection limit is about 10,000 cells; however, recent studies have shown that much lower densities can be detected using microassay techniques (Hazen, 1997). The extraction process for the lipids has also been used to simultaneously extract nucleic acids, providing a method to relate sequence information (e.g., as determined by DNA probing) and lipid signature compounds from the same environmental sample.

We will use this approach to relate sequence-specific information (e.g., T-RFLP mapping, DNA probing) to lipid profiles in the mine borehole samples, the DSDP samples and the in situ borehole experiment. The combined analyses will provide a way to associate populations identified by sequence with molecular signatures associated with physiological status.

The metabolites, dissolved organic and inorganic compounds and complexes, will be measured by ICP-MS and IC-MS and the dissolved gas species by GC and Kappa 5 (low H_2 and CO concentrations).

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PLAN FOR STRENGTHENING THE ASTROBIOLOGY COMMUNITY

INTRODUCTION

The Indiana-Princeton-Tennessee Astrobiology Institute (IPTAI) entitled "Detection of Biosustainable Energy and Nutrient Cycles in the Deep Subsurface of Earth and Mars" focuses on subsurface microbial communities that have been sequestered from the surface photosphere for tens to hundreds of millions of years. Using a combination of field and laboratory experiments, we proposed to characterize environments supporting deep-subsurface microbes on Earth. Observational and experimental data will be used to design innovative instruments, datalogging systems, and algorithms for differentiation of non-biotic (sterile) from biotic biogeochemical cycles on Earth and, potentially, on Mars. We seek specifically to analyze microbe/mineral and microbe/ion interactions; to determine isotopic signatures of organic and inorganic species present in gaseous, aqueous, and solid phases; to document genomic diversity and metabolic strategies; and to study protein expression and origin of metabolites in deep microbial ecosystems. This information is crucial for the development of life-detection approaches that can be tested on Earth and can be deployed as flight-capable instruments on future Mars drilling missions.

Education and Public Outreach (EPO) activities in IPTAI are designed around three areas of emphasis. First: educational workshops for undergraduates and high school teachers where participants actively collect and interpret data from laboratory and field experiments. Second: public outreach through a web site with premiere-quality digital media including animations and video that illustrate how and why scientists conduct research in deep mines. Third: mentoring undergraduate and graduate research at Indiana, Princeton, and Tennessee universities. These astrobiology students will work with faculty to design a series of web-based quantitative and investigative activities for all pre-college students but highlighting the diverse careers of leading women on the IPTAI team.

Inclusion of collaborators from the School of Fine Arts, IU Instructional Support Services, and University Information Technology Services at Indiana University is an unusual aspect of this proposal. High-resolution digital video/audio materials will be collected during field experiments and will be use in both research and educational components of the IPTAI. Videos produced by scientists will document research methods in a substantially different way from conventional commercial films. We hope to capture examples of both set-backs and advances in research resulting from unanticipated and challenging conditions in deep mines. Given severe time and access constraints in deep mines, digital documentation of the physical conditions and the configuration of instruments is essential for interpretation of experimental results (Fig. 1).

EDUCATION AND PUBLIC OUTREACH

The science and engineering fields within the United States are lacking in minority participation especially African-American and American Indian students. In the Science and Engineering Indicators 2000 submitted by the National Science Board to NSF, representation of Black, Hispanic and American Indian individuals among scientists and engineers is substantially lower than their respective proportions of the total U.S. population. Demographics on terminal degree also show disparities. In 1999, 61% of black engineers and scientists had bachelor's degrees as their highest degree compared to 56% of all engineers and scientists. Furthermore, the fields of social sciences, computer and mathematic science rather than life or physical sciences are the more prominent fields for black scientists and engineers. These differences are attributed to diverse factors including lack of funding, guidance/mentorship, and opportunity. Given that the U.S. faces a dire shortage of engineers and scientists for educational,

industrial, and governmental programs, it is important to foster participation by currently underrepresented groups. Aggressive recruitment may be necessary for development of new intellectual

Figure 1. Pratt checking flow on a massive filtration apparatus connected to a borehole valve located at a depth of 3.3 km below the surface in Kloof 4-41 Mine, South Africa.

capital in critical areas such as economy, environment, health and security. Developing underutilized human resources can lead to a competitive advantage for the U.S. in a global economy.

The established scientific community must find better ways to motivate minority students towards graduate-school education. Many explanations are posited for the relatively small numbers of students of color entering PhD programs: today's sluggish market for college instructors, the high costs of graduate education, limited financial support, and more lucrative opportunities in other professional fields (Davidson and Foster-Johnson, 2001). Projections by the Bureau of the Census, however, show that the population of elementary school children in the United States in 2030 will be equally divided between non-Hispanic whites and all other racial/ethnic groups combined (NASA, 1996). If more minorities are encouraged to attend graduate school and pursue academic careers, they will serve as tomorrow's role models and mentors for our increasingly diverse population.

Purpose and Goals

Educational and public outreach in the IPTAI proposal is targeted on recruiting, encouraging, and facilitating retention of underrepresented or minority undergraduates and teachers. Our efforts will be mainly within the U.S. but likely will include participants from Canada and South Africa. We will specifically design materials for pre-college girls and for workshop participation by undergraduates and high-school teachers. The annually hosted workshops will be the most visible and costly component of our EPO effort. Our past experience indicates, however, that a one-on-one approach is the most effective way to foster genuine engagement with future scientists and science educators. Workshops will target U.S. minority undergraduate students and will be supervised by an international group of mentors. Internships and workshops will be conducted primarily at laboratories in the U.S. but also will be

conducted in tandem with field experiments at mines in the U.S., Canada, and South Africa. Our over-arching EPO goals are:

- To engage students and teachers in multidisciplinary team research using internship and workshop activities. Particular effort will be devoted to minority and female students.
- To involve undergraduate students, graduate students, and post-doctoral researchers in field experiments at deep mines. Field research will be supervised by faculty members with extensive underground experience in order to ensure attention to issues of training and safety.
- To increase student awareness of career opportunities in astrobiology and in related growth industries such as geomicrobiology, biotechnology, bioremediation and environmental engineering.
- To recruit undergraduate majors and graduate students in astrobiology and geomicrobiology.
- To foster transfer of innovative technologies between academic, government and industrial research groups through collaborative geomicrobiological research.

The proposed educational experiences will explore the unusual geochemical and microbial environments that exist within and around the world's deepest mines. Astrobiology internships and workshops will encompass environmental field techniques, microbial and geochemical laboratory exercises, and tutorials on a broad range of topics including controversial aspects of environmental remediation, origins of life, and genetic engineering.

Graduate Education and Post-Doctoral Training

The single largest component of funding in the proposed NAI is advanced educational training in the form of graduate assistantships and post-doctoral associateships. A total of six graduate assistantships and four post-doctoral associateships are included in our budget under a combination of NASA-requested and institutional-matching funds). External funding for student positions is a critical step in getting academic administrations to consider new degree and certificate programs in astrobiology. Proposals for astrobiology and/or geomicrobiology degree programs are currently under consideration at Princeton University and Indiana University. Regardless of the degree title, a substantial number of new graduate degrees will result from the proposed investigation, and these young scientists will identify themselves as members of the astrobiology community. Pratt and Onstott have successfully advised a total of 23 graduate degrees of which four are female PhD's and three are female MS's. It is notable from the perspective of role models that the proposed NAI includes three women scientists (Pratt, Pfiffner, and Sherwood-Lollar) in leading roles.

Pratt, Onstott, Pfiffner, and Sherwood-Lollar have all demonstrated their ability to supervise students under demanding and difficult conditions in deep mines. Post-doctorate and graduate students training was essential to the NSF LExEn research project "Collaborative Research: South African Ultradeep Mines - Long-Term Sites for Interdisciplinary Studies (LSLIS) into the Extreme Environment of the Deep Subsurface@ (EAR-99788267; Ben-Ari, 2002). Students involved with the South African project came from university and DOE laboratories in the U.S. and from academic institutions in Canada, Japan, and Germany. More than twenty undergraduate students, graduate students, and post-doctoral associates participated in joint field exercises and co-authored publications. These students and post-doctorate researchers were trained in geochemical, isotopic, microbiological and molecular field methods. We believe that the proposed NAI field experiments offer a similar extraordinary opportunity for participation on a multidisciplinary research team. With incorporation of EPO as an integral part of the NAI proposal, we will be able to effectively link programs in pre-college outreach, undergraduate research mentoring, graduate research advising, and post-doctoral supervision. We believe that public outreach should go hand-in hand with student mentoring and scientific research if we want to successfully recruit under-represent groups to careers in science and engineering.

Workshops for Undergraduates and Teachers

We propose to model our internships and workshops on previously successful efforts in South Africa. The University of Tennessee, Knoxville was funded by NSF to hold two consecutive workshops in 2001 and 2002 (http://geomicro.utk.edu/) entitled, "The South African Field Laboratory Workshop: Experience for Minority Undergraduates (Christen, 2002) and Life in Extreme Environments and Biotechnological Applications." In these workshops, thirteen to sixteen minority U.S. and previously disadvantaged South Africa (S.A.) students participated in a series of hands-on, field exercises and laboratory tutorials over a five day period under the guidance of U.S. and S.A. mentors in S. Africa (Fig. 2). Over 80% of attendees would recommend this experience to someone else and over 75% of the attendees have enrolled, are applying, or plan to apply when they complete their bachelors degree, to advanced degree programs or internships in research laboratory (NIH, CDC). The overwhelming success of these workshops and the experience we gained from conducting these educational outreach efforts has encouraged us to formulate a 7-week long program under the National Science Foundation's (NSF) Research Experience for Undergraduates (REU) program (proposal pending) with realistic goals.

Figure 2. Undergraduate Science Majors sampling biofilms in a deep South African Gold Mine. Photo taken during 2001 University of Tennessee Workshop.

Students training to be engineers and scientists need a sophisticated understanding of the situations in which they will be working. As either practitioners or researchers, most individuals holding a science degree do not work exclusively within technical and scientific spheres. It is important for students to realize that they will work in complex social, political and international contexts. Research and study abroad provides an effective way to add ethical and humanistic dimension to the scientific enterprise. Research shows how overseas educational experiences lead to increased awareness about ethical concerns that face society as a whole, increased interest in international affairs, world-mindedness, and cross-cultural empathy as a result of overseas educational experiences. Also, students returning from overseas generally demonstrate a change in behaviors, including increased involvement with organizations that focus on international issues and participation in activities with the goal of increasing international understanding. An abroad opportunity to collaborate with mentors and students in inquiry-based learning allows students to broaden their understanding of their career goals during transition from formal education to profession. The deep-mine workshops proposed in the Indiana-Princeton-Tennessee NAI

will give students a social and cultural context for scientific research. Issues of intellectual property, patents, biodiversity, and national and/or planetary resources will be considered in the context of scientific research. Sociological research indicates that vocational exploratory behavior may occur in studies abroad, often leading to an increase in career options. (Hannigan, 2001; De Winter, 1997; Handelsman, 2002).

In the U.S. and many other countries, earth science education in high school is minimal. Subsequently, students are unprepared to pursue geology, earth science, and environmental sciences at the university level. To address this need, we plan to hold a weekend workshop for secondary teachers during years 2 through 5. These training workshops would assist in curriculum ideas related to astrobiology and geomicrobiology. We think these workshops will help teachers capitalize on the excitement of exploring for life on other planetary bodies. We will use existing materials on NASA web sites and we will develop new materials based on our research activities in deep mines.

Relationships with Minority Institutions

The Center for Environmental Biotechnology at Tennessee has a long history of research collaborations with Florida A&M University (FAMU) which is a historically black college or university (HBCU). We have successfully involved FAMU students in our previous South African workshops. Dr. Pfiffner and Ms. Davis have an open seminar invitation from the Environmental Sciences Institute at FAMU. We will use that opportunity to actively recruit college students in astrobiology, geomicrobiology, and educational outreach in the proposed astrobiology institute. In addition to FAMU, we have expanded our HBCU interactions to include Hampton University, Xavier University of Louisiana, and the University of Puerto Rico. Word of mouth through student participants at workshops has been, by far, our most effective form of advertising. We plan to continue emailing flyers to numerous universities and posting workshop notices in journal outlets like Geology, EOS, and the University Faculty Voice.

Outreach Science for Pre-College Girls

Pratt and Pfiffner have considerable experience developing hands-on and quantitative science exercises for pre-college girls. For five years in the mid-1990's, Pratt coordinated the geological and environmental components of Brownie Math and Science Day at Indiana University. More than 300 girls in first and second grade attend this event each year. Pfiffner has worked extensively with the "SHaring ADventures in Engineering and Science" (SHADES) colloquium is for 6th and 7th grade females and their math and science teachers in East Tennessee counties. The SHADES program consists of highly interactive, demonstration-oriented presentations and exhibits on science and engineering topics. The goal of the program is to show students that mathematics, physical sciences, life sciences, chemistry, and the engineering disciplines are "fun" and interesting and that career options are very diverse. The students attend a presentation (25 minutes) with hands-on activities for each the five disciplines and then participate in a team design competition. Presenters are engineers and scientists from the local professional societies, academia, and corporations who are interested in motivating young people to pursue careers in science and engineering. Career planning information is included in the registration packet. The program is offered one Saturday every Spring and Fall with the location moving through local schools and schools in neighboring counties.

Both the Brownie Math and Science Day and the SHADES program are designed for any girl who has an interest in and aptitude for science and math. These programs do not target the best students but serve a broad spectrum of young girls who might be motivated to take the high school math and science courses allowing them to major in technical fields in college. Sessions for teachers are planned as a part of the SHADES colloquium, and future plans include in-service credit for participating teachers. Students and teachers who attended the previous conferences have been very enthusiastic about it. SHADES is sponsored by the Greater Knoxville Math/Science Coalition. Coalition member organizations include the American Association of University Women (AAUW), Association for Women

in Science (AWIS), Society of Women Engineers (SWE), American Nuclear Society (ANS), and the Tennessee Society of Professional Engineers (TSPE). A similar program is called Broaden Your Horizons.

As part of the EPO activities in the proposed NAI, we will develop specific presentations to be used in Brownie Math and Science Day at Indiana and in the SHADES colloquium at Tennessee. The exercises developed and tested at local science events will be refined and loaded on the IPTAI web site for use by other elementary and junior high-level schools. Astrobiology graduate students will work with faculty on the development and refinement of these materials. Specific attention will be given to illustrating these exercises with photographs showing women scientists in diverse roles. We think it is important for young girls to see photos of actual women scientists and to have links to career biographies for these women. The proposed IPTAI has several women in leading roles who would be appropriate subjects for career biographies.

Programmatic Evaluation

Evaluation of the EPO program would be conducted as one part of the overall science program assessment. At the end of the workshop abroad or in the U.S., the students, teachers, and mentors will fill out an exit survey form that will solicit a written evaluation of the program and suggestions for improving this program. A follow-up questionnaire will be included as suggested by NSF's "Looking Beyond the Borders" handbook and the "2002 User-Friendly Handbook for Project Evaluation" (Frechtling, 2002; Loretz, 2002). Additionally, we plan to track the students' career development for four years by communication with the student and their advisors. Students who have participated in the South African workshops have routinely asked the mentors for advice on graduate schools or career advancement programs, and for letters of recommendation to the aforementioned. To date, we have had maintained good communication with undergraduates that participated in the NSF 2001 and 2002 workshops.

Annual reporting will include information on the overall efforts from student recruiting and selection through the abroad and stateside research and educational training internships / workshops as part of the EPO program for the proposed NAI. This information will include the undergraduate students' demographic information, year of schooling completed, their home institution and its highest degree granted in the student's field of study. Additional information about the students will include their research activities and findings with respect to specific research results and the training and development they received during the program. The training and development may include special skills learned, opportunities to present the research work within the NASA Astrobiology Initiative and in professional meetings, and efforts made with respect to graduate school and career advancement. Students will be encouraged to present their research at their home institutions, regional and national meetings, and to publish their research findings. One of the U.S. undergraduate participants, Kristi Coleman, of the South African workshops has presented a poster at the 2002 American Geophysical Union International Fall Meeting in San Francisco, CA (Coleman, et al. 2002). Another student, Jonesta Nolan will participate in a poster presentation at the American Society for Microbiology International Annual Meeting in Washington, DC, May 2003 (Nolan, et al. 2003). These types of presentation and publications will be documented and reported. Outreach activities to the international universities and mining companies will be described, as well as the impact of these international collaborations toward enhancing a diverse, competitive, and internationally networked workforce.

Summative program evaluation will be used to measure the success of the program. Measures to consider in the evaluation are student completion of their undergraduate programs, matriculation to a graduate program in the sciences or engineering, and recruitment in science and engineering careers as compared to a control group. Additional assessment will be garnered from the student and mentor surveys, questionnaires and evaluations of the EPO program for this NASA Astrobiology Initiative program. The program evaluation will include a cost assessment element. The UT Center for International Education will assist with program evaluation, data analysis, and assessment of this proposed EPO.

The technical/educational advisory committee whose members will be comprised of outside educators and scientists along with some NAI team investigators. This committee will review and evaluate the program progress, provide feedback for refinements for future activities.

DEVELOPMENT OF COURSES AND PROGRAMS RELATED TO ASTROBIOLOGY

At Princeton University, Onstott will develop a sophomore level course on Astrobiology supported by the University's Sophomore Initiative. This course will draw upon the expertise of faculty from Geosciences, Chemistry, Engineering and Astrophysics departments. This course will provide the basis for the developing a curriculum in Astrobiology. Many courses are already offered that are relevant to Astrobiology, including Freshman Seminar 160w-Terraforming Mars (Onstott-Geosciences), FRS-Origin of Life (Dismukes), FRS153 "Elements of Life" (Stiefel), CHM544/ENV544 "Metals in Biology" (Stiefel). A new Geoscience course-A tour of the Solar System is being developed by Duffy. Ge201 "Earth and the Terrestrial Planets" taught by Suppe. The ultimate goal is to have an undergraduate certificate in Astrobiology at Princeton University by 2008.

At Indiana University, Pratt is developing a new graduate course in geocmicrobiology. This course will be offered for the first time in Spring 2004 and will draw on faculty from the Geological Sciences Department, the School of Public and Environmental Affairs, and the Biology Department. For the past eight years, Pratt and associate instructors have taught a freshman-level course entitled "Earth: Our Habitable Planet." This course is taken by more than 200 students each the fall semester and more than 100 students each the Spring semester. Habitable Planet is designed to introduce students to the complex interactions among atmosphere, biosphere, hydrosphere and geosphere. A major component of Habitable Planet is current hypotheses concerning the origin of life and presence of life on other planetary bodies. Planets and Meteorites, taught by A. Basu, is another fully enrolled introductory science course taught in the Department of Geological Sciences. Pratt gives guest lectures based on her deep-subsurface research in an introductory Physics course entitled "Physics of Extraterrestrial Life." which is taught by Charles Horowitz. Pratt and Horowitz are working collaboratively with Chemist Donald Burke on the development of an introductory astrobiology course. We are designing this course for high enrollment (+200 students per semester) and for rotating faculty instructors from Geological Sciences, Biology, Physics, and Chemistry. If successful, this new course will be the first step toward a certificate or minor in astrobiology at Indiana University.

SUPPORT FOR ELECTRONIC COMMUNICATION AT INDIANA UNIVERSITY

Recognizing the importance of emerging communication and collaboration tools to teaching, research, and administrative activities, specific action items regarding videoconferencing, media streaming and collaboration were included in the Indiana University Information Technology Strategic Plan (ITSP) 1998-2003. The ITSP provided funding for service establishment and enhancement of existing centrally-funded management and operations. Through strategic plan initiatives, the University provides services and support for videoconferencing, media streaming and collaboration, without fee to academic, administrative and research users.

For the proposed IPTAI, Indiana University is able to provide institutional commitment to a wide range of communication and collaboration technologies through an extensive support organization. Indiana University Digital Media Network Services operates one of the largest University videoconferencing networks, provides media streaming services, and is in process of establishing an institutional data collaboration service. The vision is to make these three services function seamlessly - any event involving participants in distributed locations can take advantage of any or all of the services on a reserved or ad-hoc basis depending on their particular need.

Videoconferencing

Videoconferencing is used extensively throughout Indiana University's eight-campus system in support of teaching, research, and administration. NASA-deployed videoconferencing systems are identical to the systems currently supported at Indiana University. The technology currently used on campus is standards-compliant H.323, Internet-based. Approximately 145 group systems (conference rooms and classrooms) and 250 desktop systems are represented. Group systems are Polycom ViewStation and desktops are Polycom ViaVideo models. Two carrier-grade Polycom MGC100 videoconferencing bridges, provide for multipoint conferences (more than two parties in a single conference call), and can support approximately 200 simultaneous 384kbps endpoints in conference. Gateway bridging to ISDN H.320 videoconferencing is provided. The Radvision ECS200 gatekeeper supports 1000 registrations and 200 simultaneous calls in routed mode. Indiana University participates in the Internet2 Commons, ViDeNet and Global Dialing Scheme (GDS), allowing for easy dialing via E.164 "telephone-like" numbers to participating sites throughout the globe. Within the University, dialing is supported through a Global Address Book service. Key infrastructure components, such as the conferencing bridge and gatekeeper, are duplicated to provide fail-safe operation. Funds are requested in the IPTAI budget for two additional Polycoms that can be deployed in offices of the Institutional PI's at Princeton and Tennessee.

Media streaming

Media streaming services at Indiana University will support the encoding, storing and delivering of a variety of digital formats, including Real, Windows Media, QuickTime, MP3, MPEG-1, and MPEG-2. MPEG-4 support is in development. Current infrastructure for storage and delivery includes a Real Helix server with 1 TB of storage and capacity for 400 concurrent streams. The Helix server supports Real, QuickTime, Windows Media and MP3 formats. A VideoCharger server provides storage and delivery of MPEG-1 and MPEG-2 formats. Content-provider access to the streaming storage and server is provided via accounts on the institutional web publishing server. This method makes the process to publish streaming content intuitive, and familiar to anyone already publishing web content on the institutional web environment.

Implementation of a re-architected system is in progress that will significantly enhance the media streaming infrastructure. The new architecture is based on a 4TB network-addressable storage (NAS) server that provides a common storage service for a diverse collection delivery severs, supporting the range of delivery formats. The storage will be mirrored on identical systems on our Indianapolis and Bloomington campuses, interconnected by a University-owned fiber intranet between the two campuses. A third snapshot server will take periodic snapshot backups.

In 2003, an activity will be completed to establish a digital asset management system. Currently, all assets are managed as file objects with unique user spaces. The digital asset management system will provide methods to preserve and manage resources, through the use of standards-based video metadata and catalog, and will provided expanded access through search and retrieval systems. Commercial systems will be evaluated, as well as implementation of open-standards profiles such as the ViDe Application Profile for Digital Video. Also in 2003, in conjunction with the deployment of a digital asset management system, a digital rights management system will be implemented. Currently, assets can be quasi-secured through obfuscation. A comprehensive, secured method for providing rights management will be implemented.

Videoconference-to-streaming gateway

Videoconference-to-streaming gateway service to record and stream videoconferences was brought into production in the fall of 2002. The service, referred to as IStream, is built around Polycom Viewstations whose audio/video output feeds directly into streaming encoders; plus a comprehensive software system built to provide scheduling, operations, and user access to the content. The service allows any H.323 videoconference, occurring anywhere in the world, to be easily recorded and streamed, live or

on-demand. Real Networks and MP3 formats are supported. The service currently has capacity to capture eight concurrent videoconferences, and is scaled according to demand.

The University has installed videoconference endpoints in auditoriums at the IUB and IUPUI campuses specifically to take advantage of the IStream service. Lectures are recorded and optionally streamed live. The recorded format can be audio and video using Real Networks, or audio-only using MP3. The MP3 option has proven extremely popular - students can download lectures directly to desktop PC's or portable MP3 players. During the fall 2002 term, 30 classes utilized the I-Stream service. In addition to classes, guest lectures and one-time events are streamed using IStream, and administrative meeting have made use of IStream to establish meeting records.

Data collaboration

Data collaboration taxonomy can describe categories of remote presentation, interactive collaboration, and persistent shared virtual workspace. Remote presentation, typically used in conjunction with audio or video conferencing, provides for a member of a conference group to share, in real-time, the display of a computer desktop or application, with other conferees - and to share the information at native computer resolution, rather than say, at reduced resolution though in-stream display in a videoconference. Interactive collaboration provides for the live sharing of a computer desktop or application among a group - with any member of the group able to take control of the application. Interactive data collaboration tools can be specialized, such as web co-browsing or whiteboard, or may be generalized to share any application or desktop. Persistent shared virtual workspaces (PSVW) have the feature that information and work are captured and persistent over time in the shared space. PSVW tools range from simple threaded discussion lists, to complex environments that support tool sets such as threaded discussion, file sharing, project planning, outliners, meeting management, document review, calendar and others.

Remote display and interactive data collaboration are considered to the critical next step in establishment of electronic collaboration services at Indiana University. Several products are in pilot test at the University. A production implementation is planned for early summer 2003. Remote display and interactive data collaboration services will be established using easy-to-use web-based tools, hosted on University servers. Shared virtual workspaces are being explored in specific projects rather than enterprise service context. Videoconference classrooms and conference rooms are being equipped to permit concurrent display of videoconference and remote data display images.

Service Support and Cost

The Indiana University Digital Media Network Service unit is charged to provide infrastructure services and comprehensive user support for videoconferencing, media streaming and collaboration services. In addition to the infrastructure services described, DMNS provides support for acquisition, training, use, facility and resource scheduling, management, operations and help desk - staffed to provide instantaneous response to user service problems and requests. The DMNS operations center utilizes advanced commercial and custom tools to monitor and operate the network, including the Polycom Global Management system. DMNS is staffed with three videoconferencing engineers, two media streaming engineers, a data collaboration engineer, a systems administrator, a scheduler, a help desk coordinator, hourly help desk staff and a unit manager.

Members of the Digital Media Network Services unit are deeply involved in national and international digital video activities and collaboration technology groups and activities. The DMNS manager is currently co-chair of the Internet2 Digital Video Initiative; chair of the Video Development Initiative (ViDe), and is past chair of the Committee on Institutional Cooperation (CIC) Video Working Group.

The videoconferencing, media streaming and data collaboration services and support organization are provided as centrally-funded IT services. The services and support are provided without fee to academic, research and administrative users. Users are obliged to purchase their own end-systems, such

as Polycom videoconferencing terminals, but the support services, and central systems services such as multipoint conferencing bridging, streaming storage and servers, and data collaboration servers are provided without fee.

PRODUCTION OF PREMIERE-QUALITY DIGITAL MEDIA FOR WEB AND VIDEO DISTRIBUTION

Scientists and students on the IPTAI team will benefit substantially from collaboration with a professional staff of artists and digital media consultants. This production collaboration will enable IPTAI to develop premiere-quality materials for use on the web site and at conferences and workshops. We believe this collaboration will result in educational videos that illustrate how and why scientists conduct research under difficult and dangerous conditions such as in deep-mine locations. Video material of this type is unlikely to result from commercial television programs or magazines where the emphasis is on scenery and drama rather than scientific goals.

Digital Media Services (DMS) is a unit within University Information Technology Services (UITS) at Indiana University. DMS provides premiere-quality digital media solutions including Macromedia Flash animations, World Wide Web site design, production and maintenance, Digital Video Production including acquisition and post-production using the next generation of video standards including High Definition Television (HDTV), high-quality 3-dimensional computer animation, interactive Digital Versatile Disk (DVD) production as well as duplication services for the various media.

DMS is especially well-positioned for producing materials for the educational outreach and visualization components of the IPTAI proposal. Producing both short-form and long-form video programming for documentary-style materials can be applied to a variety of purposes including broadcast documentaries, short video packages, provision of material for public information entities, use in museum and other various educational venues, interactive DVD and streaming media via the Internet.

Acquisition and Animation

DMS maintains the position that capturing the visual and aural experiences of any situation in the highest quality format allows for the material to be skillfully manipulated to fit a wide variety of specific informational, educational and display purposes. To accomplish this, DMS acquires motion content using advanced digital imaging cameras. Typically, content would be shot using 720p or 1080i High Definition Television equipment. DMS is currently evaluating a number of products for purchase including the Sony CineAlta and the Panasonic AJ-HDC27F. For shooting conditions which could be potentially hazardous, DMS will rely on cameras such as the AG-DVX100.

Digital Media Services utilizes several 3-dimensional computer animation packages including Alias/Wavefront Maya, Discreet 3D Studio Max and Softimage XSI. Besides utilizing professional university staff to produce 3D content, DMS also capitalizes on skilled students from such schools and departments as the IU School of Informatics and the IU Department of Telecommunications.

Compositing, Postproduction and Distribution

Digital Media Services employs several talented in-house digital artists to acquire still images and to manipulate those images along with motion content and graphics with a variety of tools including Adobe Photoshop, Adobe Illustrator and Pinnacle Commotion.

The Avid DS|HD High Definition Nonlinear Edit System is the postproduction tool of choice for Digital Media Services. The Avid DS|HD allows for total image and motion control, extensive layering and compositing and advanced audio mixing. Materials edited on the DS|HD allow the editor to create productions in full HDTV but also allows for the finished project to be exported in a variety of frame rates and resolution sizes including the current NTSC standard. In effect, productions are “future-

proofed" allowing for use with today's display and transmission standards as well as the standards which will be in place within the next few years.

Selected Production Tools
1) Rimage Autostar II 2)Sonic Scenarist 3)Pioneer PRV-9000 4)Pinnacle StreamGenie 5)Helix Producer 6)Discreet Cleaner 7) Nikon Super Coolscan 4000ED 8) Epson 1640XLGA 9) Olympus Camedia E-20 10) Panasonic DVC Pro Camera 11) Media 100 NLE 12) Avid DS|HD NLE 13) Avid Xpress DV NLE 14) HDTV Cameras (Product Review Under Way – Purchase by May 2003)

Digital Media Services distributes content using a wide variety of methods. Distribution modes include Real Media and Windows Media streaming via dedicated streaming servers maintained by Indiana University Digital Media Network Services, digital video tape, Betacam SP, CD-R and DVD duplication using the Rimage Autostar II with Everest printing. Productions can be made available on most professional broadcast media for over-the-air transmission.

World Wide Web and Interactive DVD

In addition to the video capabilities mentioned above, whether it is acquired in the field, studio or by purely electronic methods, content produced by Digital Media Services is ideally poised to export material to the World Wide Web using Macromedia Dreamweaver and Indiana University's robust web hosting environment and/or to interactive DVD using Sonic Scenarist.

Summary

By acquiring, compositing, editing and distributing content completely within the digital realm, content produced by Digital Media Services maintains the highest possible technical quality while ensuring compatibility with current and future technologies. This allows the content to be shareable, scalable and adaptable thus protecting the time and financial resources spent.

We anticipate writing media grants for supplemental funding to cover costs for production of a broadcast documentary in Years 4 and 5 of IPTAI. We estimate that production of a one-hour video including audio collection, audio mixing, video editing, and 3-D animations will cost about $120,000. Jasiak and Droppo (see CV's for non-funded collaborators) will take the lead on writing grants for video production if the IPTAI is funded.

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FACILITIES AND EQUIPMENT

Indiana University: Indiana University supports the IU Stable Isotope Research Facility (SIRF) by providing salary and benefits for Mr. Steve Studley (professional-rank technician). Additional technical expertise is provided by Mr. Jon Fong (lab technician), and Ms. Terry Stigall (electronics expert). Currently, SIRF maintains five isotope ratio mass spectrometers:

1. Finnigan 252 GC-C-IRMS (gas chromatograph-combustion-isotope ratio mass spectrometer)
2. Finnigan 252 dedicated for sulfur isotopic measurements, with dual-inlet, multiport inlet, continuous flow elemental analyzer, and laser fluorination system.
3. Nuclide 6-60 IRMS (soon to be retired)
4. Finnigan Delta E Mass Spectrometer
5. A new Finnigan Delta Plus with gas chromatography-combustion-elemental analyzer interfaces has recently been ordered as a result of the funding of an NSF equipment proposal. The new instrument will be dedicated to H and O isotopic analyses.
6. Finnigan MAT TSQ 700 (gas chromatograph-molecular mass spectrometer)
7. Perkin Elmer 5100 Atomic Adsorption Spectrophotometer with flame and graphite furnace capabilities as well as hydride generation
8. Jobin Yvon ICP JY-38

Indiana SIRF is one of the few laboratories in the U.S. with a modern isotope mass spectrometer (Finnigan MAT 252) dedicated to running sulfur samples. This instrument has an elemental analyzer (EA) inlet that is well calibrated for samples containing between 0.1 and 1 micromole of sulfur in the form of $BaSO_4$ or AgS. Our reputation for high accuracy and precision of sulfur isotope measurements using the EA is validated by numerous requests for access to the SIRF mass spectrometers. The institutional affiliation of our collaborations and contracts for $*^{34}S$ data over the past two years is as follows: Max Planck Institute for Marine Microbiology (Germany), Odense University (Denmark), University of Cincinnati, University of Missouri at Columbia, American Museum of Natural History, Harvard University, NASA Ames, Carnegie Geophysical Laboratory, Northern Illinois University, Kanazawa University (Japan), Iowa State University, University of Missouri, and Pennsylvania State University. In addition to routine $*^{34}S$ measurements, Ripley and Pratt are collaborating on the design and calibration of a laser-assisted fluorination chamber for determination of $*^{33}S$ and $*^{36}S$ on samples of AgS converted to SF_6. We currently are able to produce pure F_2 in our reaction line and we are able to detect all four sulfur isotopes in the form of SF_6. We anticipate having the fluorination chamber connected directly to the mass spectrometer via a cryogenic trap within two months. We have a waiting list of more than twenty researchers who have requested rare sulfur isotope measurements when the fluorination apparatus is completely installed. Many of our outside requests for rare isotope measurements are from members of currently funded Astrobiology Institutes.

Princeton University:

Laboratories**:** The Geomicrobiology lab resides in the basement of the department and houses fume hoods, crushing, grinding, powdering, and separation equipment. The lab also includes chemical stock room, two HEPA filtered hoods, UV sterilization hood, -80°C freezer, -20°C freezers, refrigerators, anaerobic glove bag, incubators, water baths, high temperature ovens, centrifuges, UV spectrometer, Robocycler for DNA amplification and electrophoretic gels. UV thermal printer is available in Ward lab for printing DNA gel runs. The lab's analytical facilities include Perkin Elmer ICP-AES, LICOR CO_2 analyzer and Dionex ICMS. The lab's optical microscopy and fluid inclusion section houses two microthermometry stages, cathodaluminocsope and two high quality optical microscopes. A digital, color CCD and analog B/W CCD camera and image analyzer is connected through the local computer network for uploading digital photomicrographs. The BMX60 microscope is equipped with epifluorescence imaging with UV cubes and filter sets appropriate for DNA and protein stains and FISH and phase contrast objectives. The department's machine shop resides on the same floor several lathes and drill presses included a fully-programmable lathe, which is used to manufacture our field sampling equipment. DNA sequencing facilities, FACScan flow cytometer, Molecular Dynamics Imager for mapping

radioactive samples and the laser confocal facility are readily available on the same floor in the Molecular Biology Department. The Geosciences X-ray diffraction lab resides on the same floor and houses two Scintag PAD spectrometers, one of which possesses a high temperature stage. The lab also makes use of CEBIT's ICP-MS also resides on same floor. The electron beam facility at the Princeton Materials Institute and houses a Phillips XL30 FEG SEM, a CAMECA SX50 EPMA, a Phillips CM200 FEG TEM, a Zeiss 910 TEM. GC-MS, HPMS and MALDI- TOF facilities for detailed organic characterization are available in the Mass Spectrometry Facility of the Chemistry Department.

University of Tennessee: The Center for Environmental Biotechnology at The University of Tennessee, Knoxville occupies 26,000 sq. ft. in the newly constructed Science and Engineering Research Facility on the main campus of the University of Tennessee, Knoxville and 8,000 sq. ft. at the Pellissippi Facility. CEB has skilled technical personnel and extensive capabilities for aerobic and anaerobic microbial cultivation, analytical and radiological methods used in microbial ecology, biodegradation, and molecular biology. This laboratory contains all the necessary basic equipment needed for microbial experiments including shakers, incubators, waterbaths, autoclaves, laminar flow hoods, laboratory and field anaerobic glove bags, liquid scintillation counters, centrifuges and spectrophotometers. The laboratory contains 8 capillary or packed column gas chromatographs equipped with detectors (TCD, FID, ECD, PID and GPC) and autosamplers, and a Hewlett Packard bench-top 6890 GC/MS and a VG Platform II HPLC electrospray, atmospheric pressure chemical ionization, GC/MS. The HP system is also configured with the Entech autosampler and serial preconcentrators for VOC's. Analytical computer systems are used for data management. Additional equipment available to the project include a Dionex ASE-200 Accelerated Solvent extractor with a 24 station autosampler, HPLC with fluorescence, UV-Vis, electrometric, conductivity, and radiochemical detectors, supercritical fluid capillary chromatograph, and Fowler cells. Microscopes include the Laser Precision Analytical triple-beam ATR/FTIR with an Analect IR Microscope, Zeiss Axioplan microscope, and a confocal microscope and Hamamatsu C4200-77 photon-detecting camera with the Argus-10 imaging system. In the molecular biology laboratory, a Perkin-Elmer-Cetus thermal cycler, bioimaging analyzer for gels (Alpha Innotech Image analyzer / densitometer the data from is Window 95/97 compatible), conventional gel electrophoresis units, "Fast-prep" bead beater (Bio 101) and a DGGE unit with a TGGE converter (BioRad), Beckman Oligo 1000 DNA Synthesizer, GSI Lumonics ScanArray 5000, Engineering Services, Inc. DNA Micro-Arrayer (SDDC), BioRad iCycler QPCR. The Department of Microbiology has a flow cytometer and the Department of Geological Science has an isotope ratio mass spectrometer. In addition, the University of Tennessee has a dedicated Molecular Biology Resource Facility (MBRF) with an Applied Biosystems 373A Automated Sequencer. The University also maintains the Biology Services Facility with machine and electronics fabrication/repair services.

Oak Ridge National Laboratory: *ORNL Environmental Sciences Division* has an extensive array of instrumentation.

Seafloor Process Simulator and Deep Subsurface Process Simulator. The high pressure biogeochemical laboratories contain tow major high pressure devices, a 72 liter 3000 psi seafloor process simulator and a <1 liter 5000 psi subsurface sediment analysis system. Both systems are computer instrumented and controlled and are in explosion proof vented facilities. All safety and health consideration are fully implemented and highly trained scientific personnel are operators. Facilities are used for geochemistry, biogeochemistry, isotopes studies, methane and carbon dioxide hydrates and supercritical carbon dioxide studies in complex native sedimentary materials.

Microbiology and Molecular Biology. The Microbiology Laboratory has extensive facilities for work with aerobic and anaerobic microorganisms. A variety of controlled temperature incubators and chambers, autoclaves, freeze-drying facilities, storage facilities, refrigeration, and a wide range of state-of-the-art analytical equipment are available. The laboratory contains sterile transfer hoods, autoclaves, spectrophotometers, gas chromatographs, automated plating equipment, automated bacterial identification systems, laser colony counting equipment, fume hoods for work with volatile contaminants, gassing stations for the preparation of anaerobic media, two anaerobic glovebags (Coy Laboratory, Inc.), electrolytic respirometers, flow cytometry, an automated GeneTACÔ colony picker (Genomic Solutions),

a robotic liquid handler (RoboSeqÒ 4204 SE), HiGro System (GeneMachines), and Bioflo 110 modular benchtop chemostats (New Brunswick). Trained laboratory technicians keep this equipment operational. The Molecular Laboratory also includes 24 PCR thermocyclers, an iCycler Thermal Cycler (Bio-Rad) for real-time quantitative PCR, an AKTA FPLC (Amersham Pharmacia Biotech) for protein purification, a denatured gradient gel electrophoresis system, controlled temperature water baths and incubators, several high-speed centrifuges, and numerous microcentrifuges, an electroporator, and a UV crosslinker for DNA hybridization studies. A variety of electrophoresis equipment, power supplies, Oriel photomultiplier with collimating beam probe and fluorescence spectrometer are available. UVP digital photograph systems equipped with CCD camera for image capture are also available.

Geochemistry Laboratory. The Mineral Characterization Laboratory contains a Scintag XDS 2000 X-ray diffractometer (XRD), a FTIR spectroscopy (Nicolet Magna-IR 760 spectrometer), an Atomic Force Microscope (Digital Instruments) and a JEOL JSM-35CF Scanning Electron Microscopy (SEM), utilizing both secondary electron imaging and backscattered electron imaging in conjunction with energy-dispersive and wavelength-dispersive x-ray spectroscopy. The ESD has laboratory facilities designed for the detection and quantification of the elements relevant to FRC research that are available for use by this project. There are dedicated gas chromatographs with TCD, FID, ECD and MS detectors and high-pressure liquid chromotographs (HPLC) for the analysis of organic compounds and dissolved gases. There are ion chromatographs (IC) equipped with conductivity and spectral array detectors for anion analysis and the detection of chelated metals; capillary electrophoresis equipment with Ultraviolet/Visible detectors for anion, cation, and chelated metal detection; atomic absorption spectrophotometers with graphite furnace for the analysis of metals and an inductively coupled argon plasma spectrophotometer for determination of metals and some nonmetals (*e.g.* S, P

University of Toronto: In the past decade, compound specific isotope analysis (CSIA) has revolutionized the application of stable isotopes to understanding biogeochemical cycling. Continuous flow analysis provides an increased sensitivity of analysis of 4-5 orders of magnitude and is the key to routine application of isotopic analysis to compounds present at the kinds of low levels common in the natural environment. The Stable Isotope Laboratory at the University of Toronto played a leading international role in the application of this new area of technology to dissolved organic contaminants in hydrogeological studies and to naturally occurring gases in deep groundwater systems. and scientific research. The laboratory is equipped with a high sensitivity Finnigan MAT 252 continuous flow gas source mass spectrometer for compound specific carbon isotope analysis and dual inlet CHON analysis. In addition in 1999 the laboratory installed one of the first Delta+-XL Finnigan MAT continuous flow mass spectrometers for compound specific hydrogen isotope analysis. The laboratory has extensive experience and well developed sample preparation systems (vaccum lines, carbonate prep systems, CHN sealed quartz combustion lines, Toepler gas transfer systems) for both gaseous and dissolved organic contaminants. A full time research associate (Dr. G. Lacrampe-Couloume) and part-time technician ensure the highest level of sample throughput, standardization and QAQC. In addition the laboratory hosts 5 gas chromatographs equipped with FID, TCD and micro-TCD detectors and cryogenic capabilities, laminar flow hoods and an anaerobic chamber. In addition to the facilities of stable isotope analysis, in cognant departments the University of Toronto houses abiotic and biotic microcosm facilities, microbiological growth chambers, GC and LC, and equipment for isolation and characterization of micro-organisms and microbial DNA and. Through Dr. Sherwood Lollar's status as Adjunct Professor in the Dept of Chemistry we also have access to the Environmental Analytical Chemistry "Analest" Facility incorporating a wide range of sophisticated chromatographic, spectroscopic and GC-mass spectrometry instrumentation.

Pacific Northwest National Laboratory: Since 1970, Pacific Northwest National Laboratory has been a leader in studies of subsurface microbiology. While, most of this work has been directed toward the resolution of environmental problems, a great deal of fundamental investigation has been conducted both on-site and off- by PNNL staff members. Bench to field-scale research activities have formed the basis for understanding the subsurface fate and transport of radionuclides, metals, anions and organic contaminants, as well as microbial community structure and the ecological energetics of the subsurface

biosphere. These activities have been sponsored by DOE, DOD, EPA, NSF and private industry.

Environmental Microbiology Laboratories. Environmental Microbiology Group maintains research facilities at PNNL and has extensive capabilities for studying aerobic and anaerobic microorganisms and the processes they catalyze. This equipment and instrumentation includes anaerobic glove bags, custom-mix gassing stations for the preparing anaerobic media and culturing fastidious microbes, incubators, bioreactors of various sizes and configurations, confocal laser scanning and epiflourescent microscopes, flow activated cell sorter, kinetic phosphoresence analyzer (for U), liquid scintillation counter, HPLC, GC, FPLC, and reduction gas analyzer. The group maintains fully-equipped molecular biological facilities including thermocyclers for PCR (including real-time Taqman PCR), pulse-field gel electrophoresis equipment, a Perkin Elmer model 373 automated DNA sequencer, and GeneScan software package for Terminal-RFLP analysis.

Diversa Corporation. Diversa possesses the technology to encapsulate cells in gel microdroplets, run the growth columns, and has four MoFlo Cytomation flow cytometers for analysis and sorting of GMDs. Diversa is a medium-sized company (ca. 400 employees) with cutting-edge molecular biology capabilities and technologies. Diversa has invested tens of millions of dollars in their high-throughput cultivation system, which is now being used in a high-throughput manner for small molecule discovery.

Lunar and Planetary Institute, Universities Space Research Institute:

The Lunar and Planetary Institute is a focus for academic participation in studies of the current state, evolution, and formation of the solar system. The LPI maintains a resident research staff of 20 scientists whose main tasks are to provide the planetary expertise necessary for the Institute to achieve its goals, and to maintain their scientific proficiency through peer-reviewed research activities. The Computing Center at LPI is built around a local area network that connects SUN Ultra SPARCstations, an 8 node Beowulf Linux Cluster and over 100 Pentium IV PCs and G4 Macintoshes with a combined online disk storage of over 1 terabyte. The network is connected to the Internet via a 3 Mbps ATM link.

The LPI's Center for Information and Research Services (CIRS) collection includes over 50,000 monographs, journals, slide sets, maps, videos, and CD-Roms. The subject emphasis of the collection is astronomy and geology, with limited collection development extending into secondary support fields of computer sciences and remote sensing.

As a NASA Regional Planetary Image Facility (RPIF), CIRS curates data and provides a facility for researchers to examine and interact with data from planetary missions. The RPIF data collections contain images, maps and CD-Roms that have been obtained from the Surveyor, Ranger, Lunar Orbiter, Apollo, Magellan, Galileo, Voyager, Viking, Clementine, Mars Pathfinder, and Mars Global Surveyor missions. CIRS is also a member of AMIGOS, a network of over 700 regional libraries that provides continuing education classes, support services, resource sharing, and access to the Online Computer Library Center (OCLC), an international network of over 40,000 libraries sharing resources, cataloging, and cooperative development work.

Lawrence Berkeley National Laboratory: **LBNL Center for Environmental Biotechnology Core Facility:** The core microbiology facility of LBNL is in the Center for Environmental Biotechnology, located in building 70 and 70A. The seven-laboratory unit occupies a total area of 2,950 square feet. The laboratories are set up for class II, type A/B3 molecular- and microbiology work. Level 1 quality and safety assurance procedures are in place. The following work-specific equipment and instruments are available in the laboratories:

- Lietz Laser Con Focal Microscope with digital imaging;
- Affymetriz Gene Microarray Processor 3000
- SterilGARD II 6-foot vertical laminar-flow, biological safety cabinet (Baker);
- 2 Avanti J-25 high performance centrifuge (Beckman);
- 2 Coy Anaerobic Chambers one double wide with microscope and incubator
- DU 640 UV/VIS scanning spectrophotometer (Beckman);
- Ultra-low temperature freezer (Revco);
- 2 Axioskop RLF for DIC, phase contrast, and epifluorescence with microphotography (Zeiss

- Integrated SpeedVac (Savant);
- GeneAmp PCR system 9600 (Perkin-Elmer);
- Expedite 8909 DNA synthesizer (PerSeptive Biosystems);
- Model 377 ABI Prism automated DNA sequencer (Perkin Elmer);
- CHEF DRII pulsed field electrophoresis equipment (Bio-Rad)
- MIDI identification system (Hewlett Packard);
- High sensitivity MSD mainframe for the HP 6890 GC (Hewlett Packard).
- BIOLOG microbial identification system (BIOLOG);
- Environmental shakers with photosynthetic light banks (New Brunswick);
- Alliance HPLC system with a 996 photodiodphotodiode array detector and a 474 scanning fluorescence detector (Waters).

The core microbiology facility preserves and maintains laboratory strains and wild-type strains isolated from environmental samples, microbial genomic DNA, plasmids and cloned genetic material. We also have 3 glove boxes and incubators that allow us to work with anaerobic microorganisms and microaerophiles.

VIMSS-GTL Experimental Core Facilities: The Environmental Molecular Microbiology Facility (ORNL, LBNL, U. Washington, Diversa Inc.), Environmental Simulation and Culture Facility (LBNL, U. Washington, Diversa Inc.). Technologies and facility resources are under development at LBNL and Diversa for culturing hard to grow organisms in controlled, reproducible environments. LBNL is also developing defensible environmental model chambers at different scales for better mimicking the natural environment of the microorganisms under study. These facilities will be fully integrated with the Advanced Light Source Microscope Beam Lines (1.4.3, 4.0.1-2, 6.1.2, 7.0.1, 10.3.1, 10.3.2) to take advantage of its unique analytical capabilities for environmental and biological samples, (infra red and x-rays); the Center for Isotope Geochemistry and its ability to analyze environmental samples for stable isotopes, (isoprobe); and the Center for Environmental Biotechnology with it's facilities for PLFA and nucleic acid analyses from environmental samples, soil columns, bioreactors, SLCM imaging, and biological safety level 2 laboratory. The LBNL National Center for Electron Microscopy will also enable other studies of environmental and biological specimens using state-of-the-science electron microscopes, (scanning transmission electron microscopy). This integration of unique instrumentation and facilities at LBNL with the VIMSS facilities at University of Washington for flow cytometry, bioreactors, functional microarray construction, and the Diversa environmental culture, isolation, archiving facilities, make this facility a unique and valuable resource for DOE. These facilities are also key to obtaining the highest quality and quantity of biological material for the other experimental facilities and research cores in VIMSS.

Goddard Space Flight Center
Greenbelt, MD

Origin and Evolution of Organics in Planetary Systems

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The complete NAI Goddard Team proposal is not available because it contains proprietary information

Executive Summary: NASA Goddard Space Flight Center

Planetary Systems form by collapse of dense inter-stellar cloud cores (Frontispiece). Some stages in this evolution can be directly observed when stellar nurseries are imaged (Figure ES.1), while other stages remain cloaked behind an impenetrable veil
of dust and gas. Yet to understand the origin of life on Earth, we must first develop a comprehensive understanding of the formation of our own planetary system.

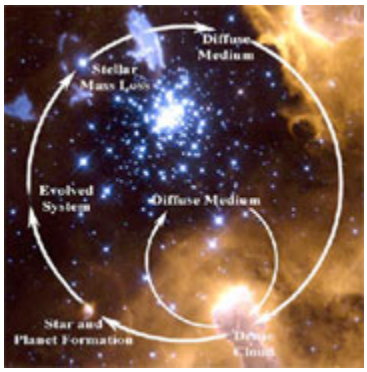

Figure ES.1 HST image of NGC 3603 showing the life cycle of material (including carbon) in a star-forming region.A cycle of stellar birth and death leads to the synthesis and evolution of organic compounds. Carbonaceous material ejected from dying stars enters the diffuse medium and then is cycled into dense clouds. The collapse of a dense cloud forms an evolved stellar system (see the Frontispiece for more detail) where these organic compounds can be delivered intact to planetary surfaces and mixed with those produced endogenously. As the lifetime of the evolved system comes to a close, stellar mass loss recycles material to begin the process anew.

Dense cloud cores are very cold (10-50 K), and their dust grains are coated with ices comprised of water and organic compounds.Many of these organics have potential relevance to the origin or early evolution of life,if delivered to planets.

The survival of these organics through the violent birth-phase of a star is less certain. Properties of the young star (its mass, spectral energy distribution, whether it formed in isolation or as a multiple star, etc.)play a key role in controlling the evolution of organic material in the proto-planetary disk. The location within the disk is important since the nature and effectiveness of such processing depends strongly on distance from the young star, on distance above the nebular mid-plane,and on time. The ultimate delivery of these primitive organics to young planets and their moons also evolves with time, as the bodies grow in size and as the nebula clears.

We propose to investigate the origin and evolution of organic compounds in planetary systems, and their delivery to young planets.

The proposed research addresses the heart of Goal 3 of the Astrobiology Roadmap:
Understand how life emerges from cosmic and planetary precursors.

The central question is this: Did delivery of exogenous organics and water enable the emergence and evolution of life?

The investigation is divided into four Themes:

Theme 1: *Establish the taxonomy of icy planetesimals and their potential for delivering pre-biotic organics and water to the young Earth and other planets.*
Theme 2: *Investigate processes affecting the origin and evolution of organics in planetary systems.*
Theme 3: *Conduct laboratory simulations of processes that likely affected the chemistry of material in natal interstellar cloud cores and in proto-planetary disks.*
Theme 4: *Develop advanced methods for the in-situ analysis of complex organics in small bodies in the Solar System.*

We seek to better understand the organic compounds generated and destroyed in the interstellar and proto-planetary environments, through observational, theoretical, and laboratory work. We will examine the potential for and limitations to delivery of exogenous pre-biotic organics to planets, examining factors that enhance or restrict this potential.

We will, for the first time, investigate the effect of astrophysical X-rays on the evolution of exogenous organics in proto-stellar disks. We will follow these factors over time, from the natal cloud core through the

end of the late heavy bombardment (~4.1 Ga). We will evaluate the possible role of exogenous organic material in terrestrial biogenesis.

The proposed research will significantly improve our understanding of the nature of organics in other planetary systems, the processes affecting them, and the potential for delivering pre-biotic organic compounds to planets.

The Management Plan: An Integrated Research Approach

The proposed research is interdisciplinary and it involves researchers at multiple institutions. This is both an intellectual asset and an organizational challenge. The effectiveness of a Team is demonstrated when its total output exceeds the sum of its individual parts. We have developed a management strategy that we believe will enable this objective.

- *Internal collaboration will be enhanced by bridging post-doctoral associates and students across projects within a Theme.*

- *Theme-Based "Expeditions" will be mounted to ensure that our students receive hands-on experience in techniques used in all Themes.*

- *Students and post-docs will be encouraged to explore other aspects of Astrobiology at luncheons every two weeks.*

- *An Executive Scientist will ensure smooth operations of the Node, and timely reporting to NAI Central and to NASA Headquarters.*

- *An Executive Committee will review the scientific progress and activities, monthly. An independent Board of Visitors will assess progress on an annual basis.*

- *An Education and Public Outreach Lead will ensure that our E/PO plan is smoothly executed.*

Education and Public Outreach

GSFC and the Minority Institute Astrobiology Collaborative (MIAC)will implement a multifaceted program based on Astrochemistry and focused on organics in the solar system. We will develop curriculum materials, conduct teacher professional development workshops,and bring observational cometary research into middle and high school classrooms. We will support MIAC institutions in the professional development of K-12 educators in under-served communities and build upon existing MIAC, GSFC and UMCP (Deep Impact EPO) programs and educator networks.

Lead Institution Commitment

NASA 's Goddard Space Flight Center has long-established scientific expertise in all four Theme areas. The proposed research draws upon large and highly productive ongoing programs in areas of Laboratory Astrochemistry; Planetary Systems research; Interstellar, Stellar, Planetary, and Cometary spectroscopy; and Flight Instrument Development.

The Center has made an advance commitment to the Astrobiology Program by hiring Drs. Jason Dworkin and Michael DiSanti as civil servants, and providing infrastructure support to them. Goddard also plans to fill additional civil service positions in the areas of nebular and cometary chemistry.

The Center has recently devoted an NAS-NRC Resident Research Associateship to Astrobiology and is actively seeking a candidate to fill this position. If our Node is selected, the Executive Scientist will relocate to Goddard. Visiting faculty, post-doctoral associates, and graduate students will be supported to augment the already significant scientific complement at the Center.

Leverage

This proposal heavily leverages the existing research programs of the individual Investigators. Our Investigators have access to advanced laboratories and observatories through their existing institutional arrangements and partnerships.

Principal Objectives

Theme 1: *Organics in Icy Planetesimals: A Key Window on the Early Solar System*

A. Comet taxonomy via specific molecules and isotopes

1. Measure abundances of parent volatiles
2. Measure the ratio HDO/H2O
3. Measure abundances of chemically related molecules

B. Perform detailed theoretical studies of the molecular chemistry of proto-stellar disks

1. Model infall for specific chemical changes in the major volatile components
2. Determine how trace cometary organics can also be formed at the accretion shock
3. Model interstellar deuterium fractionation as ISM material incorporates into the nebula

C. Model dynamical transport of icy planetesimals in the early Solar System

1. Model organic flux into Oort cloud, terrestrial region, and out of the Solar System
2. Model 1 including giant planet migration
3. Model 2 including the formation of Uranus and Neptune
4. Simulate the growth of grains followed by settling to the mid-plane

D. Determine isotopic compositions and abundances in Lunar breccias

1. Determine the signature(s) of highly siderophilic abundances in Lunar breccias
2. Connect signature(s) to materials exposed to early Solar System processes
3. Determine if the composition of the late influx changed with time
4. Connect siderophilic impactors with those of organic-rich chondrites

Theme 2: *From Molecular Cores to Planets: Our Interstellar Heritage*

A. Study the evolution of material in molecular clouds

1. Map chemical abundances to determine the physical conditions within a molecular cloud
2. Search for new interstellar organic molecules

B. Determine the initial conditions for planet formation

1. Understand the growth of grains prior to and during incorporation in the disk
2. Observe grain growth and document opacity loss as material gets incorporated mm scale bodies
3. Determine the relationship between cometary and interstellar chemistry

C. Connect the X-rays and UV from young stars to formation and destruction of organics

1. Compare abundances of organics around young stars with models and lab simulations
2. Measure the emission and ionization state of molecules near young stars
3. Measure changes in the chemical abundances as a consequence of strong X-ray and UV flaring

D. Search for organic signatures in the IR spectra of transiting extra-solar gas-giant planets

Theme 3: *Organic Material from Laboratory Simulations of Astrophysical Environment*

A. Analyze complex organics in grain-catalyzed reactions

1. Investigate organics in hydration and thermal metamorphism ala various meteorite types
2. Compare results with astronomical observations, meteorites, and Earth-return samples

B. Analyze complex organics in UV, X-ray, electron, and proton processed ices

1. Follow formation and destruction of selected organic compounds in detail
2. Look for the formation of particularly interesting biological molecules
3. Compare results with astronomical observations, meteorites, and Earth-return samples

C. Analyze more complex simulations

1. Combine materials and techniques of 3A and 3B
2. Investigate organics in residues in various aqueous environments
3. Follow the reactions of residues mixed with grains
4. Follow the reactions of residues induced by additional ion or photon processing

Theme 4: *Advanced Analysis of Primitive Material*

A. Determine how to measure the history and the chemical state of organics in situ

1. Evaluate a number of possible chromatographic mass spectral techniques
2. Compare bulk pyrolysis and laser and ion beam volatilization for this evaluation (4A.1)

B. Evaluate, minimize, and manage thermal perturbations to Earth-return samples

1. Determine how to preserve the structure and isotopic composition of relevant organics
2. Optimize method to determine the original composition of compounds before heating

C. Utilize lab analogs to develop and calibrate instruments

Marine Biological Laboratory
Woods Hole, MA

From Early Biospheric Metabolisms to the Evolution of Complex Systems

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1.

PROPOSAL TITLE PAGE

From Early Biospheric Metabolisms to the Evolution of Complex Systems

Submitted by: The Marine Biological Laboratory at Woods Hole

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3. EXECUTIVE SUMMARY

Life flourishes within a thin veneer that corresponds to no more than 0.05% of Earth's diameter and it represents less than 10^{-10} of our global mass. Yet, through metabolic and biogeochemical processes, biology imposes an overwhelming force on planetary change. Whether biological systems similar to those on Earth ever occurred or continue to function on other planets or large satellites is unresolved. Given the imprint of life on Earth's geological record, the presence of comparable biological activity would have a profound effect on the landscapes and atmospheres of other solar system bodies. Recognizing the potential role of biotic systems in modifying planetary environments, astrobiological imperatives include delineating the evolutionary history of life, exploring the nature of early metabolic processes, and defining the limits of the "habitable zone". When and where did life originate? How did biological complexity develop from emergent properties of living organisms? Which metabolic processes were significant drivers in modifying early Earth? What are the limits of extreme environments compatible with life? Answers to these questions will have a direct impact on how to organize and target exploration in search of living organisms beyond our own biosphere.

We cannot be certain about when or how many times prebiotic chemistry crossed the threshold to a microbiological world. Impact histories (4.1-3.8 Ga) constrain the persistence of the earliest evolutionary lineages to the end of the period of intense bombardment although deep subsurface chemoautotrophs at kilometer depths could have survived even the largest impact events. Current paradigms trace the evolutionary history of all extant organisms to a common ancestor or population of ancestors that was exclusively microbial. Cyanobacterial-like fossils suggest that life is at least 3.45 Ga-old [1, 2], but the biogenic origins of these structures are under renewed scrutiny [3]. The chemical record documents prokaryotic metabolisms that may have existed 3.47-3.85 Ga [4, 5] and eukaryotic biosignatures that may be 2.7 Ga-old [6]. Yet, these interpretations are subject to artifacts that might be attributable to microbial contamination and they do not set absolute limits on the possible origins of life on Earth. Much later in Earth's history, the multicellular world emerged [7]. The paleontological record teaches us that microbial life can thrive in diverse environments devoid of multicellular organisms [8]. In contrast, the survival of macroscopic organisms is completely dependent upon the transformations afforded by complex microbial communities. If life exists beyond Earth, compelling arguments hypothesize such a world had its origins and continued manifestation in a microbial world.

Microorganisms of untold diversity are usually members of complex communities that dominate every corner of our biosphere. They orchestrate key processes in geochemical cycling, biodegradation and in the protection of entire ecosystems from major environmental shifts. Their effects can be global in scale. A single group of minute cyanobacteria, the *Synechoccoccus* / *Prochlorococcus* clade, with a planet-wide biomass on the order of one billion metric tons, is responsible for some 10 to 50% of the ocean's primary productivity [9]. Microbial carbon re-mineralization, with and without oxygen, maintains the carbon cycle. Microorganisms control global utilization of nitrogen through nitrogen fixation, nitrification, and nitrate reduction. They drive the bulk of sulfur, iron and manganese biogeochemical cycles [10]. Rarely studied bacterial mutualists provide essential nutrients and other compounds to diverse plant and animal hosts, and thus have a pervasive impact on the distribution, productivity and diversification of multicellular organisms. Processes and activities mediated by microbial populations have continuously imprinted the geological record, which provides a means to date major environmental shifts. Similarly, the genetic record offers insights about biological evolution, the invention of new metabolic capabilities, and increases in biological complexity that led to the emergence of the multicellular kingdoms of plants, animals and fungi.

The MBL/Woods Hole Astrobiology team seeks to understand patterns and mechanisms of genome evolution and metabolic variation that allowed diverse microorganisms to adapt to new environments, generate novel phenotypes, and evolve processes that led to environmental changes on a global scale, some of which can be detected through remote sensing. In addition to providing increased knowledge about evolutionary history of living organisms on Earth, these studies will provide a basis for interpreting telemetry data and developing life detection technology for planetary exploration. Our general strategy emphasizes the integration of molecular approaches to evolutionary biology with studies of metabolic activities in environments that are likely to reflect conditions on early Earth. The physiological and microbial diversity studies will generally focus on the hydrothermally altered sediments of Guaymas basin in the Gulf of California, the acidic, heavy metal laden Río Tinto of southwestern Spain, and isolates from the Juan de Fuca Ridge. Four major research themes (**EARTH'S EARLY BIOSPHERE; TERRESTRIAL ANALOGUES FOR EARLY MARS; BIOSIGNATURES AND LIFE DETECTION** and **EVOLUTION OF GENOME ARCHITECTURE IN PROKAROTES AND EUKARYOTES**) provide a framework for interdisciplinary research. Several of these projects represent new initiatives, while others are logical extensions of efforts supported by our initial membership in the NAI. We also describe an education and outreach program that capitalizes on our strengths in microbiology, molecular evolution and genome sciences.

EARTH'S EARLY BIOSPHERE: EVOLUTION OF MICROBIAL PHYSIOLOGIES. (A. Teske, S. Sievert). Classical, ribosomal (rRNA) phylogenies alone cannot inform us about the early evolution of microbial metabolisms and pathways. Through studies of genes for conserved enzymes with well-defined physiological roles within anaerobic and/or autotrophic pathways, we will explore the physiological history of early microbial life in the context of the early biogeochemical evolution of the biosphere. Individual projects include:

- Exploring the origin and diversification of ancient pathways: sulfate reduction and methanogenesis
- Determining the evolution and diversity of autotrophic CO_2 fixation pathways including reductive TCA pathways in anaerobic and extremophilic microorganisms in modern and paleo-environments.

TERRESTRIAL ANALOGUES FOR EARLY MARS: a) Iron Oxidation – Shaping past and present environments, b) Microbial diversity and population structure studies in the Río Tinto and **c) Life detection through remote sensing.** (L. Amaral Zettler, M. Sogin, K. Edwards, A. Teske, J. Mustard, J. Head). Banded iron formations (BIF) dating back to the Archean and the broad phyletic distribution of proteins with FeS centers indicate the importance of iron and Fe cycling for early life forms. Our astrobiology team will explore microbial Fe metabolism in prokaryotes from metal sulfide deposits at Juan de Fuca Ridge and determine microbial population structures in the acidic, Fe-rich environment of Río Tinto; both are potential analogues for an early, wetter Mars. An interdisciplinary team of biologists and geologists will incorporate new information from these studies into the design of life detection through remote sensing. We will also complete expression profiling experiments for oxygenic cyanobacteria in microbial mats with the goal of understanding the molecular basis of adaptation to extreme environments and application of these techniques to studies of microbial communities in extreme environments. Individual projects include:

- Investigating the fundamental biology of Fe oxidation in mesophilic and extremophilic microorganisms, and establishing the linkages to geochemical processes.
- Community microbial diversity surveys of the microbiota from Río Tinto based upon SAGT ("Serial Analysis Gene Tags", a newly developed technique for high-throughput analyses of rDNA sequences)
- Coupling diversity with biogeochemical data with emphasis on iron oxidation

- Use of minerals from iron-rich, acidic environments as biosignatures for remote sensing
- Expression profiling and the formation of biogeochemical gradients in cyanobacterial mats and in extreme environments

BIOSIGNATURES AND LIFE DETECTION: DETECTING ANCESTRAL PEPTIDES AND PROTEINS (M. Riley, N. Wainwright, R. Gast). Members of protein super families share ancestral conserved functional domains. We will adapt enzyme cascade reaction technology, which detects the presence of bacterial lipopolysaccharides, to develop a miniaturized instrument for the detection of ancestral peptides and nucleic acid sequences. Projects and focus areas include:

- Identifying appropriate key ancestral sequences in *E. coli* gene families
- Designing appropriate probe sequences, hybridization and detection assays
- Developing a miniaturized instrument for amplified life detection

EVOLUTION OF GENOME ARCHITECTURE IN PROKARYOTES: Genome-Genome Integration: Symbiosis, genetic assimilation, and evolutionary innovation. (J. Wernegreen). Beyond single point mutations, genome-genome interactions have been a driving force in the evolution of early life, the origin of eukaryotes and the evolution of multicellular complexity. These interactions foster lateral gene transfer between organisms and the remodeling of genome architecture. Using a genomic scale approach, we will study changes in genome structure and expression patterns for endosymbiotic associations between bacteria and eukaryotic hosts.

- Identifying changes in genome architecture, including lateral gene transfer, that catalyze endosymbiont-host interactions and contribute to their current diversity.
- Examining plasticity of gene expression in genome-genome interactions.

EDUCATIONAL AND PUBLIC OUTREACH PROJECTS AND ENRICHMENT OF THE ASTROBIOLOGY COMMUNITY (L. Olendzenski, D. Patterson, M. Sogin). This Astrobiology proposal will support courses and fellowships in ongoing MBL programs: *Workshop on Molecular Evolution, Advances in Genome Technology and Bioinformatics,* and *Living in the Microbial World*. We are in the final stages of forming a joint Brown-MBL graduate training and research program that will support 30-40 year round students at the MBL. When this new initiative begins (September, 2004), we anticipate accepting PhD students who will pursue astrobiology-relevant research topics in molecular ecology, evolutionary biology and genome science. Our continuing WEB site **micro*scope** will also develop tools for archiving and integrating image-rich data from habitats currently being studied by astrobiologists, including Río Tinto.

The institutional commitment includes dedicated laboratory space, capital equipment for laboratory research and facilities for theoretical analyses. The MBL, WHOI, Brown and UNC are committed to full participation in the virtual Astrobiology Institute. If funded, we intend to cooperate fully with other programs in Astrobiology in terms of sharing resources and new information gained from the proposed investigations. The management team will include PI M.L. Sogin (chair), Lorraine Olendzenski (EPO coordinator at MBL), and Co-Is from the collaborating institutions: Andreas Teske (UNC), Katrina Edwards (WHOI), and John Mustard (Brown University).

4. SUMMARY OF PERSONNEL

Personnel

Mitchell Sogin, PI	MBL
Linda Amaral Zettler, Co-I	MBL
Katrina Edwards, Co-I	WHOI
Rebecca Gast, Co-I	WHOI
James Head, Co-I	Brown
John Mustard, Co-I	Brown
Monica Riley, Co-I	MBL
Stefan Sievert, Co-I	WHOI
Andreas Teske, Co-I	UNC
Norman Wainwright, Co-I	MBL
Jennifer Wernegreen, Co-I	MBL
Lorraine Olendzenski, Co-I	MBL
David J. Patterson, Co-I	MBL

**** Institution Association**

MBL: Marine Biological Laboratory, Woods Hole, MA
WHOI: Woods Hole Oceanographic Institution, Woods Hole, MA
Brown: Brown University, Providence, RI
UNC: University of North Carolina at Chapel Hill

5. RESEARCH AND MANAGEMENT PLAN

This Astrobiology proposal has four primary themes. The first (**5.1**) **EARTH'S EARLY BIOSPHERE: EVOLUTION OF MICROBIAL PHYSIOLOGIES** explores patterns of early microbial physiologies as inferred by analysis of microbial populations, mostly from extreme environments. Technologies and samples are common threads for two projects that will explore sulfate reduction and methanogenesis, and ancient CO_2 fixation. The second theme (**5.2**) **TERRESTRIAL ANALOGUES FOR EARLY MARS: a) Iron Oxidation – Shaping past and present environments, b) Microbial diversity and population structure studies in the Río Tinto,** and **c) Life detection through remote sensing** focuses on isolates from metal sulfide deposits at Juan de Fuca ridge and the acidic, heavy metal environment Río Tinto of southwestern Spain where we will study iron oxidation, microbial diversity and population structures using a novel technique, SAGT (Serial Analysis Gene Tags). We will link this work with life detection studies based upon remote sensing technology. We also describe our progress in an ecogenomic-based study of expression profiling for cyanobacteria. The very same technology will be adapted to studies of microbial populations in extreme environments. The third theme (**5.3**) **BIOSIGNATURES AND LIFE DETECTION: DETECTING ANCESTRAL PEPTIDES AND PROTEINS** will take advantage of our expertise in computational biology and the rapidly expanding genome data bases to design life detection protocols that can either be used for life detection experiments on samples returned to Earth from other solar system bodies or as part of an instrumentation package for *in situ* analyses on other planets or large satellites. The final theme **(5.4) EVOLUTION OF GENOME ARCHITECTURE IN PROKARYOTES: Genome-Genome Integration: Symbiosis, genetic assimilation, and evolutionary innovation** addresses how symbioses influence evolution of genome architecture.

5.1 EARTH'S EARLY BIOSPHERE: EVOLUTION OF MICROBIAL PHYSIOLOGIES.

Life on early Earth developed within an anaerobic environment and evolved physiologies and metabolisms that were ancestral to modern-day sulfate-reducing, methanogenic, and methane-oxidizing microbes. Through molecular evolution studies, most commonly analyses of RNA constituents of the translation apparatus, we have gained important insights about the remarkable diversity of microbial populations in extreme environments where these metabolisms shape biogeochemical processes. These microbial ecosystems may resemble conditions of early Earth. The next chapters about the evolutionary history of early life will emerge from studies of metabolic diversity, distribution and the genetic adaptations of microbiota that participate in sulfate reduction, methanogenesis, methane oxidation, iron oxidation and processes that resembled ancient autotrophic carbon fixation pathways. Through the collaborative use of high-throughput DNA technology and high performance computing, we will explore the molecular evolution of coding regions for proteins that must have been of pivotal importance in the origins and evolution of these early metabolic pathways.

5.1a Origin and diversification of ancient pathways: sulfate reduction and methanogenesis (A. Teske).

Prokaryotic metabolic pathways underlie the biogeochemical cycles that affect geological history and planetary evolution. The anaerobic pathways of sulfate reduction, methanogenesis, and methane oxidation were predominant in ancient microbial communities; their isotopic imprints are pervasive in the carbon- and sulfur-isotopic record, from the present back to the Archean-Proterozoic transition [11]. Anaerobic microorganisms, their physiological interactions and geochemical activities are good model systems for early microbial ecosystems that thrived before the oxygenation of the Earth's biosphere, and for microbiota on planets that never evolved an oxygenated atmosphere or water column. The antiquity and evolutionary significance of

these microbial metabolisms are shown in the high degree of phylogenetic conservation of the key genes of sulfate reduction, methanogenesis, and anaerobic methane oxidation. By comparative sequence analysis of key genes, we can retrace the evolution of these anaerobic microbial pathways and enzymes, and the diversification of microbial life under the conditions of the early biosphere.

Isotopic evidence for widely expressed microbial sulfate reduction, in the form of ^{34}S-depleted sedimentary sulfides, goes back to the middle and early Proterozoic, 2.2 to 2.3 Ga. Only small isotopic fractionations (generally under 10% between sulfate and sedimentary sulfides) occur in sediments older than 2.5 to 2.7 Ga [12]. Laboratory experiments with sulfate-reducing bacterial populations show that, in order to suppress the isotopic fractionation signal of sulfate reduction, the sulfate level of the archean ocean must have been under 200 μM [13]. At this sulfate concentration, methanogenesis becomes the dominant anaerobic microbial process. Thus, it is very likely that methanogenesis predated the onset of sulfate reduction in the biogeochemical evolution of the Earth and dominated the archean biosphere; greenhouse warming of the archean Earth by methanogenesis could have compensated for the lower luminosity of the "faint young sun" [13]. The carbon isotopic imprint of biomass produced by methanogenesis, in the form of highly ^{13}C-depleted kerogen ($\delta^{13}C$ • -60%), is found in late Archean and 2.8 Ga-old Proterozoic kerogens [14]. This isotopic record was originally interpreted as evidence for biomass accumulation by aerobic, bacterial oxidation of biogenic methane [15]. Anaerobic oxidation of biogenic methane and subsequent incorporation into microbial biomass is a more likely explanation, since evidence for the stepwise and pervasive oxygenation of the proterozoic biosphere appears much later, ca. 2.2 Ga [16].

To search for deeply-branching and (possibly) ancestral representatives of sulfate-reducing, methanogenic and methane-oxidizing microorganisms and their key genes in modern environments, we focus on hydrothermal vents and deep subsurface habitats. Hydrothermal vents are some of the earliest and best-protected microbial habitats. They may have survived repeated impact frustration of early life and might occur on other planets with oceans and active plate tectonics, volcanism or tidal heating. On Earth, hydrothermal vents sustain complex microbial ecosystems that utilize inorganic energy sources, such as sulfide, hydrogen, and reduced metals, and geothermal sources of carbon, such as methane, CO_2 and geothermally synthesized low-molecular weight organic compounds. The deep marine and terrestrial subsurface is a vast microbial habitat that houses pervasive microbial life, especially anaerobic bacteria and archaea. Fermentative and sulfate reducing bacteria as well as archaea, and methanogens have been detected down to at least several hundred meters of oceanic sediments [17], and microbial texture alteration has been detected for the basaltic ocean crust [18].

To identify the bacteria and archaea that constitute these diverse methanogenic, sulfate-reducing and methane-oxidizing prokaryotes in hydrothermal vents and in marine deep subsurface habitats, analyses of rRNA sequences are complemented with sequencing surveys of highly conserved marker genes for sulfate reduction and methanogenesis. In this way, it is possible to focus a genomic survey on these specific classes of microorganisms. Screening a microbial community for the highly conserved key genes of these processes, the dissimilatory sulfite reductase (*dsr*) and coenzyme M methyl reductase (*cmr*) genes, results in a diversity census that shows the composition and complexity of this microbial community. At the same time, a survey of these genes in environmental samples shows the evolutionary divergence that has accumulated in these genes since the early Proterozoic or the Archean, and the phylogenetic depth of these metabolisms in the bacterial and archaeal tree of life. With a growing database, homologous and ancestral traits of these genes can be defined that can be significant for an understanding of enzyme evolution and its functional constraints.

Sulfate reduction and *dsrAB* genes. The key gene of dissimilatory sulfate reduction, (*dsrAB*), codes for the alpha and beta subunits of the enzyme dissimilatory sulfite reductase, which catalyses the reduction of sulfite to sulfide, after previous activation and reduction of sulfate to sulfite. Consistent with an early origin of the sulfate-reducing pathway, the *dsrAB* genes are phylogenetically conserved in several deeply branching bacterial and archaeal phyla, and to some extent consistent with 16S rRNA phylogenies [19]. With a growing database of *dsr* genes, 16S rRNA and *dsr* phylogenies turned out to be discordant for some sulfate reducers. For example, the dsr genes of thermophilic members of gram-positive genus *Desulfotomaculum*, and of the thermophilic genus *Thermodesulfobacterium* are apparently related to those of delta-Proteobacterial sulfate reducers, suggesting lateral gene transfer [20]. Since these discordances are known, and limited to a few cases, the phylogeny of the *dsrAB* genes allows a simultaneous phylogenetic and metabolic identification of evolutionary divergent sulfate-reducing microorganisms [19, 20]. The α and β subunits of the *dsr* gene are most likely the results of an early gene duplication, as shown by considerable homology among the two subunits. The homologous subunits include sequence regions harboring the catalytic center that consist of a $[Fe_4S_4]$-cluster coupled to a siroheme prosthetic group [21]. Paralogous gene trees of the two subunits can be used to identify deeply-branching *dsr* genes. At present, the deepest lineage seems to be the thermophilic bacterium *Thermodesulfovibrio yellowstonii,* and not the archaeal genus *Archaeoglobus* [20], which would argue for a bacterial origin of sulfate reduction. More deeply branching dsrAB sequences of ancestral sulfate reducers could be isolated from habitats that are known to harbor a great diversity of sulfate reducers.

We will focus on hydrothermal vents and the deep subsurface, as promising candidate environments for unusual and ancestral sulfate reducers. For example, the Guaymas Basin in the Gulf of California, the northernmost extension of the East Pacific Rise mid-ocean ridge, is covered by organic-rich, hydrothermally altered sediments that show very high sulfate reduction rates over wide temperature ranges [22-25]. The Guaymas sediments harbor highly diverse sulfate-reducing bacterial and archaeal populations, including novel, mesophilic and thermophilic fatty acid oxidizers[26], hyperthermophilic archaea of the genus *Archaeoglobus* [27], and members of the propionate-oxidizing, acetate-producing family *Desulfobulbaceae* [28]. Our recent *dsrAB* gene survey in Guaymas sediments detected *dsrAB* genes related to well-known proteobacterial sulfate-reducing genera (*Desulfobacter* and *Desulfobacterium*), but also a novel, deeply-branching *dsrAB* genes that are unrelated to *dsrAB* genes of any cultured sulfate-reducing microorganism [29]. A phylogenetic examination of the two paralogous *dsrA* and *dsrB* subunits of these deeply-branching Guaymas genes has started. We plan to extend this search for novel, deeply-branching sulfate reducers and their *dsrAB* genes to other hydrothermal and deep subsurface environmental samples, including deep subsurface samples obtained from the Ocean Drilling Program (leg 201, Equatorial Pacific and Peru Margin), microbial mats from Guaymas, sulfidic hydrothermal vent chimney rock, and geothermal fluids from basaltic ocean crust at the flanks of mid-ocean ridges.

Methanogenesis, methane oxidation, and *mrcA* genes. Coenzyme M methyl reductase (*cmr*) is the key enzyme of methanogenesis, which catalyses the terminal and highly exergonic step of the methanogenesis pathway, the reduction and release of the coenzyme-M bound methyl group as free methane. The *cmr* gene occurs exclusively in methanogenic archaea; in contrast to other genes of methanogenic pathways, it is not known to be affected by lateral gene transfer to other prokaryotes, for example aerobic, methanotrophic bacteria [30]. The α subunit (*mrcA*) of the Coenzyme M methyl reductase gene is highly conserved, accessible by PCR, and generally consistent with 16S rRNA phylogenies [31-33]. Thus, the major lineages of methanogenic archaea can be identified on the basis of their *mrcA* genes.

At present, it is not known whether *mrcA* genes also participate in the anaerobic, sulfate-dependent oxidation of methane to CO_2 [34], which is carried out by members of at least two

phylogenetic lineages of recently identified archaea, ANME-1 and ANME-2 [35-38]. Anaerobic methane oxidation by archaea must either proceed through an energy-intensive reversal of this process using *cmr*, or bypass this step completely and have no need for this enzyme [39]. Screening the environmental DNA samples from sites with known anaerobic methane-oxidizing activity by cloning and sequencing will help to decide two different possibilities: Coenzyme M methyl reductase may be restricted to phylotypes of well-known, cultured methanogenic archaea, and is not involved in anaerobic methane oxidation. Alternatively, novel phylogenetic lineages of the *mrcA* gene that do not match known methanogens, and are found specifically in samples with methane-oxidizing activity or 16S rRNA signatures, could play a role in anaerobic methane oxidation. Current genome sequencing studies of purified ANME-1 and ANME-2 archaea from environmental samples are testing whether these methanotrophic archaea carry *mcr* genes [38].

The search for novel *mrcA* genes will focus on anaerobic environments that harbor diverse methanogens, and methane-oxidizing archaeal populations. Guaymas sediments are a promising target; their methanogenic populations include hyperthermophilic, autotrophic methanogens of the genus *Methanococcus* [40, 41] and the deeply branching *Methanopyrus* [42], as well as members of the formate-utilizing, mesophilic or moderately thermophilic family Methanomicrobiales [28]. Methane produced by these diverse methanogenic communities combines with the methane pool originating from pyrolysis of organic matter buried in the Guaymas sediments; the resulting methane concentrations in the Guaymas vent fluids are orders of magnitude higher than at non-sedimented, bare lava vent sites [43]. In this geochemical setting, anaerobic methanotrophs thrive; 16S rRNA analysis and ^{13}C-isotopic analysis of taxonomically informative archaeal lipids has shown that the anaerobic methanotrophic communities in the Guaymas Basin include both ANME-1 and ANME-2 archaea [28].

Methane cycling and sulfate reduction in the deep marine subsurface. Large and very valuable sample sets for this project have become available through collaboration with the Astrobiology group at the University of Rhode Island, led by Stephen D'Hondt. The deep marine subsurface is an ancient microbial habitat of global dimensions that harbors a large proportion of the prokaryotic biomass of the planet; its physiological and evolutionary biodiversity is just beginning to be explored [17]. Marine deep subsurface sediments differ in many respects from hydrothermal vents. They are characterized by low temperatures; the microbial populations are several orders of magnitude less dense and active; suitable electron donors and carbon sources for microbial metabolism are scarce; the geochemical and thermal gradients that are compressed into a few cm at vents are extended over several 100 m [17]. These extended porewater sulfate and methane gradients strongly suggest that sulfate-reducing and methane-cycling microbial populations (analogous to Guaymas) are dominant community members that determine the overall biogeochemical activity patterns in the deep subsurface [44]. Deep subsurface sediments were collected through the Ocean Drilling Program (ODP) cruise Leg 201 in the Equatorial Pacific and on the Peru Margin (ODP sites 1225 – 1231; see preliminary cruise report on www-odp.tamu.edu/publications/prelim/201-prel/201toc.html). For each site, detailed geochemical profiles (sulfate, methane, reduced metals, hydrogen) and cell counts are available; process rates (methanogenesis, sulfate reduction, hydrogen and acetate turnover) are being determined by different cruise participants. The geochemical profiles show clear zones with sulfate reduction, methanogenesis, and the sulfate reduction/methanogenesis transition layers. From each site, selected samples from the methane maximum in the methanogenic zone, from the anaerobic methane oxidation layer, and from the sulfate reduction zone will be examined. First priority is given to sites 1229 and 1230. Site 1229 is located on the Peru Margin at 150 m depth (extending to 200 mbsf) and shows a unique feature, a deep subsurface incursion of sulfate-rich evaporite brines that replenishes sulfate at ca. 90 m depth and thus creates an additional sulfate reduction/methanogenesis transition zone, in addition to the near-surface (ca. 36-38 mbsf) transition [45]. The organic-rich site 1230 is located in 5086 m

water depth in the Peru Trench (extending down to 280 mbsf), and shows a very steep gradient from the sulfate-reducing to the methanogenic layers which was sampled in high resolution. In contrast to the high-organic rich Peru Margin and Peru Trench sites (1227, 1228, 1229, 1230), the organic-poor Central Pacific sites are characterized by low process rates, lower bacterial numbers, and highly extended geochemical gradients. The coexistence of methane and sulfate over several 100 m of sediment suggests slow, concomitant sulfate reduction and methanogenesis in this area (sites 1225, 1226, 1231), contrary to our understanding of the sequential order of microbial redox reactions according to energy yield [46].

In general, the survey for *dsr* and *cmr* marker genes in these subsurface samples has a dual focus. Besides the evolutionary implications of finding novel and deeply branching marker genes for sulfate reduction and methanogenesis (as spelled out for Guaymas), the precisely defined geochemical gradients and process rates will allow the identification of geochemical controls (organic carbon input, *in situ* carbon substrate and hydrogen concentrations) that govern the composition of the sulfate-reducing, methanogenic, and methanotrophic communities. During the course of this project, additional samples will become available, from anaerobic methane-oxidizing sediments of the Hydrate Ridge offshore Oregon (ODP leg 204), deeply buried cretaceous black shales from the Demarara Rise (ODP leg 207), and off-axis ridge flank subsurface fluids made accessible by a corked borehole. These subsurface samples will be investigated in collaboration with the NAI group at URI, which includes biomarker studies (compound-specific isotopic analyses), process rate measurements, and geochemical analyses.

METHODS. In spite of continuing primer development [20], it is not certain whether PCR-based approaches will reliably detect all environmental *dsrAB* and *mrcA* genes of interest, including the most ancestral and deeply-branching lineages that are of particular interest to Astrobiology. New versions of these key genes with non-conserved primer sites would be missed. Primer site conservation can never be taken for granted, as shown by the finding that even 16S rRNA sequence motifs that were regarded as universally conserved showed substantial variation between different bacterial lineages [47]. Therefore, the survey of *dsrAB* and *mrcA* genes will be complemented by alternative approaches.

A possible method is gene cassette PCR [48]. Genes that have been acquired by horizontal gene transfer in bacteria are flanked by integrons, 59-base pair sequence motifs with highly conserved inverted repeats at both ends. The units of DNA captured by integrons, gene cassettes, can accumulate in microbial genomes; multiple insertion events lead to the formation of multicassette arrays. By combining a forward primer for a key gene sequence motif with a reverse primer targeting a neighboring integron inverted repeat, it is possible to circumvent the requirement for two conserved target sites within a key gene, to broaden the amplification range of existing primers, and to sequence terminal regions of genes that would otherwise remain inaccessible [48]. The downside of this approach is that it will selectively detect and amplify genes that have undergone at least one integron-catalyzed gene transfer event.

Shotgun cloning and fosmid library construction for deep subsurface bacteria and archaea could circumvent PCR entirely. To isolate high-quality, non-degraded DNA from deep subsurface sediment material, we will start with established approaches for genomic library construction from soil that include gentle detachment of prokaryotic cells from particles as the first step for library construction [49]. Practical experience with marine environmental samples indicates that fosmid libraries are easier to generate and to screen than BAC libraries, in particular for highly complex communities (California coastal sea water and Antarctic picoplankton; [50]). Inserts that contain phylogenetic anchors can be identified using a multiplex PCR-denaturing gradient gel electrophoresis approach [51]. Just as rRNA sequences can describe phylogenetic context when present in larger DNA fragments, key genes of known function and distribution can serve as “phylogenetic anchors” to identify the source of large

DNA inserts cloned from natural poulations of bacterio- and archaeoplankton [52]. Novel genomic information is not limited to *dsrAB* and *mrcA* genes, but may include functional and structural genes that correspond to physiological demands and environmental stresses of deep subsurface and hydrothermal vent habitats, and allow a better understanding of microbial survival strategies and adaptations to these ancient habitats.

Extensive surveys of ancestral and extreme microbial habitats (hydrothermal vents, deep subsurface environments) for the genetic markers of sulfate reduction, methanogenesis and methane oxidation, will yield new insights into the evolution of these pathways and their key genes. The environmental diversity of key gene sequences and amino acid sequences, their secondary structure motifs and conserved sites, contains information about environmental and functional constraints that have shaped the evolution of these ancient microbial pathways. Deep subsurface and hydrothermal vent environments are good places to start such a survey, for two reasons: They mirror several characteristics of the early Earth, the absence of oxygen, and the necessity for protection against an inhospitable surface environment characterized by cosmic radiation, inclement weather and meteorite bombardments. Further, they provide good analogs to subsurface life under extraterrestrial conditions where a permanently inhospitable surface environment might have kept microbial life underground, and never allowed its evolution towards complex, multicellular life within a phototrophic, oxygenated biosphere [53].

5.1b The evolution and diversity of ancient CO_2-fixation pathways in anaerobic and extremophilic microorganisms: Clues to the early evolution of life on Earth (S. Sievert).

Despite research advances in studies of microbial diversity, life in extreme environments and prebiotic chemistry, the origin of life on Earth remains an enigma. Key unresolved questions include: When did life first appear? What was the nature of the first organisms? and Did early life have a heterotrophic or an autotrophic origin? [54, 55] Carbon isotope data suggest that autotrophic organisms, which preferentially fix isotopically light carbon, may have existed >3.7 Ga ago [56-58], indicating that autotrophy was one of the earliest evolved metabolisms on Earth. Many consider the reductive TCA cycle to be the most ancient autotrophic carbon fixation pathway [59-62]. This cycle occurs in phylogenetically-diverse prokaryotes whose growth conditions fit well with our view of the primitive Earth. One of the two key enzymes of this pathway is ATP citrate lyase (ACL). Limited gene sequence data are available for this enzyme and we have no information about the diversity, distribution and importance of organisms using the reductive TCA cycle in nature. This information is required in order to understand the carbon cycle on our planet today and in the geological past, and the evolution of this ancient carbon fixation pathway.

The objective of this project is to examine the occurrence of the ACL gene in organisms where this activity is known to be present, and whether it occurs in other prokaryotic autotrophs where information about CO_2 fixation pathways is lacking. Newly-acquired sequence data will be used to explore phylogenetic patterns generated by ACL for insights into the evolution of this ancient pathway on Earth. This represents the first step in developing a better understanding of the environmental significance of this potentially important metabolic pathway.

CO_2 fixation and the early evolution of life on Earth. An autotrophic origin of life posits that metabolism evolved before all else [59, 60, 62-65]. Recent data suggest that the atmosphere of early Earth was weakly reduced and contained mainly carbon dioxide, nitrogen, and only trace amounts of CO, H_2, methane, reduced sulfur gasses, and possibly O_2, making it an unlikely environment for the formation of a prebiotic broth that might have fueled the growth of heterotrophic organisms [55, 66, 67]. However, the possibility exists that increased concentrations of organic molecules appeared in evaporating ponds [54] or that selective absorption to minerals led to the concentration and polymerization of active monomers [68]. In

both cases, organic molecules might have been synthesized abiotically on Earth or delivered by meteorites [64]. The first life forms may have been thermophiles [69]. Temperatures on the surface of the early Earth probably had cooled to 100˚C or less by 4.4 Ga [70]. Although these surface temperatures may have existed for only 20 My [71] (a potentially longer time frame for the subsurface), they could have provided suitable conditions for the evolution of thermophilic autotrophs (organisms that use inorganic carbon as the sole source of carbon) [55, 64, 69, 72]. Phylogenetic analyses of 16S rRNA and whole genomes place autotrophic hyperthermophiles at the base of the tree [69, 73-75], although this view has been challenged [76, 77]. Submarine hydrothermal vents might have been sites for the origin of (thermophilic) life or provided refugia for thermophiles to survive the heavy bombardment by meteorites prior to 3.8 Ga, which frequently sterilized the surface of the Earth and vaporized large parts of the ocean [54, 78, 79].

Today, representatives that are able to grow autotrophically exist in almost all major groups of prokaryotes [80]. These organisms play essential roles in ecosystems by providing a continuous supply of organic carbon for heterotrophs. The Calvin cycle may be the most important extant autotrophic carbon fixation pathway [81], but despite its global significance, it has a rather narrow phylogenetic distribution (**Figure 1**). It does not occur in Archaea or

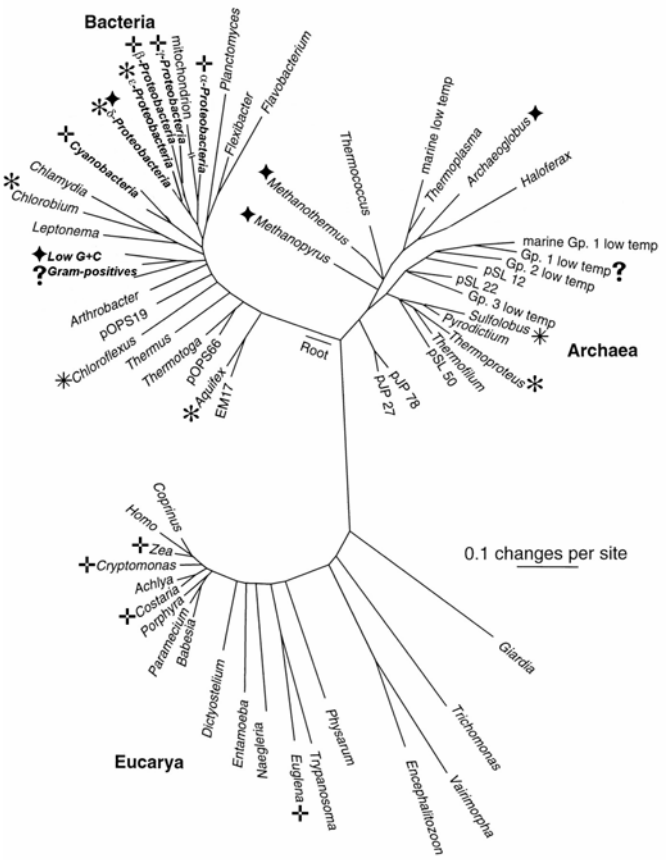

Figure. 1. Universal tree based on 16S rRNA sequences showing the phylogenetic distribution of the different carbon fixation pathways. = Calvin cycle; = reductive TCA cycle; = Hydroxypropionate pathway; = reductive acetyl-CoA pathway **?** = unknown

hyperthermophilic organisms and among the Bacteria has only been identified within the α, β, and γ-Proteobacteria and cyanobacteria, from which the chloroplasts of plants are derived [80]. This phylogenetic distribution argues against the Calvin cycle as being an ancient carbon fixation pathway. Furthermore, microorganisms present in extreme environments that resemble

conditions on the early Earth, e.g., high temperature, anaerobic or acidic conditions, utilize different CO_2-fixation pathways [80, 82]. At present there are three alternative pathways known [80], the reductive tricarboxylic acid cycle, the reductive acetyl-CoA pathway, and the hydroxypropionate pathway. Although fewer organisms are known in which these pathways operate compared to those that utilize the Calvin cycle, the phylogenetic distribution of these alternative pathways is much broader, as they have been identified in several major lines of descent in the bacterial and archaeal domains (**Figure 1**; **Table 1**). In addition, the fact that these pathways occur mainly in anaerobes and (hyper)thermophiles [82-88] supports the idea that these carbon fixation pathways are ancient and were of much greater importance in the geological past before the rise of oxygenic phototrophs about 2.3 Ga, or during large anoxic events such as during the Cretaceous [64, 89-91]. These lines of evidence suggest that if microorganisms exist on other planetary bodies with reducing environments, these pathways are likely to also be present.

Table 1. The phylogenetic distribution of different CO_2-fixation pathways.

		CO_2-Fixation pathway			
Phylogenetic group	**Representative species**	**Calvin**	**Reductive TCA**	**Reductive Acetyl-CoA**	**Hydroxy-propionate**
Proteobacteria					
α	*Rhodosprillum rubrum*	+	-	-	-
β	*Rhodocyclus tenuis*	+	-	-	-
γ	*Thiomicrospira crunogena*	+	-	-	-
δ	*Desulfobacter hydrogenophilus*	-	+	-	-
	Desulfobacterium autotrophicum	-	-	+	-
ε	*Candidatus* Arcobacter sulfidicus	-	+	-	-
Cyanobacteria	*Synechococcus* sp.	+	-	-	-
Chloroflexus	*Chloroflexus aurantiacus*	-	-	-	+
Low G+C Gram-positives	*Chlostridium aceticum*	-	-	+	-
	Ammonifex degensii	?	?	?	?
Chlorobium	*Chlorobium tepidum*	-	+	-	-
Aquificales	*Aquifex pyrophilus*	-	+	-	-
Thermoproteales	*Thermoproteus neutrophilus*	-	+	-	-
Sulfolobales	*Metallosphaera sedulans*	-	-	-	+
Pelagic *Crenarchaeota*	Uncultivated	-	?	-	?
Archaeoglobus	*Archaeoglobus lithotrophicus*	-	-	+	-
Methanogens	*Methanocaldococcus jannaschii*	-	-	+	-
Eukaryotes	Chloroplast containing organisms	+	?	-	-

Reductive TCA cycle. It has been proposed that the first autotrophic pathway was akin to the reductive tricarboxylic acid cycle (TCA) operating in extant autotrophs [59-62]. It has the characteristics of an autocatalytic cycle and leads to a complex cyclic reaction network from which other anabolic pathways could have evolved [59, 60]. The reductive TCA cycle may have preceded the oxidative TCA cycle [61, 92], and based upon biochemical and isotopic analyses appears to operate in phylogenetically diverse autotrophic bacteria and archaea, including anoxic phototrophic bacteria (*Chlorobiaceae*) [93-95], sulfate-reducing bacteria (*Desulfobacter hydrogenophilus*) [83], microaerophilic, hyperthermophilic hydrogen-oxidizing bacteria (*Aquificales*) [84, 85], and hyperthermophilic sulfur-reducing crenarchaeota (*Thermoproteales*) [84, 96]. The two latter groups are important in biogeochemical processes in extant hydrothermal habitats. Hydrothermal systems have prevailed throughout Earth's history and they might resemble sites where life originated. They also may be analogs for extraterrestrial environments where active volcanism occurs [59, 64, 97-99]. Preliminary evidence also suggests that the reductive TCA cycle is operating in *Candidatus* Arcobacter sulfidicus, a sulfide-oxidizer belonging to the ε-proteobacteria that has the unique capability to form sulfur in

filamentous form [100, 101]. Members of the ε-proteobacteria appear to be a major component of microbial communities at deep-sea vents and in the subseafloor [28, 102].

ATP citrate lyase in Prokaryotes. The reductive TCA cycle is essentially the TCA cycle running in reverse, leading to the fixation of 3 molecules of CO_2 and the production of one molecule of triose phosphate. Most of the enzymes between the two pathways are shared, with the exception of two key enzymes that allow the cycle to run in reverse: ATP citrate lyase (ACL) and 2-oxoglutarate:ferredoxin oxidoreductase (OOR). OOR catalyzes the carboxylation of succinyl-CoA to 2-oxoglutarate whereas ACL catalyzes the ATP dependent cleavage of citrate to acetyl-CoA and oxaloacetate, and its presence in prokaryotes is indicative of a functioning reductive TCA cycle. Although several prokaryotes have been shown via specific enzymatic assays to possess ACL activity, the only cultured prokaryotes for which a complete gene encoding ACL has been identified to date are the green-sulfur bacteria *Chlorobium tepidum* and *C. limicola* [103, 104]. The heteromeric ACL of *Chlorobium* is encoded by two separate genes, *acl*A and *acl*B, which are adjacent to one another [104, 105]. The same gene arrangement occurs in eukaryotes such as fungi and plants, whereas in animals a homomeric ACL is encoded by only one gene [104, 105]. The *acl*BA of *Chlorobium*, fungi, and plants show similarity to the N- and C-terminal halves of the ACL from animals, and it has been suggested that ACL in animals is a product of a gene fusion event that occurred early in the evolution of that kingdom [103]. It has been proposed that aclB may have evolved through gene duplication and diversification from the β-subunit of succinyl-CoA synthetase (SCS), whereas aclA may have arisen from a fusion event between the α -subunit of SCS and citrate synthase (CS) after a previous gene duplication and diversification [103, 105] (**Figure 2**). By using primers based on the conserved regions of the gene, a partial ACL sequence from *Candidatus* Arcobacter sulfidicus

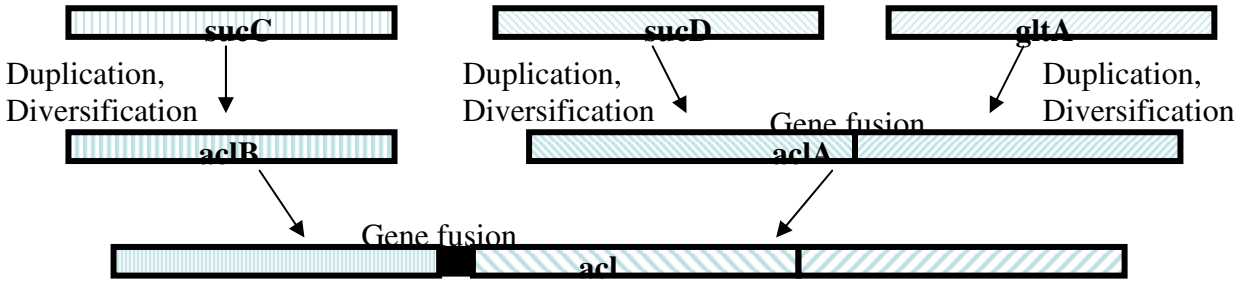

Figure 2. Schematic representation of the possible evolutionary history of ATP citrate lyase. sucC = gene encoding β-subunit of Succinyl-CoA synthetase (SCS); sucD = gene encoding α-subunit of SCS; gltA = gene encoding for citrate synthase; aclB = gene encoding ATP citrate lyase β-subunit; aclA = gene encoding ATP citrate lyase α-subunit; acl = one gene encoding for homomeric ATP citrate lyase in animals. See text for further explanations. Modified after Fatland et al. [103].

was amplified by our laboratory. Amplification products of the expected size were also obtained from *Desulfobacter hydrogenophilus*, but have not yet been sequenced [106]. Recently, two ATP citrate lyase genes were identified on two fosmids also containing the 16S rRNA of the main ε-proteobacterial epibionts of *Alvinella pompejana* [107], a polychaete living on sulfide structures at deep-sea hydrothermal vents on the East Pacific Rise. On these fosmids, the genes corresponding to *acl*A and *acl*B were adjacent to each other in an arrangement identical to that of *Chlorobium*. An ATP citrate lyase has also been purified from the hyperthermophilic hydrogen-oxidizing bacterium *Hydrogenobacter thermophilus*, which is a member of the *Aquificales*, however, no sequence information is available [108]. Although the whole genome of *Aquifex*

aeolicus has been determined [109], no ACL genes similar to the one in *Chlorobium* could be identified in this organism despite detected ATP citrate lyase activity in the closely-related *A. pyrophilus* [84]. The presence of a highly divergent ACL gene in *A. aeolicus* [110], or different metabolic properties of the two *Aquifex* species might explain these observations. Low sequence similarity with *Chlorobium* ACL would be in line with *Aquifex* being a deeply-branching bacterium. Recently, it has also been determined that the genome of *A. aeolicus* does not contain a suitable candidate for OOR and that previous reports indicating its presence are based on an incorrect annotation [109, 110]. However, a partial ACL sequence was recently obtained from *Persephonella marina*, [111] which is also a member of the *Aquificales* and is thought to grow autotrophically by using the reductive TCA cycle [112, 113].

ATP citrate lyase in Eukaryotes. Aside from the above-mentioned prokaryotes, ACL genes occur in yeasts, fungi, green algae, plants, animals, and in photosynthetic protists such as *Chlamydomonas reinhardtii* and the glaucophyte *Cyanophora paradoxa* [114-119]. Phylogenetic analysis places the ACL of the green sulfur bacterium *Chlorobium* basal to the eukaryotic homologues [103], suggesting that eukaryotes derived their ACL from bacteria. Interestingly, phylogenetic trees based on available ACL sequences are congruent with the evolutionary history depicted by rRNA [103], suggesting an ancient origin of this enzyme. In the eukaryotes studied so far, ACL is not involved in carbon fixation or carbon oxidation, but functions in the provision of cytosolic acetyl-CoA for biosynthesis of fats and cholesterols [103, 116], although it has been suggested that the reductive TCA cycle might operate in *Chlamydomonas reinhardtii* [120]. The best candidates for deep-branching eukaryotes include *Giardia* and *Trichomonas* but their nearly completed genomes do not appear to code for ACL. While these parasites might depend on their host for fatty acid synthesis, ACL may be discovered in free-living relatives. Acetyl-CoA, which is synthesized in the mitochondria, must be transported across the mitochondrial membrane to the cytosol where it is used for fatty acid synthesis. Because membranes are impermeable to acetyl-CoA, it must first be converted to citrate, which crosses the membrane. Citrate is then cleaved by ACL into acetyl-CoA and oxaloacetate. Amitochondriate eukaryotes such as *Giardia* and *Trichomonas* may have lost this gene since acetyl-CoA production and fatty acid synthesis are not separated in different compartments. ACL may have initially served to make carbon fixed from CO_2 available for general metabolism, and later this function might have switched with the development of respiratory capabilities, so that ACL tapped into the TCA cycle for carbon derived from catabolic reactions [103].

METHODS. Despite the fact that organisms utilizing CO_2-fixation pathways other than the Calvin cycle might contribute significantly to primary production in their respective habitats, few data exist on the diversity, distribution and importance of the organisms using these pathways in nature. This information is necessary to understand the carbon cycle on our planet today and in the geological past. We propose to focus our studies on the molecular evolution and ecological importance of the reductive TCA cycle, which is generally considered the most ancient autotrophic carbon fixation pathway [59-62].

Sequence diversity of ACL. We will investigate the occurrence of ACL coding regions in organisms known to utilize the reductive TCA cycle. Because of a lack of sequence information for ACL, it will first be necessary to identify the genes coding for the observed ATP citrate lyase activity in the different organisms known to have, or suspected to have, the reductive TCA cycle, including: *Aquifex pyrophilus, Desulfobacter hydrogenophilus, Thermoproteus neutrophilus, Pyrobaculum islandicum, Hydrogenobacter thermophilus, Desulfurobacterium, Persephonella* and *Thermovibrio*. All organisms will be obtained from existing culture collections, cultivated, and harvested for DNA and RNA for later analyses. Biomass will also be frozen in liquid N_2 for later biochemical analysis, e.g., assays diagnostic for ATP citrate lyase activity in cases where

activity is suspected but not yet demonstrated [84, 86, 104]. In addition to biochemical assays, RT-PCR will be used to confirm the expression of ACL.

A database of ACL sequences will be constructed by PCR amplification, cloning and sequencing of ACL genes using existing and newly-developed primers based on conserved regions of the gene. Degenerate primers have been designed by our laboratory based on an alignment of known ACL genes. The G+C content was kept between 40 and 60% and the total degeneracy was kept below 64-fold. The primer length was 18-26 nucleotides, Gs and Cs were preferred at the 3' end to enhance binding, and the G+C content was balanced over the length of the primer. We have used different combinations of primers and tested them under various conditions (temperature gradient, Mg concentration etc.). The primer pairs that gave positive results are depicted in **Figure 3** along with their binding positions and sequence information (aclA-F2/aclA-R5, and aclA-F3/aclA-R4). These primer pairs were designed to amplify a roughly 800 bp long fragment from *acl*A that spans the junction between the N-terminus

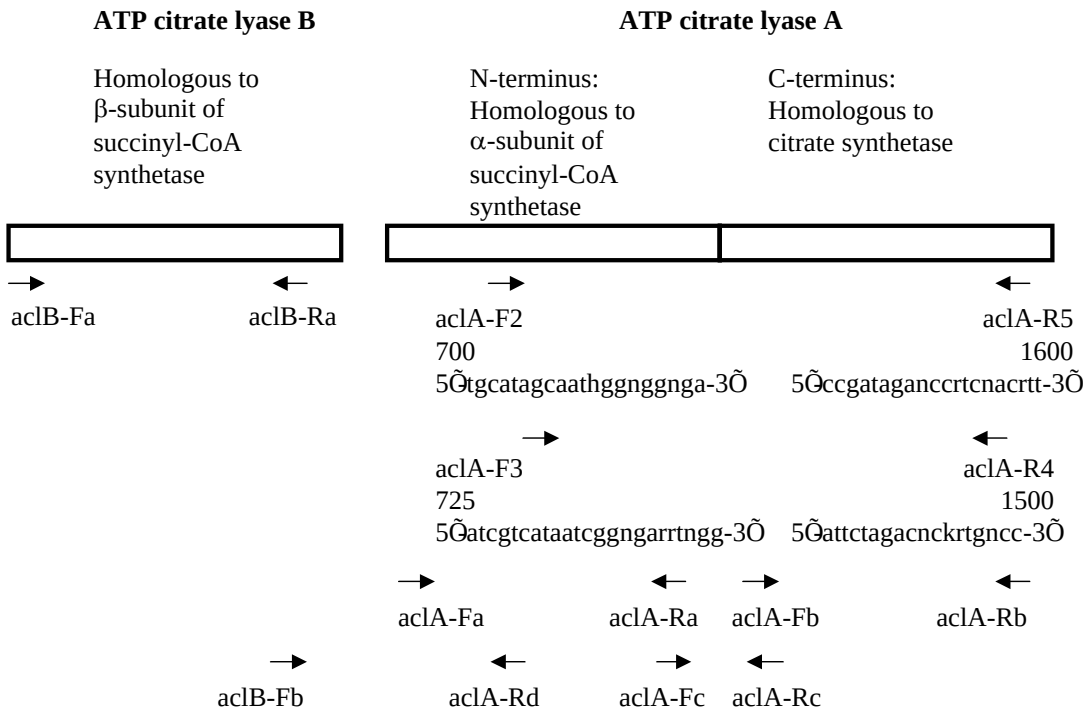

Figure 3. Domain structure of the ATP citrate lyase gene (aclBA) of *Chlorobium* sp., plants, and fungi. aclB and aclA encode for the β- and α-subunit of the ATP citrate lyase, respectively. In *Chlorobium tepidum* both genes are adjacent to each other. Depicted are also the primers with sequence and binding positions that were used to amplify a ~800 bp fragment of aclA from *Candidatus* Arcobacter sulfidicus that spans the region between the domain most similar to the succinyl-CoA synthetase and the domain most similar to citrate synthase (aclA-F2/aclA-R5; aclA-F3/aclA-R4). In addition, primers are depicted that will be used to amplify different regions of the gene(s). See text for further detail.

(homologous to α-SCS) and the C-terminus (homologous to CS). Using these primers we were successful in amplifying an *acl*A fragment from *Candidatus* Arcobacter sulfidicus. A positive amplification result with these primers for other organisms will allow us to confirm the configuration of *acl*A according to the evolutionary scheme in **Figure 3.** A negative amplification result, however, could be due to sequence divergence, and would not necessarily indicate the absence of this gene. We will employ a more rigorous PCR strategy that will first amplify each fragment (aclA-Fa/aclA-Ra, aclA-Fb/aclA-Rb), and then use custom, species-specific primers (designed from sequences for each fragment) that should generate an amplicon

that spans the junction between the N- and C-terminus regions (aclA-Fc/aclA-Rc). We will also design primers for *acl*B (aclB-Fa/aclB-Ra) and primers that should amplify the C-terminus of *acl*B together with the N-terminus of *acl*A if both genes are adjacent to each other (aclB-Fb/aclA-Rd) (see **Figure 3**).

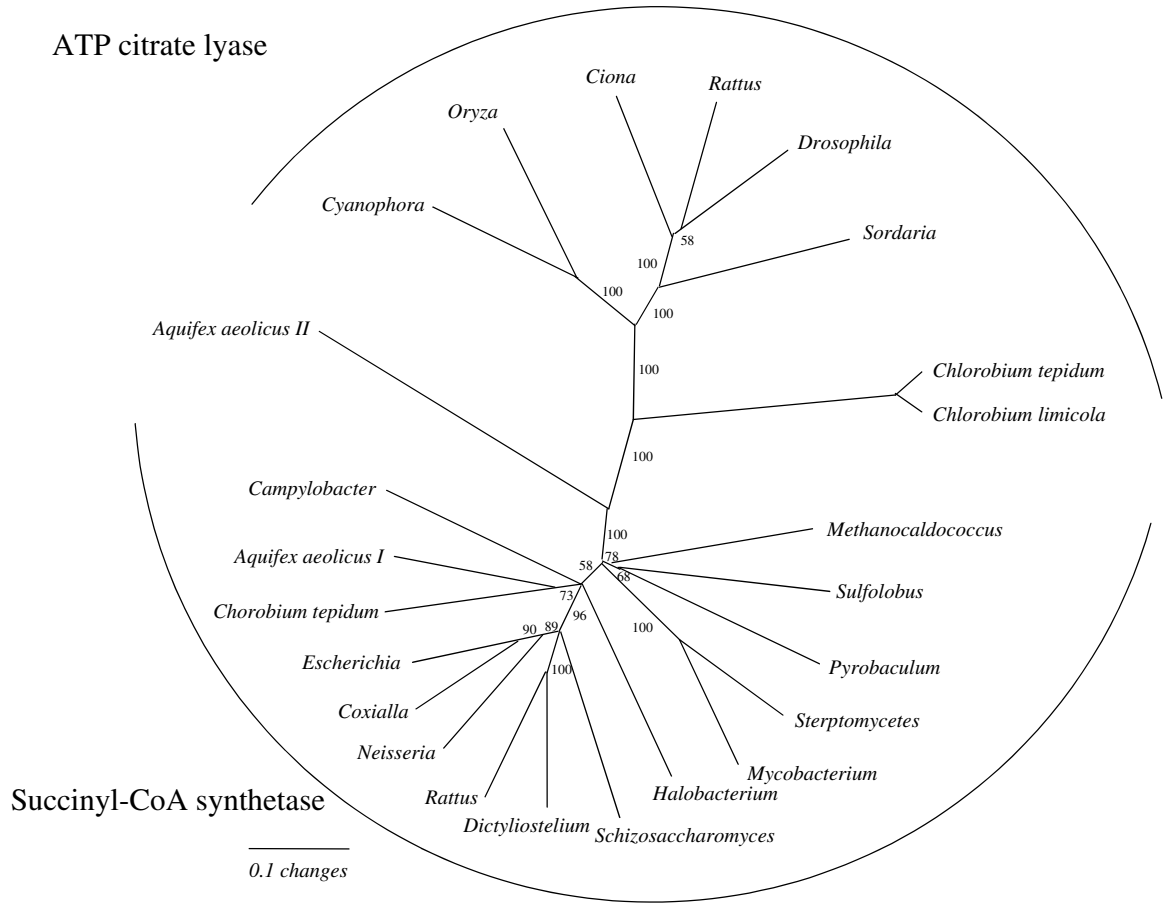

Figure 4. Neighbor joining tree showing the phylogenetic relationship between homologous domains in Succinyl-CoA Synthetase (SCS) and ATP citrate lyase (ACL). Numbers represent bootstrap values after 100 replicates. Note that the phylogenetic position of the second copy of SCS of *Aquifex aeolicus* is between those of SCS and ACL.

Aquifex aeolicus has two distinct copies of succinyl-CoA synthetase [106, 109] (**Figure 4**). Based on preliminary phylogenetic analysis using Neighbor Joining methods [121], this second copy appears to be more closely related to ATP citrate lyases, however more rigorous phylogenetic analysis will be required. It may be that *Aquifex aeolicus* uses a modified succinyl-CoA synthetase for citrate cleavage [109]. Considering the basal position of the Aquificales in phylogenetic trees based on 16S rRNA, this would be in line with the evolutionary scenario proposed by Fatland et al. [103]. First, we will verify the operation of the reductive TCA cycle in *Aquifex aeolicus* by using diagnostic enzyme assays, e.g., for ATP citrate lyase activity. At this point, it has only been assumed that *A. aeolicus* is fixing CO_2 using the reductive TCA cycle because of the operation of this cycle in the related species *A. pyrophilus*. In the case where we obtain a positive result for ATP citrate lyase activity in *A. aeolicus*, we will confirm the hypothesis of Deckert et al. [109] by over-expressing the suspected gene in *E. coli* [122].

Primers based on putative target sequences are used in a PCR reaction to amplify the target gene, which is ligated into an expression vector (e.g., pET 11a, Novagen) and transformed into *E. coli* (e.g., BL21-Gold (DE3), Stratagene). Utilizing the thermophilic nature of the overexpressed enzyme, purification will be carried out using a heating step and subsequent gel filtration.

Survey of ACL genes in other lineages. There are several autotrophic organisms in which carbon fixation pathways have not yet been examined. *Ammonifex degensii* is one of the most interesting from an astrobiological perspective. It is a thermophilic autotroph that reduces nitrate to ammonia, and rRNA sequences from closely related organisms have recently been identified as dominant sequences in surveys of microbial communities living in the oceanic crust [123].

Protists will also be screened for the presence of ATP citrate lyase. The best candidates would be free-living anaerobic or microaerophilic representatives such as *Carpediemonas membranifera*, members of the Euglenozoa, members of the ciliates, and some parabasalids (e.g. free living monocercomonads such as *Monotrichomonas carabina*). Biomass will be harvested and DNA and RNA extracted as for the prokaryotic species. Biochemical assays will be carried out to confirm ATP citrate lyase activity prior to PCR amplifications.

Phylogenetic analysis of ACL genes. We will use our expanded database of ACL sequences to study the molecular evolution of ATP citrate lyase and the reductive TCA cycle. We will determine if phylogenies based on our expanded data set of ACL genes recover well-recognized groups of prokaryotes or whether there is evidence for lateral gene transfer. If phylogenies hold well-recognized groups together, this will provide a framework for future work using ACL as a functional gene marker in specific PCR (or quantitative PCR) to study the diversity and distribution of organisms that utilize the reductive TCA cycle for CO_2 fixation in the environment. In a manner similar to analyses of rRNAs and *dsr* studies described above, samples could be analyzed from deep-sea hydrothermal vents and the deep subsurface, the acidic Río Tinto, and microbial mats occurring in the salt marshes around Woods Hole. These environments can be viewed as analogues for habitats that might have existed on early Earth or that could exist on other planets, and as a result, are likely to be inhabited by organisms with ancient metabolic traits. The discovery of novel sequences with ancestral features will also help us to elucidate the origin and evolution of the reductive TCA cycle.

Organisms capable of autotrophic metabolism were an integral part of early ecosystems. They serve an important function by making inorganic carbon available to other organisms, a central component of the global carbon cycle. The balance between the fixation of inorganic carbon and its release through heterotrophic processes is primarily responsible for the carbon dioxide and oxygen concentrations in the atmosphere. The microbial communities on early Earth most likely existed under conditions that are today confined to what one may describe as extreme environments. Organisms that thrive under those conditions primarily utilize carbon fixation pathways other than the Calvin cycle. This project has the goal to better understand the evolutionary history of one of the most ancient carbon fixation pathways and to lay the foundation for an assessment of its environmental significance. As part of this proposal, environments will be studied that have resemblance to conditions on early Earth and that can also be considered as analogs for possible microbial communities on other planetary bodies. Autotrophy is a requirement for life to exist anywhere, yet very little is known about the organisms responsible for primary production in these microbially dominated ecosystems, making this project an important component to further our understanding about the functioning of these systems.

This project will also foster the interaction between different partners involved in this proposal. Examples include but are not limited to the group of Monica Riley, whose expertise in molecular evolution and comparative sequence analyses will be very valuable. Other ties include links to the Bay Paul Center's DNA sequencing capability and their expertise in phylogenetic

analysis. There will also be extensive collaborations with the other groups working on early metabolisms, namely Andreas Teske (UNC), Katrina Edwards (WHOI), and Linda Amaral Zettler (MBL). Besides sharing samples, the interaction with these groups will lead to a better understanding of the microbial communities inhabiting these systems by combining multiple lines of inquiry.

5.2 TERRESTRIAL ANALOGUES FOR EARLY MARS:

Today, the surface of Mars seems an unlikely place for active biology. It is a cold, oxidizing environment that appears to lack liquid water, although water ice is present in polar regions, and water vapor and ice crystals occur in the atmosphere. There is evidence for a warm and wet early Mars and numerous geological features show that liquid water flowed from the subsurface out onto the surface of Mars at many times throughout its history [124]. Even more exciting are the recent results from Mars Global Surveyor and Odyssey that point to the presence of liquid water and water mobility in the very recent history of Mars. These include the gullies interpreted to represent the flow of liquid water in relatively recent periods [125], evidence for a latitude-dependent layer of water ice and dust deposited from polar regions toward the equator down to about 30 degrees north and south, apparently by recent paleoclimate changes [126-129], abundant near surface water ice detected by Odyssey spectrometers in approximately the same latitude bands [130], and geologically recent mountain glacier deposits seen in equatorial regions along the margins of the Tharsis Montes [131]. This changing view of Mars as a potential abode for life, and discoveries of life whereever liquid water is found on Earth, rekindles enthusiasm for seeking evidence of extant or past life on Mars.

The study of environments enriched in iron may provide insights into the biochemistry and metabolisms of the early Earth and ancient Mars. For example, there are many theories that include pyrite (FeS_2) as a driving force in the origin of life. Pyrite is the most common metal sulfide on Earth [132] and was thought to be involved in prebiotic Earth chemistry in its interactions with phosphate. Furthermore, pyrite is the most stable iron mineral under anaerobic conditions [133] as existed on early Earth. Wächtershäuser [134] proposed that life arose via an anoxic chemoautotrophic pathway described as a "two-dimensional chemiautotrophic surface metabolism in an iron-sulfur world" wherein the reductive citric acid cycle and other nonenzymatic chemistry of the prebiotic Earth occurred on the surface of iron sufide minerals. Russell et al. proposed a model whereby membrane-like accumulations of the iron-sulfur mineral mackinawite allowed the oxidation of hydrogen to be coupled to the reduction of Fe (III) leading to the first protometabolism and the evolution of life [135].

Our theme, **TERRESTRIAL ANALOGUES FOR EARLY MARS** explores the biology and diversity of iron oxidation, which we hypothesize to be relevant to early metabolic processes and physiologies on Earth and possibly Mars. Our studies will focus on two kinds of environments that serve as possible terrestrial analogues for Mars; basalt-rich, mid ocean ridges and iron-sulfide-rich, acidic rivers. We will study the biological basis of iron oxidation and fractionation of Fe isotopes by neutrophilic bacterial isolates from metal sulfide deposits at the Juan de Fuca Ridge and will extend our studies of iron oxidation to eukaryotic cultures (fungal and protistan) from the Río Tinto of southerwestern Spain. These efforts will complement ongoing investigations of iron oxidation by prokaryotic acidophiles of the Río Tinto currently under investigation by the Centro de Astrobiología (CAB, an NAI Associate). We will employ a new technology “SAGT” to efficiently characterize diversity and population structures of microbial communities (both prokaryotic and eukaryotic) in the water column and biofilms of the Río Tinto. These studies of microbial communities will be correlated with variations in biogeochemical parameters. Finally, we will explore linkages between microbial diversity in the Río Tinto and the Juan de Fuca Ridge and the geological processes ([136, 137] and others) that created and modified these environments, and then develop recognition criteria for key

geological settings and minerals to enhance and focus the search for such potential astrobiological sites on Mars. The ultimate objective of this interdisciplinary effort is to develop improved models and methodologies for life detection by remote sensing.

5.2a Iron Oxidation – Shaping past and present environments.

Iron (Fe) is the most abundant element in the Earth [138]. This pervasiveness underscores the importance of Fe, and Fe cycling in the geochemistry of the planet and biogeochemistry of life, including the emergence and development of life on early Earth during the Archean (3.8 to 2.5 Ga). During this eon, life proliferated and achieved extraordinarily levels of complexity in an ocean that was essentially devoid of dissolved oxygen and sulfide [139, 140]. The Archean geologic record contains abundant laminated structures such as stromatolites and banded Fe formations (BIF), both of which have been dated to 3.8 - 3.5 Ga and have arguably been considered as artifacts of the early life on our planet [141, 142], though the precipitation of Fe oxides in BIFs predates the oxygenated atmosphere. Indeed, recent studies have suggested that it is plausible, if not likely that BIFs are the product of biogenic oxidation of Fe in the absence of oxygen [143]. For example, the oldest photosynthetic bacteria were most likely anoxygenic purple non-sulfur bacteria [144] and several of these bacteria have been shown to fix carbon dioxide (CO_2) through the photosynthesis and the oxidation of ferrous iron (Fe^{2+}) rather than the oxidation of H_2O [142]. Along with photosynthesis, the sulfur and oxygen depleted Archean ocean could have favored the evolution of metabolisms that utilize alternative electron donors (e.g. Fe^{2+}, Mn^{4+}). It has even been suggested that residual Fe^{2+} respiration occurs in some *Pedomicrobium* spp. that have evolved an aerobic metabolism, perhaps hinting at an ancestor that was able to conserve energy from this now derelict pathway [145, 146].

Current theories about the Archean ocean indicate it was enriched in dissolved Fe, perhaps as much as 50 μM [147]. At present, free dissolved Fe is largely absent from the world's ocean, because an oxygenated atmosphere prevents buildup of reduced iron. However, local anomalies (e.g. Loihi Seamount, Hawaii, acid mine drainage, groundwater), where dissolved Fe is present in the water column or sediment water interface, persist. However, other sources of Fe potentially available to microorganisms during the Archean and today are present as the solid components of Earth's crust. Fe-rich rocks and minerals are abundant in both terrestrial and oceanic habitats. For example, sulfide ore deposits that are mined for economic metals as copper, gold, zinc, and others, are comprised of Fe-rich minerals such as pyrite (FeS_2), which are known to support the growth of significant populations of Fe-oxidizing bacteria in the modern Earth environment. It has also been hypothesized that Fe-rich sulfide minerals played a role in the evolution and metabolism of Earth's earliest life forms [134]. Despite the limited availability of iron in the modern biosphere, many proteins contain FeS centers. The repeated use of such centers in protein evolution may provide clues to the use of iron in the origin of life and the nature of the early biosphere. Additionally, approximately 70% of the Earth's near-surface crustal material, most of which is covered by our oceans, is comprised of basaltic rock, which is generally about 10 wt. % FeO [148]. Recent studies have described bacteria, archaea, and eukaryotes that thrive in environments similar to the Archean ocean, systems containing high dissolved Fe (e.g. The Loihi Seamount, HI, [149]; Michigan ground water, [150], Iron Mountain, CA, [151]; Río Tinto, Spain, [152, 153]) or in bare-rock systems such as the metal sulfide deposits at Juan de Fuca Ridge [154]. While many of these recent studies are among the first to demonstrate the association of Fe with life on Earth, evidence of such an association has long been recognized – if only qualitatively in many cases.

Though abundant evidence clearly exists that indicated the relative importance of Fe cycling in the early evolution of life and chemoautotrophic metabolism by comparison to any other similarly ancient metabolic pathway (e.g., sulfate reduction, Fe reduction), the fundamental biology of Fe oxidation has received little attention. This may be attributed to the recalcitrance

of Fe-oxidizing microorganisms, particularly non-acidophilic Fe-oxidizers to laboratory growth and manipulation. However, recent strides in our laboratory and several others have now made it possible to interrogate these elusive bacteria in axenic culture. Concomitant with these strides, recent technological advances in isotope biogeochemistry, specifically in the area of stable metal isotopes, enable us to similarly probe the systematics of Fe isotope fractionation in biologically produced rocks and minerals, allowing for the potential development of robust biosignatures for these organisms. Here we propose to conduct detailed laboratory studies aimed at exploring microbial Fe metabolism – from cellular processes to mineralogical and geochemical signatures, with the goals of advancing the fundamental understanding of the biology of Fe-oxidizing microorganisms, and establishing mechanistic linkages to the influence they exert on geochemical processes on Earth, and possibly on other planetary bodies.

Río Tinto. Many examples of Fe-rich aquatic environments arise as a consequence of (or are exacerbated by) industrial activities such as mining, while others occur naturally. Our study site in southwestern Spain focuses on the Río Tinto, a 100-km long, acidic, heavy metal laden river running through the world's largest pyritic belt. Environments like the Río Tinto provide a good terrestrial model for Mars because there is growing evidence that certain mineralogical and sedimentilogical features on Mars could have only formed in an acidic environment [155, 156]. The mining of the Río Tinto region is one of the longest recorded mining operations in the world. The river's name refers to the color of its waters – "tinto" meaning red in Spanish – the result of high levels of dissolved ferric iron (Fe^{3+}) kept in solution by the acidity of the water. In other parts of the river, reduced iron also occurs in high concentrations. Geomicrobiological characterizations show that the Tinto's Fe cycle is operational under both aerobic and anaerobic conditions. Although more than 5,000 years of mining activity (from Chalcolytic times to the present) has altered this region's landscape, the nature of the water – an average pH of 2.0 and heavy metal concentrations several orders of magnitude above normal– has a much longer history. Paleontological studies of iron stromatolites has shown that Fe-oxidizing bacteria existed in the Río Tinto river basin 300,000 years ago [152]. These stromatolitic structures have recorded the geomicrobiology and climate change of the Río Tinto system and are reminiscent in composition and banding pattern of the Archean BIFs. The metabolic byproducts of similar, if not identical, microbial communities maintain current acidity levels of this Fe-rich environment.

Juan de Fuca Ridge. Deep-sea environments are among the most pristine and ancient ecosystems available to study the origin and evolution of life. Recent analyses of remote sensing thermal emission data point to Mars as having an abundance of basalt and possibly basalt that was weathered under submarine conditions [157]. Mid-Ocean Ridge (MOR) systems, such as the Juan de Fuca Ridge, are bare rock ecosystems, which host organisms ranging from hyperthermophilic Archaea to giant *Riftia* sp. tubeworms. However, the biological communities associated with the spreading centers are not limited to the areas directly influenced by the hydrothermal fluids. Newly produced ocean crust, as well as metaliferrous sediments and deposits produced by precipitating hydrothermal fluids, also store an important pool of energy, which can be harvested for cellular processes. It was recently estimated that the production of new crust along the MOR could support a biomass production, from the oxidation of Fe^{2+} within the crust and associated precipitates, of 10^{11}g C /yr using oxygen as the terminal electron acceptor [158]. Much of this oxidation would occur under a fully oxygenated water column, perhaps with microenvironments approaching hypoxia or anoxia.

Evolution and diversity of oxidative Fe metabolisms in neutrophilic and acidophilic prokaryotes. Neutrophilic Fe-oxidizing bacteria (FeOB) have been recognized since the mid 19th century, although until recently evidence of their existence had been circumstantial, reliant upon the production of "diagnostic" morphologically distinct, extracellular Fe oxide minerals [159]. A variety of Fe oxide morphologies have been described and attributed to the activities of FeOB, including "stalks" (presumably from a *Gallionella-like* organism) and "sheaths" (presumably from a *Leptothrix-like* organism), which are the two most widely recognized and cited morphotypes [145]. These morphological markers are often found in habitats were anoxic water containing generous amounts of Fe^{2+} slowly mix with oxygenated water, resulting in the production of a rust-colored precipitate which can be trapped in a biofilm.

Morphological markers have long been observed microscopically in mineral deposits from deep-sea hydrothermal environments and attributed to the activity of FeOB (eg. [160-164]). These diagnostic Fe oxide precipitates have been found in rocks from ancient hydrothermal vents, and a variety of other Fe-rich ancient habitats [165, 166]. In a recent inspection of museum mineral collections and field observations, more than 140 sites were found which contained evidence of biogenic Fe-oxide formation [167]. To date, the best evidence of these distinct biogenic Fe-oxides in a modern Earth habitat comes from observations of microbial mats at the Loihi Seamount. At this site three Fe oxide morphologies have been described: the classical twisted stalks (similar to *Gallionella sp.*), straight sheaths (similar to *Leptothrix sp.*) and amorphous oxides [149]. These authors calculate, assuming the stalks and sheaths are of biogenic origin, that up to 60% of Fe oxide production within microbial mats is the direct result of microbial respiration [149]. A report of biogenic Fe-oxide production from a pure culture estimates that 90% of the oxidation is due to microbial activity [168]. However, the physiological mechanisms that enable these organisms to harness the chemical energy from the oxidation of Fe^{2+} have not yet been elucidated.

Chemolithoautotrophic FeOB have been recently isolated and described from the Juan de Fuca Ridge and shown to be phylogenetically diverse. While previously described Fe-oxidizing microorganisms have been inferred to be members of the Archaea and beta-Proteobacteria, new isolates from the Juan de Fuca Ridge have been shown to be members of the alpha- and gamma-Proteobacteria [169]. Interestingly, the closest known relatives of these Juan de Fuca FeOB are heterotrophic and some (*Pedomicrobium sp.*) display the ability to oxidize Fe^{2+} (for unknown physiological purpose) along with heterotrophic growth.

Despite the fact that evidence for Fe-oxidation was first recognized in neutrophiles, the metabolic role of Fe-oxidation has been more clearly understood, and better studied, among acidophiles. Acidophilic Fe-oxidizing organisms have been recognized in terrestrial sulfide mineral weathering habitats such as the Río Tinto, and part of the intense interest and study of these organisms is linked to the deleterious ramifications their activities have on the environment in the form of "acid mine drainage" (AMD)[170]. For example, *Acidithiobacillus ferrooxidans* generates protons via the oxidation of pyrite to ferrosulfate and sulfuric acid [171]. Fe-oxidizing bacteria such as *Acidithiobacillus ferrooxidans* (formerly known as "*Thiobacillus ferrooxidans*"; [172] and *Leptospirillum ferrooxidans* are examples of well studied acidophilic, mesophilic chemolithotrophs. The newly described Fe-oxidizing archaeon *Ferroplasma acidarmanus* also lives in extreme acidic conditions (pH=0-1) and is thought to be the key player in the mining process. All of these genera can be found in and some of them have been isolated from the Río Tinto [152] and are currently the focus of intense study by members of the CAB.

Iron metabolism/regulation in acidophilic eukaryotes. Iron is vital for life. We know that Fe is required by all living organisms except lactobacilli and a few other bacteria. Fe-associated proteins include hemeproteins, iron sulfur proteins containing Fe/S clusters, mononuclear non-hemeproteins, and diiron oxo-bridged proteins [173, 174]. In humans, excess Fe can be lethal

while Fe deficiencies lead to anemia as well as cognitive impairment and immunodeficiency [174]. Excess Fe is toxic because of its capacity to produce hydroxyl radicals via Fenton chemistry. Organisms like *E. coli* possess bacterioferritin to cope with this problem [175]. Yet, typical environments on Earth have low concentrations of bioavailable Fe and so organisms have developed elaborate ways to scavenge Fe (e.g. bacterial siderophores and phytosiderophores).

What happens to organisms living in Fe-rich environments? We know little about the interactions of acidophilic eukaryotes with Fe, particularly under the high concentrations present in the Río Tinto. Fe oxidation in eukaryotes has never been explored systematically, but observations suggest that this process may occur, particularly among fungi, some of which have been found to oxidize Mn [176]. Mn and Fe oxidation appear to be linked in many environments among prokaryotes, but the same has not been shown yet for eukaryotes.

METHODS. We will examine neutrophilic and acidophilic Fe-oxidizing microorganisms to fully explore the cellular processes and mechanisms that enable microorganisms to harness energy from the oxidation of Fe^{2+}. We currently have 16 axenic cultures of neutrophilic Fe-oxidizing bacteria from the Juan de Fuca Ridge (Edwards Lab). Basic physiological and phylogenetic studies of these strains are complete [169], as well as some field studies that have focused on environmental, geochemical, and mineralogical aspects of their physiology and activities [146, 154]. We are presently well poised to launch studies to examine in detail the biochemical mechanisms used by Fe-oxidizing bacteria and to develop a framework with which to understand the mineralogical and isotopic consequences of their activities that may be recorded in the Earth's rock record. We will generate a 2-4X shotgun genome sequence of a gamma-Proteobacteria neutrophile that we successfully culture in our lab (strain F16). This strain groups phylogenetically with other neutrophiles that we have isolated and with the acidophile *Acidithiobacillus ferrooxidans* (*Thiobacillus ferrooxidans*) whose genome sequence is near completion (http://www.tigr.org/). By comparing the metabolic pathways in neutrophilic and acidophilic strains, we aim to identify the molecular basis of functional and physiological linkages between these two types of Fe-oxidation metabolism. Physiology studies on acidophilic prokaryotic cultures from the Río Tinto are being carried out by our Spanish colleagues at the CAB (R. Amils).

The mechanism of Fe-oxidation has been studied mostly in *A. ferrooxidans* where a complex Fe oxidase system consists of the protein components: Fe (II) oxidase, cytochrome c4-, cytochrome c, rusticyanin and cytochrome oxidase. All these are required for complete Fe oxidase activity. The components may differ in different acidophilic iron oxidizers. For example the Fe oxidase system of *A. ferrooxidans* differs from that of *L. ferrooxidans* and BC1, a moderately thermophilic gram positive Fe-oxidizing bacterial strain [177]. *Leptospirillum ferrooxidans* does not have rusticyanin, but instead has a soluble acid stable cytochrome a. Strain *BC1* also lacks rusticyanin but possesses a novel membrane bound yellow chromophore. These findings suggest a diversity of Fe-oxidation enzyme systems in Fe-oxidizing bacteria. Despite this putative diversity, components of the electron cascade of different acidophilic Fe-oxidizing bacteria may be similar. In *A. ferrooxidans* a periplasmic Fe (II)-oxidase has been purified and the gene cloned [178, 179]. Cytochrome c protein, rusticyanin and cytochrome oxidase have been purified, and the rusticyanin gene has been cloned and sequenced. [180-183]. The cytochrome oxidase protein has also been crystallized in *Paracoccus denitrificans* [184]. Less is known about the molecules involved in Fe-oxidation carried out by neutrophiles. In the sheath-producing neutrophile mentioned above, *Leptothrix discophora*, Fe-oxidation has been attributed to a 150,000 kDa protein [185].

We will also explore the potential for Fe oxidation in acidophilic eukaryotes that we have isolated from the Río Tinto. With the levels of Fe found in the Río Tinto, one would expect that if Fe-oxidizing eukaryotes exist, the Río Tinto is a model environment in which to search for

them. Dr. Ricardo Amils at the CAB has agreed to do prescreening of 1,200 fungal cultures for pH stability and Fe tolerance and will then send us 10 representative cultures to screen for Fe-oxidation activity. We will also screen protist cultures for evidence of iron oxidation, regulation and other Fe interactions. We currently have pure cultures of a chlamydomonad, an euglenid, a chlorellid and a vannellid amoeba (Amaral Zettler culture collection). Purification of mixed cultures of diatoms, ciliates and several phylogenetically distinct amoeboid groups is underway (including a nucleariid amoeba, a vahlkampfiid amoeba, a gromiid, and a heliozoan).

The overarching goal of the proposed work is to understand the evolution and diversity of oxidative iron metabolisms in microorganisms from extreme environments and their relationship to mineral structures that may serve as biosignatures. The aims of this study are as follows:

1. Determine the fractionation of Fe isotopes using neutrophilic cultured FeOB
2. Study the Fe oxide morphology formed by neutrophilic iron oxidizers
3. Identify proteins and genes common to both the acidophilic and neutrophilic FeOB using biochemical and genomic techniques
4. Examine the role of acidophilic eukaryotes in Fe oxidation/regulation

Isotopic fractionation of Fe. The role of biology in cycling of metals in the environment may exert significant control on the fractionation of isotopes. While the fractionation of light isotopes, for example C, N, O, S, is influenced by abiotic processes including changes in temperature and phase transitions, intermediate mass elements like Fe may not be fractionated significantly by abiotic processes as a result of smaller relative mass differences between isotopes [186]. However, the small relative mass difference between ^{56}Fe and ^{54}Fe may result in a significant fraction if metabolic processes incorporate a number of steps at which a fractionation could occur.

Natural ^{56}Fe/^{54}Fe isotopic ratios have been shown to vary in nature over a small range (3.5 per mil) necessitating high precision methods for determining relative ratio changes due to various factors [187]. Recent advancements in the methodology of Fe isotope determination have increased the precision of measurements enabling the analysis of mass dependant fractionations. Fe isotope determination has been measured using both Thermal Ionization Mass Spectrometry (TIMS, eg. [186, 188] and Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS, e.g. [187]. Because of the high ionization efficiency and stable mass bias of the latter instrument, low detection limits are attained. However, MC-ICP-MS co-eluding interferences from the argon plasma (primarily ArN and ArO interfere with ^{54}Fe and ^{56}Fe respectively) or other metals (eg. Cr, Ni) can plague the measurement of the Fe isotopes. These difficulties can be circumvented through use of a higher resolution scans, changes in the typical (nitric) acid matrix, using large quantities of Fe (5-25ug) or by adding a reactive collision gas to the plasma [187]. Beard et al. ([187], in press) report the complete removal of ArN through the mixture of Ar and H_2 gas in the collision chamber. These amendments to the technique allow for high precision (±0.3 to ±0.05 per mil) determination of the Fe isotopic ratios.

To date, very few studies have examined biological fractionation of Fe isotopes and include the study of (1) magnetite biomineralization by magnetotactic bacteria [188] (2) fractionation due to Fe(III) reduction by *Shewanella alga* [186, 187] and (3) mineral dissolution by siderophore producing soil bacteria [189]. These studies report negative fractionation from the reduction of Fe(III) of –1.3 and –0.8 per mil ^{56}Fe/^{54}Fe from the *Shewanella* and siderophore experiments respectively. Fractionation from the formation of the oxide mineral magnetite was not demonstrated due to the high detection limit (0.3 per mil) of the method used (TIMS) and pools measured. However, subsequent review of the work concluded that further knowledge of the equilibrium fractionation between the dissolved Fe and the magnetite could reveal a net biological fractionation, which opposes the direction of an equilibrium fractionation [187].

The effect of biological and chemical Fe-oxidation on the Fe isotope ratio will be determined through a set of experiments using strains from our collection of neutrophilic FeOB. Our justification for focusing on the neutrophiles for this portion of the work is in part related to the work described in the next section (**Fe oxide morphology**), and is thus elaborated on there. Experiments will involve growing bacteria in a minimal mineral media using synthetic $Fe(CO_3)_2$ [169] as an Fe^{2+} source. The bacteria will be inoculated into a dialysis bag (10,000 MW), which allows for exchange of dissolved species while containing particulate matter (both cells and Fe oxides) within the bag. The bag and its contents will be harvested and the contents acidified in with 0.5% SeaStar grade nitric acid in a clean Nalgene bottle. The aqueous solution will be treated identically. Relative isotope ratios will be determined using a Thermo Finnigan Neptune MC-ICP-MS using the method of Beard and Johnson [190] including using H_2 gas to eliminate the ArN and ArO co-eluding peaks. Measurement will be taken simultaneously on Faraday collectors using 10^{11} Ω resistors for ^{54}Fe, and ^{52}Cr and 10^{10} Ω resistors for ^{56}Fe. Chromium interference of ^{54}Fe is corrected using the relationship $^{54}Cr/^{52}Cr=0.0282$ [190]. Subsequently, the Fe isotope ratio will be determined under changing culture conditions including temperature, Fe source ($FeCl_{2(aq)}$, basalt, pyrite), pO_2 and pH.

Fe oxide morphology. As noted above, a long standing hallmark of the activities of the neutrophilic FeOB has been the production of morphologically distinct mineral particles. In addition to the differences in pH conditions that are used to classify the two types of Fe-oxidizers we have discussed here, i.e., the acidophiles (generally thriving at pH •4) and the neutrophiles (most often found occurring at pH •6), there are notable differences in the morphologies of Fe oxide particles that are produced as a consequence of their activities. In general (though certain exceptions occur), the activities of acidophilic Fe-oxidizers such as *A. ferrooxidans* and *L. ferrooxidans* result in precipitation of amorphous or poorly crystalline oxyhydroxides that are morphologically indistinct. Occasionally these particles may encase the bacterium that produces them, resulting in a cell "cast" that may be preserved in oxide deposits, but often the Fe oxide deposits that result are largely devoid of recognizable or distinguishable features. In contrast, many if not most neutrophilic FeOB produce morphologically and sometimes mineralogically distinct Fe oxide particles, as discussed above. In keeping with this generality, we have found that strains in our culture collection of neutrophilic FeOB produce a wide variety of Fe oxide morphotypes, some of which are similar to those previously described [145], but many of which appear not to have ever before been observed in laboratory growth cultures [169]. SEM and TEM studies of laboratory cultures and field studies of rocks from which our cultures were derived reveal particle morphologies that include long (20-30 μm), narrow (1-2 μm) angular particles, irregularly coiled particles, and cohesive bundles of straight particles that are composed of individual oxides in the range of 20 nm or less in diameter [154, 169]. Mineralogically, all morphotypes appear to composed of "2-line ferrihydrite" [191], a very finely crystalline form of ferrihydrite [154].

To date we have not undertaken systematic studies of the Fe oxide morphologies we observe in culture, but preliminary observations suggest that the oxide morphologies vary both as function of culture type (species/strain) and culture conditions. We hypothesize that careful study of both the conditions and culture type that gives rise to specific Fe oxide morphologies may be diagnostic to the environmental conditions that give rise to similar particles that we, and many others, have observed in modern and ancient depositional environments on Earth, as well as potentially serve as biomarkers on other planetary bodies. This would be a particularly powerful tool if used in conjunction with diagnostic isotopic signatures, such as proposed above. For these reasons we will focus our mineralogical/morphological studies proposed here, and the Fe isotope work proposed above, entirely on our neutrophilic FeOB culture collection.

In parallel with the Fe isotopic studies designed to explore isotopic fractionation as a function of changing culture conditions (temperature, Fe source, pO_2 and pH), we will conduct

light (Differential Interference Contrast, DIC) and electron (SEM and TEM), and Atomic Force (AFM) microscopic studies on Fe oxide particles produced in culture compared with abiotic controls. The DIC and AFM will be conducted using a Digital Instruments Atomic Force Bioscope. The AFM head is used simultaneously and in-line with the light optical components of an Inverted Zeiss Axiovert 100. This equipment is available and maintained in the Edwards lab. TEM and SEM studies will be conducted at the MBL microscopy facility, which is equipped with a Zeiss 10CA transmission electron microscope and a JEOL 840 scanning electron microscope (supported by a multitude of preparative and ancillary equipment).

Identification of Fe oxidizing genes and proteins in neutrophilic prokaryotes. The important role that FeOB play in geochemical processes is controlled at the cellular level by the genes and ultimately the proteins that participate in terrestrial sulfide dissolution, AMD, rock weathering in the deep-sea, and Fe redox cycling in a variety of aquatic Earth habitats. We propose to conduct molecular biological studies to identify Fe-oxidizing proteins and genes in select strains of neutrophilic FeOB from our Juan de Fuca Ridge culture collection. We will conduct protein purification studies on representatives from α- and γ- proteobacterial lineages with high growth rates and yields. Methodologies for growth and harvest of the neutrophiles will be as described [169]. In Edwards et al. 2003 [169] we detail aerobic growth on several different Fe substrates. For our work here, we plan to use ferrous carbonate, as it appears to facilitate most rapid and abundant growth as well as iron oxide formation.

Physiological studies of *Leptothrix ochracea* and *Gallionella* dominate the research that has been carried out thus far on neutrophilic FeOB. Nothing is known about the genes and little about the proteins involved in Fe-oxidation in neutrophilic FeOB. We propose to use a two pronged approach to identify the proteins and genes involved in neutrophilic iron oxidation. We believe this approach provides the most robust combination of methodologies for answering some of the key questions regarding the basic mechanism of Fe-oxidation.

We propose to initiate our protein work using the methodology of Corstjens et al. and Takai et al. [185, 192]. The former use SDS-PAGE followed by an assay to detect Fe-oxidation activity in the gel. The assay consists of rinsing with 10 mM MES at pH 6.0 for 45 min., followed by incubation in 2 mM $(NH_4)_2Fe(SO_4)_2$ in 10 mM MES at pH 6.0. Development of brown or yellow color indicates Fe-oxidation activity. Using this fairly simple colorimetric assay, it was possible to identify an Fe-oxidizing factor of 150,000 kDa from the neutrophile *Leptothrix discophora*. We will use both SDS and native PAGE to identify the iron oxidizing factor in our study. In a study by Takai et al., several components of the Fe oxidase activity from the cell membrane fraction were detected on SDS PAGE after purification, with masses of 46 kDa (α), 28 kDa (β), 24 kDa (γ), 20 kDa (δ), and 17 kDa (ε) [192]. However, with native PAGE, the homodimeric enzyme was estimated to be 263 kDa $(\alpha\beta\gamma\delta\varepsilon)_2$. We will use a similar approach to isolate the total cell membrane proteins of the neutrophilic cultures. Since the technique that Takai et al. developed was designed for use with enzymes from acidophiles (with low pH optima), we will have to standardize the chromatography for purification of neutrophilic membrane proteins using common gel filtration and ion exchange chromatography (Pharmacia). Purified proteins will then be sent out for N-terminal sequencing to a commercial facility (Rockefeller University). In order to identify the components of the iron oxidation system, we can use the protein sequence to degenerate DNA probes for Southern hybridization or for PCR amplifications. PCR amplicons or genomic fragments that anneal the DNA probes will be excised, cloned, and sequenced to obtain the entire gene sequence of interest. The information generated provided by DNA sequencing will allow us to examine if there is a connection between the phenotype (a given Fe-oxide morphology) and genotype of Fe-oxidizing organisms in our current culture collection – and among any Fe-oxidizers yet to be discovered. This technology is intellectually straight-forward and routine in molecular biology laboratories but we are well aware that this approach can be labor intensive. As an alternative, genomics based

approaches can accerate acquisition of similar kinds of information. When combined with even minimal protein sequence information, it becomes possible to identify relevant coding regions and entire pathways.

We will use information gained from the above work in combination with comparative genomics to determine genes and pathways involved in Fe-oxidation. We will sequence draft coverage (2-4X) of the genome of a relatively well-characterized neutrophile that we successfully culture in our lab (the strain F16). This analysis will provide the first genomic data of a neutrophilic Fe-oxidizing bacterium, and will make a significant contribution to the field of microbial genomics. Among the various neutrophiles we have isolated in our lab, we selected F16 for genome sequencing based on three criteria. First, on a practical level, we selected F16 due to the relative ease of culturing this strain in the lab and our ability to obtain high quality DNA for genomic libraries. Second, F16 is closely related to several other neutrophiles in the gamma-Proteobacteria, and thus may be representative of a group that is abundant in the environment. Third, F16 is a member of a phylogenetic assemblage of γ-proteobacteria that includes the acidophilic Fe-oxidizer *Acidithiobacillus ferrooxidans* (*Thiobacillus ferrooxidans*) whose genome sequencing is near completion (http://www.tigr.org/) and several *Pseudomonas* spp. whose sequences are completed (*Pseudomonas putida* KT2440 and *Pseudomonas aeruginosa* PAO1) or near completion (*Pseudomonas syringae* DC3000, http://www.tigr.org/). This availability of full genome data for closely related lineages will facilitate annotation and will allow us to compare the metabolisms of acidophilic and neutrophilic Fe-oxidizing bacteria. Through those comparisons, we hope to identify any common mechanisms for Fe-oxidation and differences that may reflect their distinct habitats and physiologies.

First, we will determine the genome size of the neutrophilic strain F16 through Pulsed Field Gel Electrophoresis (PFGE) using the Biorad CHEF Mapper XA in the Bay Paul Center. Using this approach, bacterial cells will be embedded in agarose plugs, in which intact genomic DNA will be prepared and subsequently digested with rare cutting enzymes. The resulting large genomic fragments will be sized using PFGE and summed to calculate total genome size. If F16 is greater than ~4 Mb, we will measure chromosome sizes for the six other close relatives of F16 in our culture collection of neutrophilic oxidizers, and select an isolate with the smallest size genome for shotgun sequence analysis. The calculations below assume a genome size in the range of 4 Mb, which is typical for γ-Proteobacteria. We will then use conventional recombinant DNA techniques to construct a genomic library [193] and sequence 10,000 randomly selected, recombinant clones using the 3730 ABI sequencer at our high throughput sequencing facility. Estimating an average genome size of 4 Mb and read lengths of ~900 bp, we will collect a minimum of ~2 X coverage of the genome. If the sampled sequences are Poisson distributed, this coverage will reveal >85% of the genome's coding capacity. Sequences from both ends of each insert will be assembled using ARACHNE [194, 195]. Contigs plus singletons will be analyzed by similarity searches against GenBank using NCBI BLASTX [196-198]. Besides searching for similarities to genes identified as being involved with Fe-oxidation, we will use software such as Pathway/Genome Database (PGDB) designed to help infer gene function, metabolic and genetic networks [199]. For example, in *A. ferrooxidans*, the genes encoding the two c-type cytochromes, cytochrome oxidase and rusticyanin occur on the same operon [200]; the same may be true for the neutrophilic pathway. Depending on the results of this study, future work could include microarray experiments to study upregulation and downregulation of genes during growth on alternative minerals such pyrites and siderites. Genome information will also aid in elucidating other metabolic processes in these extremophiles.

Screening acidophilic eukaryotes for iron oxidase activity. Whether acidophilic eukaryotes living in high iron environments can harness the energy of iron-rich substrates has never been systematically studied. Such metabolic capacities in eukaryotes most likely occur in environments where iron is not a limiting nutrient but these conditions are relatively rare in

contemporary ecosystems. The Río Tinto is unusual in this regard because it is iron rich and supports a broad diversity of eukaryotic protists and fungi. There is a strong correlation between Mn and Fe oxidation. Studies of fungi suggest iron oxidation may occur in eukaryotes. Structures formed in the presence of Mn-oxidizing fungi resemble "*Metallogenium*" particles that are observed at the aerobic-anaerobic interfaces in natural environments where Fe and Mn are deposited [145, 176]. These particles were first described as organisms (hence the genus name) but subsequently, no detectable biological membranes or nucleic acids associated with these particles were found and it was later suspected that they may be formed by diffusible Mn-oxidizing factors secreted by the fungi. Formation of "*Metallogenium"*-like particles is of astrobiological interest because microfossils resembling these structures have been described in precambrian rocks ([145] and references therein). Furthermore, any particles that have direct biogenic links would be important to explore for potential biosignatures.

Unlike the prokaryotic iron oxidizers, assays for iron oxidation in eukaryotes have not been reported in the literature. The potential for photosynthetic activity to catalyze oxidation of Fe^{2+} as seen with Mn^{2+} in *Chlorella* sp. could lead to false positives in assays for iron oxidation [201]. But, low pH may retard such activity. Obviously this potential interference is of little concern for studies of nonphotosynthetic species. Dr. Ricardo Amils at the CAB will prescreen fungal cultures and provide us with ten isolates for further testing. We will include examples from our culture collection of acidophilc protists isolated from the Río Tinto in a systematic survey of iron oxidation. We will survey for proteins involved in possible iron oxidation in eukaryotes using biochemical assays of proteins resolved by SDS- PAGE as described above for neutrophilic prokaryotic iron oxidizers. If we discover evidence of iron oxidation, we may explore options of isolating and characterizing proteins involved in iron oxidation.

With the completion of this work, we will gain a better understanding of some of the microbial physiologies that leave behind a geochemical record. Earth's extreme environments such as the Río Tinto and the Juan de Fuca Ridge serve as possible analogs of environments like that of Mars and therefore search for biosignatures similar to those found on Earth would be of great astrobiological relevance.

5.2b Microbial diversity and population structure studies in the Río Tinto

Declared dead over four centuries ago, the River of Fire (from the Phoenician "Ur-yero"), today known as the Río Tinto, runs 100 km through the world's largest pyritic belt in southwestern Spain and empties its acidic waters into the Atlantic. A combination of microbial ecology and molecular diversity studies show that the Río Tinto is teeming with microbial life [153, 202, 203]. Chemolithotrophic microorganisms (bacteria and archaea) feeding on polymetallic sulfidic ores contribute to the low pH (1.7-2.5) and high iron concentrations in the Río Tinto. Metals including Cu, As, Zn, Cr, Ni, and Ag are present at high concentrations, sulfate can reach 15g/l and redox potentials range from +280 mV to +610 mV [204-207]. Although mining activities have altered the system during the last 5,000 years, evidence of its antiquity older than 0.3 Ma (measured using Uranium-Thorium isotopes) has been found in massive laminated iron beds in three iron rock levels at different elevations above the Río Tinto [152]. Studies of the microorganisms in the Río Tinto may provide clues to ancient pathways or metabolisms no longer used by microbes living under less extreme conditions.

Acidity contributes to the extreme nature of the Río Tinto, but high heavy metal concentrations may present a greater challenge to the microbes living in the river. Yet, chemolithotrophs have learned to harness the energy of the iron-rich substrates found in the river. Pyrite serves as an energy source for chemolithotrophic prokaryotes since it contains both iron and sulfur that can be used by sulfur and iron oxidizing microbes – both found in the Tinto. The major iron-oxidizing prokaryotes in the river include *Acidithiobacillus ferrooxidans*,

Leptospirillum ferrooxidans, and *Ferroplasma* spp. that have been isolated and cultured from different parts of the river. Quantitative fluorescence *in situ* hybridization (FISH) experiments show that *A. ferrooxidans* and *L. ferrooxidans* are the most abundant FeOB [208]. Examples of microbes capable of reductive iron and sulfur metabolisms are also found [203].

A striking aspect of the Río Tinto is the contrast in phylogenetic diversity between the prokaryotic and eukaryotic communities. While the Río Tinto does not support complex metazoan life (although rotifers and insect larvae have been observed in the river; Amaral Zettler, personal observation) unicellular eukaryotes are abundant and more diverse than prokaryotes. Along with chemolithotrophic prokaryotes, photosynthetic protists represent the main primary producers in the river, and even numerically dominate the biomass in some parts of the river [204]. Morphological studies reveal the presence of diverse eukaryotes including fungi, different kinds of algae, various unrelated amoeboid protists, various unrelated flagellated protists, and ciliates. Most recently, molecular studies based upon comparisons of 16S-like rRNA genes PCR amplified from biofilms in the Río Tinto demonstrate even greater eukaryotic diversity than seen at the light microscope level [153] (see **Figure 5**; compare clones in bold from molecular survey with underlined taxa identified directly with light microscopy). To date, molecular surveys of the Río Tinto have been restricted to domain-level investigations of prokaryotes (Bacteria and Archaea) OR eukaryotes (Eukarya) but combined studies have not been undertaken. The nature of samples studied has contributed another significant bias. Eukaryotic studies have thus far only focused on biofilms and not bulk water column samples. In contrast, prokaryotic studies have only targeted the water column. We have only begun to dissect the biodiversity present in this rare ecosystem and the factors that shape community structure in the Río Tinto.

In this project we will take an integrated approach to assess the molecular diversity of both prokaryotic and eukaryotic components of community diversity in selected regions along the Río Tinto. We will employ a new technique developed at the MBL called SAGT [209]) in combination with traditional full-length rRNA gene sequencing of novel taxa not already in our Río Tinto sequence database. We use molecular techniques to investigate microbial diversity and population structure in the water column at both spatial and temporal scales, and we will collect concomitant physico-chemical measurements using water samples taken for the molecular surveys. When possible, determination of as many *in situ* physico-chemical parameters will be made on biofilms as well, although we acknowledge the limitations imposed by microelectrodes available for field measurements. This will allow for the correlation of biological diversity information with physico-chemical parameters of the river. The answers gained through this study will give us a comprehensive view of the microbial ecology of the system, a first step towards establishing an ecological genomics project for the Río Tinto. As part of this effort we seek to answer the following questions:

1. What accounts for the remarkable eukaryotic diversity in the extreme Río Tinto environment?
2. From a molecular perspective, how homogeneous are the blooms of diatoms, euglenids and other algae that form dense communities in various parts of the river?
3. How does phylogenetic diversity compare between the water column and biofilms along the river's edge?
4. Does diversity of a given community correlate in any way with physico-chemical parameters at a given site?
5. Are environments with greater turbidity more homogeneous than less disturbed ones?
6. How are the prokaryotic and eukaryotic microbial communities linked to each other and to what extent do they interact at the gene level?

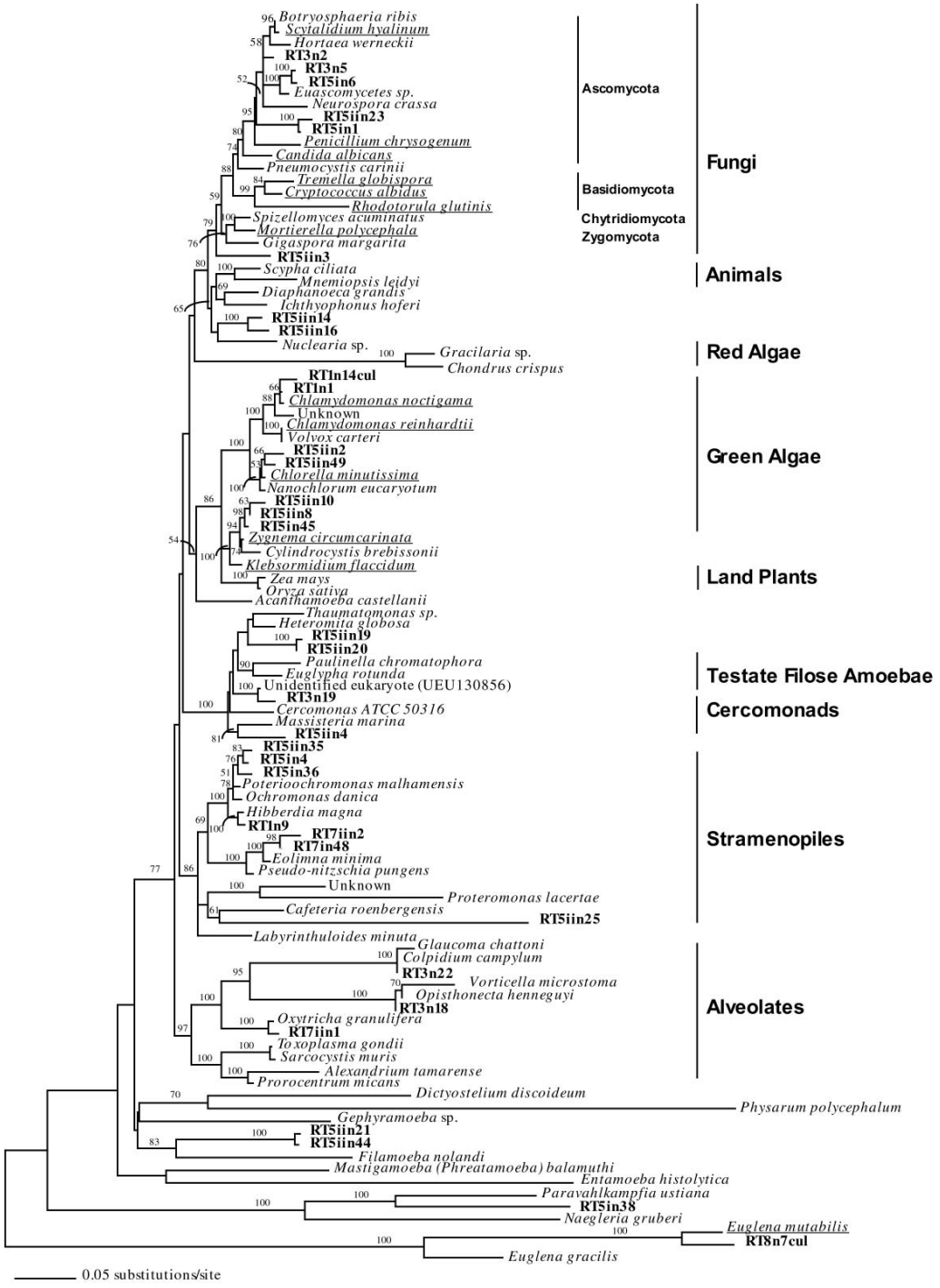

Figure 5. A minimum evolution phylogenetic tree for small subunit rRNAs using a likelihood model. Bold letters indicate environmental clones. "RT" indicates the sequence is from the Río Tinto and "cul" indicates sequences from cultured Río Tinto isolates. Underlined taxa represent genera that have been identified in the river based on microscopic observation. Sampling sites were as follows: RT1, La Palma; RT3, Berrocal Upper; RT5i, the Origin, black filamentous biofilm; RT5ii, the Origin, green filamentous biofilm; RT7i, Anabel's Garden green biofilm; RT7ii, Anabel's Garden yellow biofilm. Bootstrap support values are shown and the scalebar represents the number of substitutions per site.

Selected locations for study. We will establish the phylogenetic complexity and homogeneity of the water column of three chosen locations (henceforth designated "stations") along the river. Our studies will compare phylogenetic diversity in the water column to biofilm communities at a station and between stations along the river. We have chosen our stations based on varying concentrations and oxidation state of iron present (see http://astrobiology.mbl.edu/riotinto/ for more detailed information and photographs of the study locations). For each station we will sample three different sites, with three-fold replication. We will sample during each of the seasonal extremes of the river – one winter and one summer sampling. A brief description of the three locations and rationale for their choice are outlined below:

The "Origin" station where the river begins has several sources of water with very distinct properties and varying iron concentrations. Origin site 1 corresponds to the "Intersection" in Gónzalez-Toril, E., et al [208] where the waters from all three major sources converge before passing under the roadway (pH 2.7, 9.6 g Fe/L). Site 2 is a yellow pool below the wall and corresponds to Gonzalez-Toril site I (izquierda) (pH 2.9, 0.8 g Fe/L). Site 3 is a small deep red stream coming out from Peña del Hierro and corresponds to Gonzalez-Toril site II (izquierda, izquierda) (pH 1.8, 19.8 g Fe/L). Our eukaryotic biodiversity study of both black filamentous and green filamentous biofilms at the Origin yielded a broad range of rRNA phylotypes including sequences related to *Chlorella*, *Zygnema*, *Nuclearia*, unidentified fungi, cercomonads, different stramenopiles, valhkampfiid amoebae and other unidentified amoebae. (See **Figure 5**). We do not know which of these groups also occur in the water column. With respect to prokaryotes, FISH studies of water column samples demonstrated the presence of α–, β– and γ–proteobacteria as well as members of the Nitrospira (including the genus Leptospirillum) and representatives of the Archaea [208]. Sequence analysis of Denaturing Gradient Gel Electorphoresis (DGGE) bands from the same samples confirmed the presence of sequences that were most closely related to cyanobacteria, *Leptospirillum* species, *Acidithiobacillus* species, *Ferromicrobium acidophilus*, *Acidiphilium* species, *Magnetobacterium bavaricum,* and the archaeal species *Ferroplasma acidophilum* and *Thermoplasma acidophilum.* [203]. No attempts were made to characterize eukaryotic populations in these studies.

At the second station, Anabel's Garden, many different types of biofilm flourish and eukaryotic diversity and abundance is high. The average total Fe concentration at Anabel's Garden is 2.3 g/l and it is present in a very reduced form (high Fe^{2+}). Site 1 is in a small pool just below where the water exits from the culvert under the road; on the wall above this pool are black and green filaments attached to the wall in the rushing water. Site 2 is in a small pool to the right of site 1; it appears to fill from water seeping under the roadway and is covered with a thick mat of green algae. Site 3 is downstream of where the seep water from site 2 joins the main flow from site 1. Eukaryotic rDNA diversity studies of biofilms demonstrated the presence of diatoms and ciliates (**Figure 5).** Molecular surveys of prokaryotes in the water column using FISH and DGGE demonstrated similar kinds of species at both the origin and Anabel's Garden. [203].

The Berrocal site is just downstream of a bridge and is characterized by deep red water, localized anoxia, and dramatic concretions of river boulders cemented together with iron precipitates. The average total iron concentration here is 3.1 g/l. While there is little obvious eukaryotic biomass in this area, we have observed conspicuous blooms of *Euglena* in the shallow waters along the shore. We have also seen green and colorless flagellates, lobose amoebae, and heliozoa. Site 1 is just downstream of the rapid section below the bridge, site 2 is underneath the bridge, and site 3 is downstream of the rapids after the river reverts to a calmer flow. Different fungal sequences, cercomonad, and ciliate sequences have been obtained from biofilms at the upper portion of this station (RT3 in **Figure 5**). Water flow at Berrocal is both rapid and turbulent relative to other stations and this may have a significant impact on community structure. We will sample the water column here and localized anoxic sediments. Earlier DGGE

analyses showing domination by *A. ferrooxidans* [203] did not agree with FISH detection of α–, β– and γ–proteobacteria and members of the Nitrospiria group [208].

SAGT study of the whole microbial community in the Río Tinto. We will study the microbial diversity and population distribution in the Río Tinto using the SAGT technique [209]. This technology is modeled after serial analysis of gene expression (SAGE, [210]) which describes relative gene expression patterns. With SAGT, the PCR products from orthologous hypervariable regions in rRNA genes are ligated together to form large concatemers. A single DNA sequencing reaction of a cloned concatemer can include as many as 20-30 orthologous hypervariable regions represented in a population of nucleic acid molecules. In this way, samples loaded onto a 96-channel capillary sequencing machine can provide information about thousands of microorganisms in an analyzed sample.

The first step in the SAGT technique involves PCR amplification of small subunit rRNA genes from genomic DNA extracted from an environmental sample using conserved primers that contain a type II restriction site and flank a hypervariable region (20-100 bp) of the rRNA gene. The primer sequences are removed from the amplicons by digestion with this type II enzyme leaving a short base pair extension. Digested fragments are ligated to form concatemers that are then cloned and sequenced. The concatemer has the following structure:

[cloning vector]--[hypervariable #1]--[hypervariable #2]--...--[hypervariable #N]--[cloning vector]

The primer pairs can be designed to amplify any of the rapidly evolving regions in rRNA genes. Alternatively, we can design primers to amplify regions that are not rapidly evolving and hence restrict the granularity of the analysis, but at the same time identify population members that are more distantly related. It is also possible to design primers to target hypervariable regions from any of the three primary domains of life. Finally the technique offers a strategy for minimizing artifacts of PCR bias in analyses of microbial population structures. A population of PCR products from mixed templates will be biased if there are differences in processivity of the polymerase on different template sequences or if primers bind with different efficiencies in a template-dependent manner. A modest 10% difference in the efficiency of amplication for two starting templates present in equimolar concentrations will produce a 64-fold difference in final product concentrations after 30 rounds of amplification. Two methods for minimizing PCR bias include targeting short sequences for amplification (as inherent in the design of SAGT) and minimizing the number of amplification steps. With our SAGT strategy, we predict it will be possible to generate adequate numbers of amplicons for constructing concatemers of hypervariable regions by using only 5-10 rounds of amplification. After ligation into large concatemers, additional rounds of PCR can be used to obtain adequate concatemer copy numbers for insertion into cloning vectors. We can eliminate PCR bias introduced during this second round of amplification by restricting our data analyses to unique concatemer sequences.

To analyze the high-throughput data we rely upon a data analysis system, which uses PHRED [211, 212] to analyze the output from the sequencing machines and a signature recognition program that we will develop to identify individual hypervariable regions represented in the concatemers. We will build databases that represent the frequency of occurrence of each member of the concatemer and will use FASTA [213, 214] searches of the large rRNA gene database to identify the species to which the individual sequences correspond. We recognize that these short DNA sequences will not be sufficient for reconstructing evolutionary history but if identical or near identical sequences are represented in the large existing database for rRNA genes, it will be possible to obtain a putative identification for each member of the SAGT concatemers. If we discover completely novel hypervariable regions not represented in the databases, we will design primers complementary to these regions and in conjunction with standard conserved primers, amplify and fully sequence these novel amplicons. These novel sequences will then be included in phylogenetic analyses using methods and

programs routinely used in our center. Finally, we will use statistical methods to estimate microbial diversity and species population structure [215]. A special attribute of this technique is it can be "tuned" to address questions about population structure for different phylogenetic ranges. For example, through primer design, we can target genus-specific sequences that flank hypervariable regions. With such a strategy, the representation of amplicons within the concatemers will report relative numbers and diversity within a particular genus. The ability to quickly screen large numbers of hypervariable or moderately conserved regions provides a means to address questions about homogeneity of algal blooms, as well as ascertain the relative molecular diversity of microbial communities found in biofilm versus the water column. By combining diversity information with physical and chemical data (see below) we can explore how geochemical forces shape community structure in the Río Tinto.

Determining relevant physico-chemical parameters of the Río Tinto. The *in situ* measurements of physico-chemical parameters using specific electrodes will include pH, temperature, conductivity, redox potential, and O_2 concentration. Mass spectrometry and X-ray fluorescence will be used to measure S, Fe, Zn, Cu, Al, As, Ni, Mg, Ca, K etc. The speciation (e.g. Fe_2+/Fe_3+, sulfide/sulfate, As (III)/As (V), NO_3-) will require complementary techniques [216]. All physico-chemical parameters and biological characteristics of the river for each sampling site will then be assembled in a single data matrix. To identify the major factors that shape variation of the data we will use principal component analysis, a multivariate method that simultaneously considers many correlated variables and identifies the lowest number needed to accurately represent the structure of the data set. We will determine the significance of associations of the different variables by using the Pearson correlation test for parametric variables (i.e. all except oxygen, sulfate concentration, and redox potential values). When variables considered do not conform to a bivariate normal distribution, we will use non-parametric tests for association, which render correlation coefficients of rank association like the Spearman's Rho coefficient [217]. Our study will reveal potential physico-chemical correlations with distributions of prokaryotes and eukaryotes in the Río Tinto.

Towards ecogenomics in the Río Tinto. In most ecosystems including the Río Tinto, consortia of micro-organisms orchestrate key processes in geochemical cycling, but little is known about ecological and evolutionary responses of these communities to cyclic and transient environmental shifts. Process-oriented studies demonstrate there are feedback loops between biogeochemical gradients and structured microbial populations, yet there are no comprehensive descriptions of underlying biochemical and genetic mechanisms that govern these processes. The next advance in studies of microbial ecology will be to integrate microbial diversity, the entire spectrum of metabolic activities, and complex gene expression patterns that coordinate biogeochemical processes. DNA sequencing coupled with DNA microarrays for massive parallel expression studies or SAGE-like technologies, will provide the foundation to understand how microbial communities govern and respond to formation of biogeochemical gradients. With this advanced technology we can directly measure how microbial gene expression patterns in an entire ecosystem respond to changing chemical and physical parameters. The concurrent measurement of biogeochemical parameters, community-wide gene expression patterns and spatial descriptions of microbial populations offers a means to understand the structure and function of biogeochemical machinery at different levels of biological organization. We seek to discover the links between biological diversity and the resilience and stability of biogeochemical transformations.

Community-wide gene expression studies are a high priority for studies of microbial activities in a variety of extreme environments that are relevant to astrobiology. Examples of important environments include but are not limited to cyanobacterial mats, hydrothermally altered deep sea sediments, deep subsurface communities and iron-rich acidic biofilms and the water column of the Río Tinto. Detailed knowledge about microbial population structures as

described in this proposal are essential to the interpretation of massive, community-wide expression profiling experiments, but the cost of a comprehensive gene expression survey for any microbial habitat is still prohibitive. As an alternative, expression profiles based upon one or two species can provide important insights about how organisms change expression patterns under different conditions. Ultimately we aim to examine gene expression patterns of assemblages of organisms under different conditions in the Río Tinto (such as varying iron concentrations) to distinguish which genes are up-regulated or down-regulated. The objective would be to identify the structure and cooperative regulation of genes from different members that orchestrate biogenic interactions with iron in a microbial community.

As an example of this type of approach, we are currently working with DNA microarray experiments to measure gene expression as it relates to diel variation in microbial mats. We have constructed a genomic library of the mat-forming cyanobacterium *Microcoleus chthonoplastes* using DNA extracted from a culture of a strain collected from the Sippewissett salt marsh, in West Falmouth, Massachusetts. We have sequenced 10,241 clones with insert sizes of 650 – 1150 bp, and used this information to select the sequences to array. Selection criteria for including a gene was based on a BLASTX hit to a known cyanobacterial sequence with an e value <= $1x10^{-4}$ and a Pfam score >= 25. This filter ensured that clones on the array were from *Microcoleus* and that they contained a gene coding region. After removing redundant clones, we selected 1090 sequences to array. These sequences were PCR amplified from the vector, and the DNAs printed onto glass slides for expression profiling studies.

Preliminary experiments have used the array to examine differences in gene expression during the day and night. We took laboratory cultures of *Microcoleus*, placed them into a 14 hr light / 10 hr dark cycle for 3 days, and then collected cells after two hours of light explosure and after two hours of darkness. As expected, a number of genes directly involved in photosynthesis were expressed at higher levels in the day samples as compared to the night samples. A large number of other genes involved in growth, replication, and many other processes were also upregulated during the day. At night, we found "light repressed proteins" showing increased expression, as well as a number of regulatory proteins. These data have also shown us a number of unexpected putative results, such as an increase in expression of an iron transporter at night.

To help better understand and verify these results, we are currently in the process of assembling a time course of expression data across an entire diel cycle. This information should provide us with valuable information about the precise timing and daily expression patterns of many of these genes. Once we have collected these data on diel variations, we plan to take environmental samples directly from the Sippewissett marsh and see how closely our laboratory manipulations can match what is actually happening in the environment. Using a similar strategy, in the future we intend to initiate studies of gene expression underlying biogeochemical processes in the Río Tinto. Such studies would focus on key isolates from both the water column and biofilms as identified by the population structure studies. The goal would be to correlate gene expression patterns with physico-chemical parameter variations. Details are not provided since those studies lie beyond our immediate experimental horizon.

5.2c Life detection through remote sensing (J. Mustard, J. Head)

Deposits in natural Earth environments, such as the primary and secondary minerals at Río Tinto, provide the critical geochemical components for chemolithotrophic microorganisms feeding on polymetallic sulfides and have implications for biological processes at the root level [208]. The Río Tinto deposits and geological environments [218] are thus a unique laboratory for the study of astrobiology at the root level. Understanding the geological processes responsible for the creation and evolution of the Río Tinto environment will provide insight into formation of host environments from which such basic biology might originate, habitate or evolve [208]. The creation of the Río Tinto environment involved key primary and secondary

geological processes such as sedimentation, magmatism, aqueous interaction, mineralization, fluvial alteration, deposition and diagenesis [218, 219]. Quantitative modeling of these key geological processes will provide insight into the conditions that created this unique laboratory, and the ability to predict where other similar environments and laboratories might exist on Earth and on Mars. Recognition of these environments on Mars will inform future exploration objectives and strategies for astrobiological and sample return goals.

Recognition of these environments on Mars involves two fundamental steps: 1) Application of the geological models of the Río Tinto environment to Mars to develop a set of candidate exploration sites, and 2) Development of recognition criteria for key minerals associated with the Río Tinto environment and application to upcoming orbital mineralogical detection experiments. Thus, our proposed work involves: 1) Close biological and geological collaboration to explore the linkages and synergism between the geological conditions creating the deposits and the biological conditions and environments leading to this unique biota. This represents an important step in the dialogue between workers in the biological and planetary geosciences areas. This dialogue will certainly lead to better definition of the interactive role of geological processes and cycles, biological evolution, and the application of this to other planetary environments such as Mars and Europa. 2) Documentation of the geological environments in which this type of biological activity occurs and application to Mars. 3) Identification of remote sensing signatures for key minerals associated with this unique environment. 4) Recommendations to the Mars Exploration Program for identification of potentially similar sites on Mars, and development of strategies for surface exploration and sample return.

The Río Tinto ore body is a supergiant massive sulfide deposit associated with an ancient sill-sediment complex [218]. The magmatic intrusion of sills into wet sediments produced peperitic deposits and hydrothermal fluid/wet sediment interactions, yielding ore-forming metal emplacement (**Figure 6**). The Guaymas Basin appears to represent a somewhat comparable sill-sediment complex [220]. Similar geological environments of shallow intrusion and wet-sediment interaction are thought to be potentially common on Mars [221] (**Figure 6**). Although conceptual models exist for the emplacement events in the Río Tinto [218] and Guaymas Basin [222], detailed quantitative modeling has not been undertaken. In order to provide a better understanding of the emplacement processes at Río Tinto, and to provide a basis for predicting where similar deposits might occur on Mars, we will undertake a quantitative treatment of the interaction of magma and wet sediment, and the resulting alteration, mobilization, and ore emplacement, using as a basis the framework provided in [218] (**Figure 6**). Some treatments of aspects of the processes have been made [223-226] and we will utilize these where appropriate. We will employ techniques similar to those we have used in the study of magma ice/water interactions on the Earth and Mars [221, 227-229]. Using the criteria developed from this analysis, we will then apply them to the Mars environment to document examples of candidate geological environments that might exist there. This will involve treatment of the interaction of magmatic sills with the martian cryosphere (see [221], Figure 12) and with candidate martian oceanic sediments [129].

Specifically, using the geometry of the emplacement processes illustrated in **Figure 6**, we will develop a computer program to model the upward propagation of a dike from a chosen depth in the crust. We will then implement the coupled solution of the equations of motion for the magma in the dike and the stress distribution across the dike wall which determines the dike shape, as described for an elastic host medium [230] and for a visco-elastic host medium [231]. However, instead of solving the equations semi-analytically, which requires the simplifying assumptions that previous workers have been forced to make about the variations of magma density and crustal stress with depth, we will use a completely numerical solution that allows us to choose any magma density and any distribution of crust density and stress. This permits us to

investigate what permutation of source depth, magma density, and mantle-crust density-stress profile lead to dikes that propagate all the way to the surface or stall at some depth in sill-like or

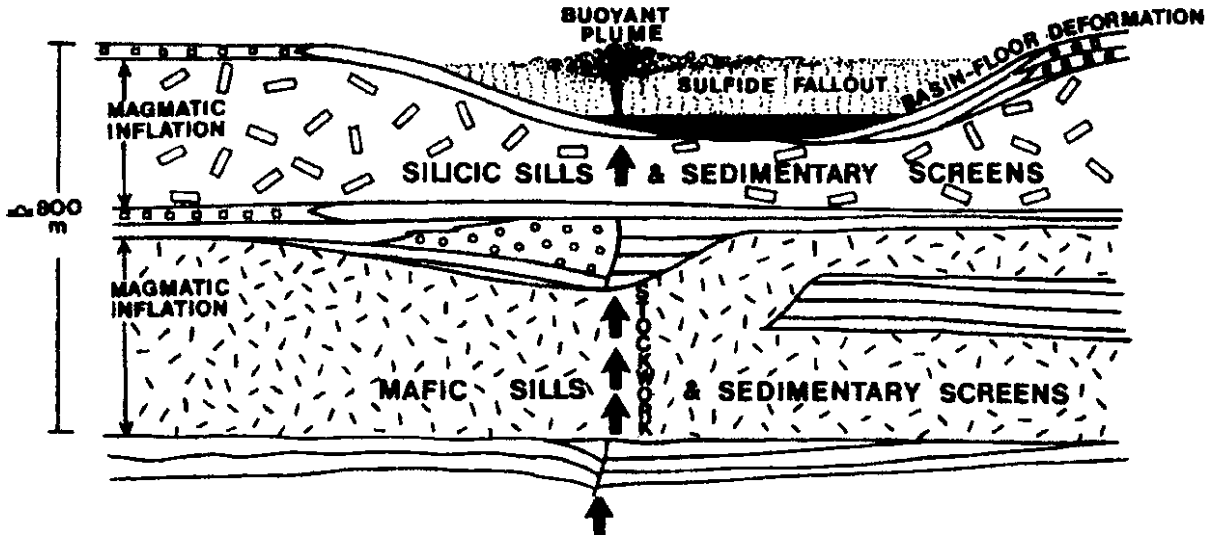

Figure 6. Schematic cross-section showing the Río Tinto deposit stratigraphic relationships and modes of emplacement in the sill-sediment complex. In this version, a major magmatic pulse creates the majority of the sill-sediment complex and basin-floor deformation traps exhalations that are driven by feeder dikes that are focused by the sill complex cap. Modified from [218].

dike-like intrusions. This approach will be particularly important for taking into account the different densities of the magma and the host rock (**Figure 6**). For dike and sill intrusions, we will also determine the final geometry using a numerical model that we recently developed to model dike and sill intrusion geometry on Mars [232]. The modeling will constrain dike and sill geometry and thickness, permiting us to assess the estimates of magmatic inflation predicted from the Río Tinto deposits (e.g., see **Figure 6**). These parameters in turn will allow us to evaluate in detail the near-surface stresses using a numerical stress simulation package (such as TEKTON) and predict in much more detail than previously possible whether features such as faults are expected to form. An additional analysis that we will perform for sill-like intrusions involves our current work on gas production during gas tip propagation. Furthermore, we will use conduction equations from this final configuration to model the mobilization of water-rich sediment to produce peperites, and to expel material to produce the buoyant plumes that are thought to produce the sulfide fallout (e.g., see **Figure 6**). For this latter aspect, we will utilize the quantitative treatment for seafloor submarine eruptions and ejecta dispersal that we have recently published [227]. In summary, the quantitative modeling of these key geological processes will provide insight into the conditions that created the unique Río Tinto deposits (**Figure 6**), and the ability to predict where other similar environments and laboratories might exist on both Earth and on Mars (**Figure 7**).

The deposits of interest in the Río Tinto region include those forming in relation to the Río Tinto itself. The Sinus Meridiani region of Mars has abundant gray hematite at the surface, possibly formed under aqueous conditions [233] and because of this is one of four sites proposed for the Mars Exploration Rovers. The headwaters of the Río Tinto have been proposed as an analog to Sinus Meridiani [219]. Groundwater mobilization of metals from the iron-rich sulfide ore bodies has created a metal-rich fluvial system. This has resulted in the highly acidic sulfate and iron-rich waters of the Río Tinto, and in the unique and flourishing biota [208]. Formation

of iron-bearing deposits in conjunction with the biota occurs presently and more ancient examples are preserved in Río Tinto river terraces. Furthermore, diagenetic alteration has locally modified the character of these deposits [219]. Finally, in the Mars environment, further alteration may occur. For example, on Earth, iron-rich oxidized caps above subsurface sulfide-bearing ore deposits are known as gossans. Gossans represent advanced states of chemical weathering in which sulfide minerals and related rocks are oxidized and leached leaving behind poorly crystalline hydrated iron oxides and sulfates [234].

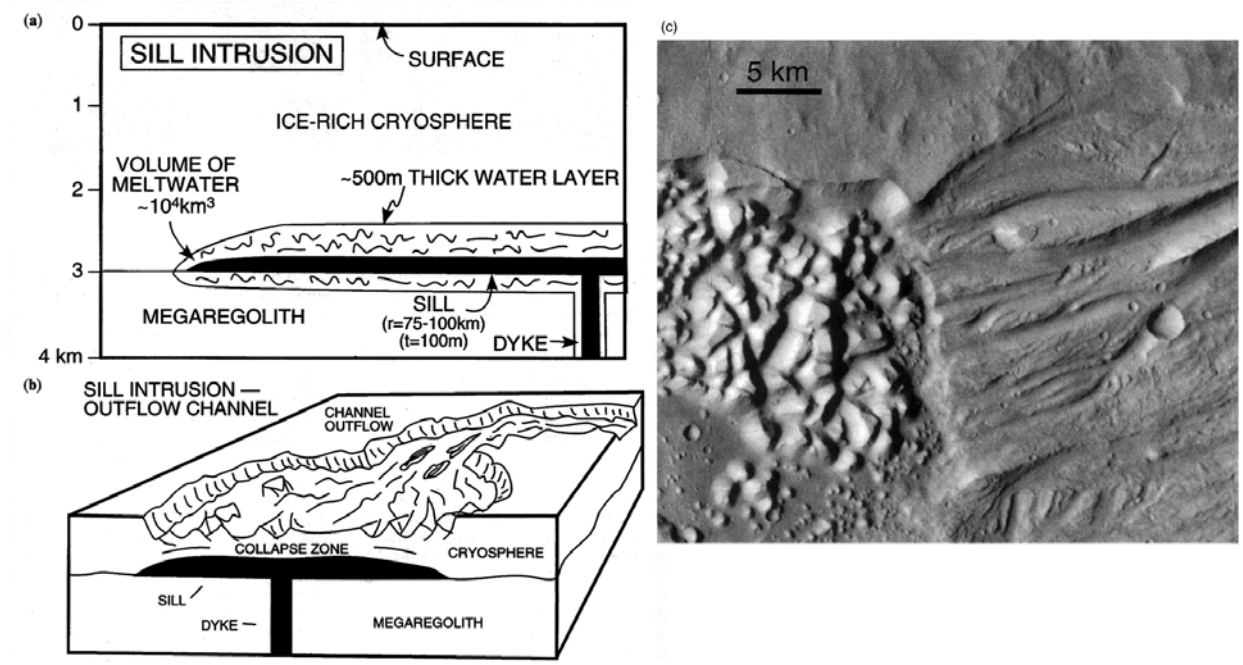

Figure 7. Interaction of a sill intrusion to produce melting, chaos formation and outflow channels on Mars. In (a) a sill intrudes into the megaregolith at the base of the ice-rich cryosphere, and conduction and melting occur, producing a significant volume of meltwater in only a few years. (b) Sill intrusion can plausibly cause surface uplift and fracturing, cryosphere cracking, significant melting, collapse, chaos formation and outflow channels. (c) Region of chaos at the head of any outflow channel on Mars that might result from sill intrusions such as those outlined in (a) and (b). From [221].

Through this investigation we propose to identify the key mineral signatures that are typical of these types of deposits and that might be recognized in future orbital and surface exploration in the Mars Exploration Program. The sulfur and Fe-mineralogy of the Río Tinto region is incredibly diverse [235] and there are multiple deposits related to the evolution of this system. Microbial activity in these environments is strongly coupled to the oxidation and reduction of iron (Fe) as well as sulfur and thus there is a suspected association between the Fe-sulfur mineralogy and biological activity [219]. Ferric and ferrous iron-bearing minerals exhibit a multitude of visible to infrared crystal field absorption features [236] which are diagnostic of mineral composition in natural environments [237]. Ferric oxides, oxyhydroxides, and oxyhydroxysulfates are particularly well characterized by their reflectance spectra (**Figure 8** Ferric oxide spectra). Our goal is to characterize the remotely-sensed signatures of iron-mineralogy in this biologically active environment to prepare a foundation for the interpretation of future spacecraft data.

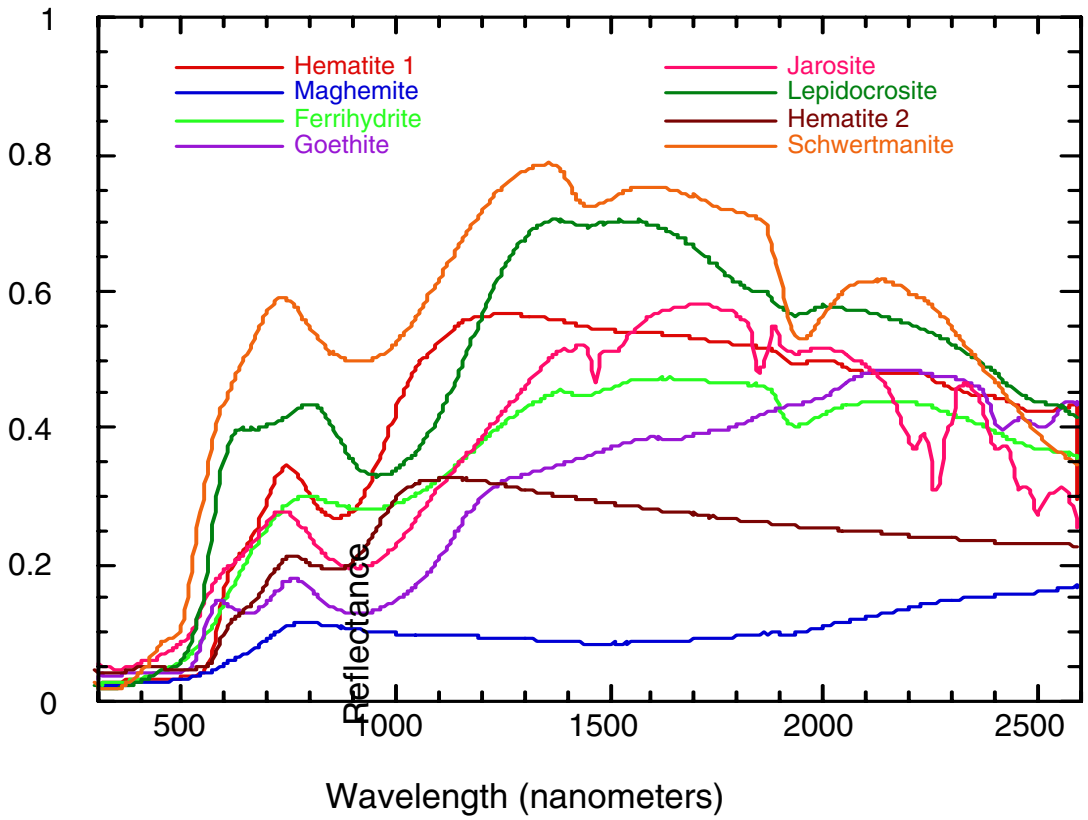

Figure 8. Ferric Spectra: Reflectance spectra of ferric oxides, oxyhydroxides, and oxyhydroxysulfates with a spectral resolution comparable to experiments on forthcoming Mars mission (e.g. OMEGA and CRISM). The position, shape, and strength of ferric and hydroxyl absorptions clearly distinguish these minerals from one another.

Upcoming Mars missions will have a number of sensors that will measure the reflectance of the martian surface in this wavelength region where Fe shows diagnostic mineral absorptions. The ESA Mars Express will have OMEGA, a visible-infrared spectrometer which will acquire global data with a 1 km spatial resolution in hundreds of spectral bands, and HRSC, a high resolution stereo camera (10 m/pixel) with multispectral coverage in the visible. The Mars Reconnaissance Orbiter (MRO) will have the CRISM instrument which will acquire data with spatial resolutions as high as 20 m/pixel and in 500 spectral bands from 0.4-4.1 µm (Mustard is a collaborator on the OMEGA experiment and a co-investigator on the CRISM experiment, Head a co-investigator on HRSC). The deposits in and around the active water courses of the Río Tinto and associated drainage basin span a wide range of iron mineralogy and appear to have distinctive properties with age [219]. Within the river system, seasonal drying and wetting results in the precipitation of amorphous ferric-bearing phases and soluble Fe-sulfate minerals (e.g. hydronium jarosite), though no associations between microbial communities and iron deposition have been established [216]. Increasing pH results in the formation of the mineral goethite and hydronium jarosite is replaced by goethite in older river deposits observed in terraces above the current river bed [219]. In still older deposits, hematite is observed to occur with goethite.

The focus for field sampling at Río Tinto is to address three key questions: What is the origin of eukaryote diversity, how are eukaryote and prokaryote communities linked, and what

relationships exist between the observed community diversity, the physico-chemical parameters and properties of a given site? We will extend the biological physico-chemical characterization of these sites with spectroscopic measurements relevant to the future exploration of Mars. This will be accomplished with both *in situ* and laboratory measurements. The *in situ* measurements will be made with an Analytical Spectral Devices (ASD) FieldSpec FR with a spectral range of 0.35-2.6 µm and a 1 nm sampling (though with coarser spectral resolution). For the laboratory spectroscopic measurements we will use NASA's RELAB facility housed at Brown University. This facility includes a bidirectional spectrometer with similar capabilities to the FieldSpec FR, but with higher precision and accuracy. There is also a Nicolet Nexus 870 Fourier Transform Infrared Spectrometer (FTIR), which can extend the spectral coverage to 200 µm. Therefore with a combination of the two laboratory instruments, the entire wavelength region critical to Mars past and future exploration with remotely sensed data can be covered.

There are four different environments that we will investigate in this context: 1) Primary mineralogy (minerals associated with the Río Tinto ore body itself), 2) secondary mineralogy (minerals associated with the groundwater migration and fluvial emplacement), 3) diagenetic alteration of fluvial deposits, and 4) gossan formation (minerals associated with intense chemical weathering). Thus the investigation tracks the process from unaltered mineralogy through the active biological zone to the older deposits with diagenetic alteration. Our initial focus will be the sites that will be analyzed in detail for microbial diversity. These sites will have associated physico-chemical measurements and we will analyze the sties with *in situ* spectroscopic measurements of reflectance from the visible to 2.6 µm. This will also include older deposits along terraces surrounding the current river bed. We will focus on terrace deposits that show evidence of the same basic depositional environment as the current active river bed. These environments will be sampled to acquire material to bring back to the laboratory for more detailed characterization. The laboratory analyses will include the collection of full spectral range and resolution measurements with the RELAB instruments as well as XRF and XRD measurements for mineralogic and elemental determinations.

This spectroscopic database will be analyzed to determine the nature of the iron mineralogy associated with the various active microbial communities, and how the iron mineralogy evolves with time. While we have measured all the expected minerals as pure specimens previously (e.g. **Figure 8**), the important part of this investigation is to sample the minerals *in situ* and in association with other components of the environment. This is necessary to determine unique associations of minerals that result in remotely sensed signatures diagnostic of the environments of interest. We will integrate these results into the geologic framework to devise a set of recognition criteria for identifying possible similar environments on Mars.

In summary, recognition of these environments on Mars involves two fundamental steps: a) Application of the geological models of the Río Tinto environment to Mars to develop a set of candidate exploration sites, and b) Development of recognition criteria for key minerals associated with the Río Tinto environment and application to upcoming orbital mineralogical detection experiments. The work described in this part of the proposal will complement other ongoing work in the NAI, specifically efforts by the CAB and at Harvard/MIT. We propose to collaborate with these teams to continue the development of Rio Tinto as a guiding terrestrial analog for the exploration of Mars hematite deposits by examining the spectral characteristics of the Río Tinto deposits and their similarities to the formation of the Meridiani hematite deposit on Mars. In our approach, we are developing quantitative models for the broad geological processes involved in the initial emplacement and subsequent evolution of the Río Tinto deposits. A common goal for several NAI teams with leadership provided by the CAB is to examine the Río Tinto deposits at the more detailed textural and mineralogical level and to make predictions that will be useful in the surface exploration of Mars, particularly those analyses performed by the upcoming Mars Exploration Rover Missions. Our effort is very complementary in that we will

be attempting to examine signatures and global geological contexts that will be useful in orbital missions that might examine the global inventory of sites and be useful in plotting out future orbital and surface exploration.

Within this NAI proposal, success in these endeavors will require synergistic collaboration between biologists and geologists to explore the linkages between geological conditions creating the deposits and the biological conditions and environments leading to this unique biota. By initiating a dialogue between the biological and planetary geosciences communities at Brown and MBL, we will gain an enhanced definition of the interactive role of geological processes and cycles, and biological evolution, and the application of this to other planetary environments such as Mars and Europa.

In addition to the benefits that will derive from the type of interdisciplinary interaction described above, this research will provide the following products: 1) Documentation of the geological environments in which this type of biological activity occurs and application to Mars; 2) Identification of remote sensing signatures for key minerals associated with this unique environment, and 3) Recommendations to the Mars Exploration Program for identification of potentially similar sites on Mars, and development of strategies for surface exploration and sample return.

5.3 BIOSIGNATURES AND LIFE DETECTION: DETECTING ANCESTRAL PEPTIDES AND PROTEINS

(M. Riley, N. Wainwright, R. Gast)

The search for signs of life in the Solar System and beyond is a high priority for Astrobiology. While remote sensing of biological signatures has the potential to identify promising sites for future missions, biomarkers provide a basis for the design of life detection experiments. We will combine our expertise in genome analysis and biochemistry to develop sensitive and rapid assay methods to quantify nucleic acid and amino acid sequences that are common to all forms of life including the most primitive organisms. The method will be applicable to both samples returned from future mission as well as miniaturization to be included as flight hardware for *in situ* analysis.

The earliest proteins on Earth would have arisen before cellular life as we know it. Many of the early proteins and fragments of proteins must have been ancestors of the complex protein world that exists today. Generating enough proteins to make a metabolically capable and self-replicating cell must have involved processes similar to gene duplication and divergence. Without assumptions about the process of duplication and divergence at those early times, we can nevertheless ask the question "How many and what kinds of ancestral proteins would be required to catalyze all the basic life processes found in all free-living organisms today?" We can also ask, "Is there a way we could detect features of terrestrial-like life that might exist on other planets?"

In the context of preparing to search for signs of life on other planets, we propose to develop a simple system for detecting either nucleotide or amino acid sequences of commonly occurring elements in proteins found throughout the living world on Earth. The elements we have chosen for detection appear repeatedly in Earth proteins. If we can detect these elements in diverse environments on Earth and if they are also used frequently in living systems elsewhere, then we may be able to detect signs of life on other planets. While we will propose specific sequences to test our methods, the strategies themselves will be flexible enough to accommodate any sequence.

We take as point of departure for the rationale, the characteristics of the proteins of the biochemically best-studied single-cell prokaryote, *Escherichia coli*. There are about 40 frequently occurring domains within *E. coli* proteins that have been characterized both structurally and functionally. Frequently occurring motifs in *E. coli* that might be examined are

listed in **Table 2.** Of these motifs, we have selected the two most frequent. The most common domain is the P-loop- containing nucleotide triphosphate hydrolase (175 instances), which occurs in many proteins that affect hydrolysis of ATP, GTP and other nucleotide triphosphates. The P-loop NTP hydrolase is the most commonly occurring ATPase type in living systems. Since this action is associated both with energy-requiring reactions and with a class of regulatory proteins, the motif is widely used for many separate reactions. The second domain we have chosen, the NAD(P)-binding Rossman fold (73 instances), appears in oxidative proteins. Oxidation is a frequently occurring type of energy-producing reaction, and NAD(P) is the cofactor most often used to capture the electrons to pass them along the electron transport chain to generate energy. The Rossman fold is the most common of several types of NAD(P)-binding sites. These functions are so universal and well-conserved that one would expect them to have evolved at ancestral times in protein development.

Table 2.

Number of Proteins	Domain description	Typical size (aas)
175	P-loop nucleotide triphosphate hydrolase	200
73	NAD(P)-binding Rossman fold	150
39	Winged helix, in regulatory proteins	100
37	Periplasmic binding, transport, type II	200
32	Actin-like ATPase domain ñ contraction	200
30	FAD/NAD(P)-binding domain	400
29	S-adenosyl-L-methionine-dependent methyl group transfer	150
28	Porins (transport)	320
26	Nucleic acid-binding proteins	60
22	Thioredoxin	100
22	Homeodomain-like ATP-binding site	50
22	His kinase of 2-component regulatory system	70
21	α/β Hydrolases	250
21	Periplasmic binding, transport, type I	270
21	ATPase domain in chaperones/DNA topoisomerase	120
21	Trans-glycosidases	200
20	Membrane proteins, all-	250

Since these two motifs appear throughout the entire sweep of living organisms on Earth, from the simplest to the most complex, they seem logical to use as probes to find if there are proteins on other planets that use these motifs. Implementing a search for fragments with either of these specificities on an unmanned mission will require a mechanical way to assay for the chosen targets and the appropriate methods to detect the targets.

Proposed method / Instrument development. Whether one is concerned with collection of data to support life or spacecraft microbial contamination detection, major desirable criteria include accurate and sensitive instrumentation, and the ability to report data near the point of collection and in near real-time. With those criteria as goals, a portable instrument was designed to have the capability of performing kinetic analysis of enzyme-related reaction development. This has been initially customized to the *Limulus* Amebocyte Lysate (LAL) assay for microbial detection; however, it is flexible enough to be adapted to a wide variety of assays that can be analyzed spectrophotometrically, including antibody and nucleic acid assays. The instrument is designed to operate independently, but to have the capability of integration via a serial data interface. The system is comprised of a heating block to control reaction temperature, a pump to

facilitate fluid movement, LED and sensor pairs to quantify optical changes during the test and on-board microprocessor and memory to control all aspects of the instrument during the test and store data results. Power requirements are 10.5 volts DC, with maximum consumption at start-up of 25 watts.

LAL is a sensitive enzyme cascade that is triggered by microbial cell wall material [238, 239]. The test has been miniaturized and run in a simple, linear micro fluidic chamber that sequentially 1) hydrates a dry, stabilized reagent, 2) incubates the sample and 3) reads color development spectrophotometrically. Data from a preliminary run is shown below in Figure 9.

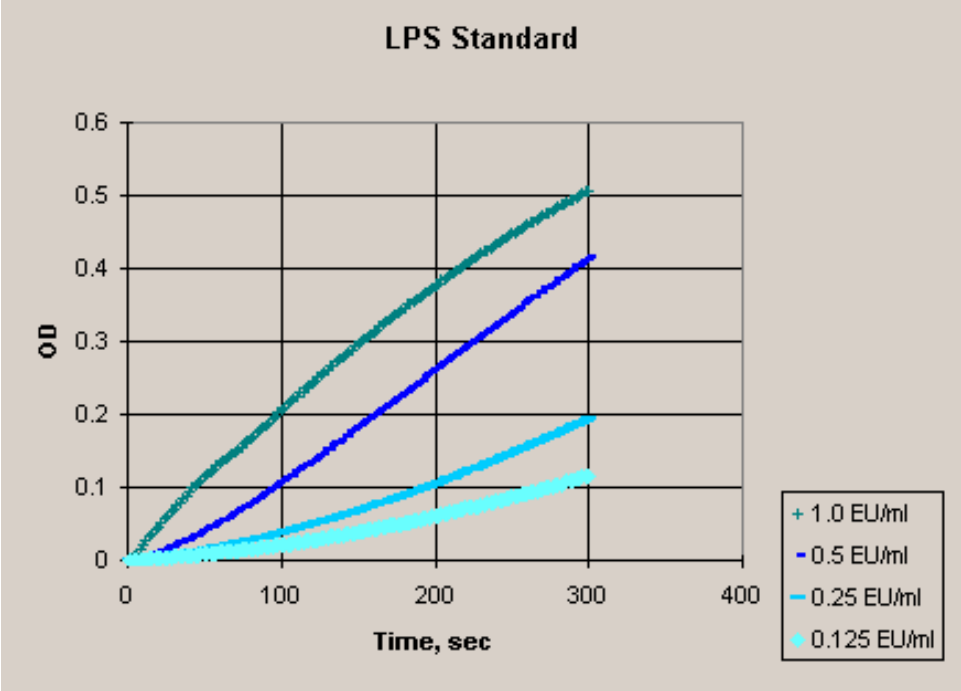

Figure 9. Each line represents the kinetic chromogenic development of one channel of a microfluidic chamber. Four channels are shown at 1.0, 0.5, 0.25 and 0.125 EU/ml (endotoxin units/ml). Data may be stored or interpreted by onboard software.

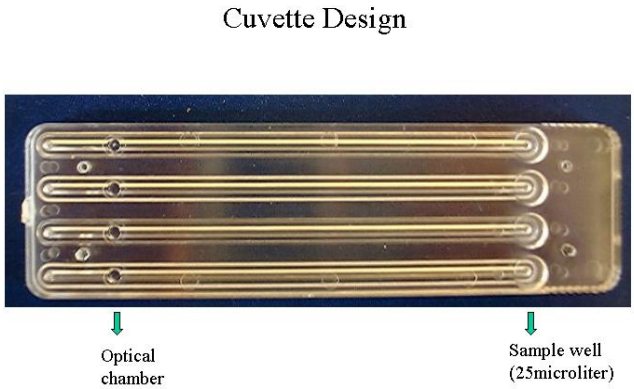

Figure 10. Microfluidic cuvette showing four parallel channels in which four separate tests can be performed. Sample is introduced on the right and is sequentially drawn over defined channel areas. Dried reagent become re-hydrated and chromogenic enzyme reactions are quantified by kinetic readings at the optical chamber.

In this study, we will develop methods specifically to:

1. Use the existing prototype instrument design to collect kinetic spectrophotometric data from competitive labeled antibody assays and competitive hybridization assays.
2. Develop the detection format initially based on fluorogenic and chromogenic antibody and nucleic acid probe label.
3. Increase method sensitivity by coupling the LAL enzyme cascade to LPS-labeled antibodies (or antigens) and nucleic acids.
4. Field test the methods with samples extracted from the environment.

Nucleic acid detection. The DNA detection method that we propose to develop is based upon the release of a labeled fragment in response to competitive hybridization with sample DNA. Research with competitive and strand displacement hybridization has displayed the use and effectiveness of these probe-target reaction systems to enhance the recovery of correctly matched hybrids [240-242]. We intend to modify this method to permit the detection of conserved nucleic acid sequences that correspond to the NAD(P)H -binding site of P-loop nucleoside triphosphate hydrolase and Rossman fold.

The method utilizes a double stranded probe DNA molecule in which the strand to be dissociated and detected is 20-25% shorter than the full-length hybrid [243], 2002). This design makes the incomplete hybrid less stable than the full hybrid, but more stable than full hybrids with clear mismatches. For our initial studies, we will label the strand to be dissociated with a fluorescent molecule. The complementary strand will be labeled with biotin to facilitate its removal from the hybridized solution after reannealing has taken place [244]. The labeled probe duplex will be mixed with double stranded tester DNA and denatured in a solution of buffer. Disassociated sequences are allowed to reanneal, the released fluorescent strand (and any hybrids) will be collected in the solution, and the solution assayed for the presence of fluorescent signal. Of the possible reannealed results in the solution phase, we will only be detecting the fluorescently labeled strands that have been released from the probe duplex by competitive rehybridization with correct matches from the tester DNA (**Figure 11**). The amount of label released can be directly proportional to the number of sequences present in the sample being tested [240].

Our probes must be synthesized to have heterogeneity at bases representing the third codon position due to the redundant nature of the genetic code. We recognize that this degeneracy can have a significant effect on the hybridization behavior of the probes. To counteract these effects, modified nucleotide bases will be incorporated to help reduce the potential melting temperature differences. P nucleotide is 6-(beta-D-ribofuranosyl)-3,4-dihydro-8H-pyrimido[4,5-c][1,2]oxiazin-7-one and it pairs with purines [245], and K nucleotide is 2-amino-9-(2-deoxy-beta-ribofuranosyl)-6-methoxyaminopurine, and it pairs with pyrimidines [246]. Both of these nucleotides can be substituted into polynucleotide sequences instead of degenerate bases, reducing the overall amount of heterogeneity by a factor of two. Most importantly, their pairing is significantly more stable than either mismatches or inosine base pairs [245-247], although it does not result in melting temperatures equivalent to the exactly matching hybrids. Primers using these nucleotide substitutions have been very successfully employed in the analysis of highly divergent sequences by denaturing gradient gel based studies[248, 249]. Such analyses are very sensitive to the change in melting temperature of duplexes introduced by even a single base change in the sequence, so the successful use of these modified nucleotides is very promising for helping to stabilize our degenerate hybrids.

```
N - N - N - N - N - N - N - N - N - N - N - N
|   |   |   |   |   |   |   |   |   |   |   |        Tester DNA
N - N - N - N - N - N - N - N - N - N - N - N

B - P - P - P - P - P - P - P - P - P - P - P - P
            |   |   |   |   |   |   |   |   |        Probe DNA
            P - P - P - P - P - P - P - P - P - P - Fl

                Mix, denature and anneal
```

Predicted hybridization products in solution phase

```
N - N - N - N - N - N - N - N - N - N - N - N
          |   |   |   |   |   |   |   |   |
          P - P - P - P - P - P - P - P - P - Fl

          P - P - P - P - P - P - P - P - P - Fl

N - N - N - N - N - N - N - N - N - N - N - N

N - N - N - N - N - N - N - N - N - N - N - N
|   |   |   |   |   |   |   |   |   |   |   |
N - N - N - N - N - N - N - N - N - N - N - N
```

Predicted hybridization products that would be removed from the solution phase

```
B - P - P - P - P -  P - P  - P - P -  P - P - P - P
  |   |   |   |   |   |   |   |   |   |   |   |
  N - N - N - N - N - N - N - N - N - N - N - N

B - P - P - P - P - P - P - P - P - P - P - P - P
            |   |   |   |   |   |   |   |   |
            P - P - P - P - P - P - P - P - P - Fl
```

Figure 11. Proposed hybridization scheme and potential products. N = tester DNA nucleotide, P = probe DNA nucleotide, Fl = fluorescent tag, B = biotin tag

We will work with a suite of three probes, each about 50 bases long, representing the entire region of interest. Although competitive hybridization assays have previously been accomplished with probes as large as 131 bp [242], we believe that the use of fragments will increase our likelihood of detecting sequences by reducing the overall degeneracy of each probe resulting in more stable hybrids. Furthermore, the detection of hybridization by any of the probes in the mixture will result in a signal, thereby potentially increasing our ability to detect even highly divergent domains or smaller fragments. Our tester DNA will initially consist of cloned sequences from various organisms. As we move into more complex mixtures and environmental samples, we will explore shearing the tester DNA into uniform pieces of less than 1000 bp (or smaller if possible). This will help to make the hybridization process more homogeneous and manageable. The probe hybridization behavior will be tested at different temperatures, with different concentrations of probe and tester DNAs, and over a range of times to determine the best set of conditions for full hybrid association. The release of the displaced strand will be monitored by accumulation of fluorescence in the solution phase and quantified relative to experimental concentration standards that we create. Our hybridization experiments will also be conducted with the potential number of environmental targets in mind so that our tester DNA will be able to drive the reaction.

We desire to link our detection method with the LAL procedure because of the probable need to increase the sensitivity when working with environmental samples. Although we are targeting the sequences of broadly conserved protein regions, the signal is potentially going to be quite low. The competitive hybridization would be accomplished in solution, followed by

detection with LAL reagents being accomplished in the prototype chamber. The detectable release of labeled probe would suggest that the domain in question was present in the sample assayed. Future work would be directed towards establishing relative numbers of sequences present.

Experiments to determine hybridization activity:

1. Probes will be synthesized with fluorescent label on the 5' end of the strand to be detected and biotin on the 5' end of the strand to be removed from solution.
2. Solution hybridization reactions utilizing varying concentrations of probe and target sequences will be accomplished and monitored to determine optimal conditions for time, temperature and limits of detection.
3. Probes will be modified to have Lipopolysaccharide attached to the 5' end of the detection strand, and LAL detection cascade will be utilized to detect specific hybridization.
4. Detection limits for LAL cascade will be determined.
5. Probes will be tested on various environmental samples.

Peptide detection. Antibodies to selected peptides will be raised either as monoclonals overlapping the sequence of interest, or as affinity purified polyclonals. Antibody detection methods will be adapted to the unidirectional instrument cuvette design in the following manner:

1. Peptide antigens will be labeled by covalent attachment of fluorescein.
2. Mixtures of labeled and unlabeled peptide will be made; some with deliberately different sequences that might bind more competitively to divergent sequences.
3. Antibodies will be immobilized by adsorption to the plastic surface of microtiter wells or instrument cuvettes.
4. Sample peptide / labeled peptide mixtures will be exposed to the immobilized antibody.
5. Conditions of competitive binding will be optimized for antigen concentration, flow rates, antigen – antibody contact time.
6. Bound versus free label will be measured spectrophotometrically and compared to standard peptide concentrations.
7. Limits of detection will be assessed.
8. Detection limits will be increased by coupling peptides to Lipopolysaccharide (LPS) to link detection to the LAL amplification cascade.

Detection limits. This study addresses the need to develop increasingly sensitive methods for life detection. Our goals include a combination of the proven methods; antibody binding, nucleic acid hybridization, with the enzyme amplifying sensitivity of LAL, to create a new method. While the initial experiments to adapt antibody and nucleic acid tests to the existing unidirectional flow of the portable cuvette will use dye labels that do not press the limits of sensitivity, the second phase of the project will use LPS as a label to tie the detection signal to the LAL cascade, gaining the amplification of the enzyme cascade. We predict this linkage will rival the sensitivity of radioactive labels in the rapid, portable format.

Our combined assay format will address the need to analyze samples returned from other bodies in the solar system as well as to be compatible with potential flight hardware for *in situ* life detection. An instrument under development currently partially funded through ASTEP (MASSE, Andrew Steele, PI) is using the proposed instrument platform with LAL enzyme cascade reagents to quantify microbial cell wall material in a variety of minerals and fossilized samples. This effort will be an excellent opportunity to collaborate with NAI partners at the The Carnegie Institute and colleagues at the Johnson Space Center.

5.4 EVOLUTION OF GENOME ARCHITECTURE IN PROKARYOTES: Genome-Genome Integration: Symbiosis, genetic assimilation, and evolutionary innovation (J. Wernegreen)

In both prokaryotic and eukaryotic microorganisms, transformations in genome architecture far surpass point mutations as an important driver of evolutionary novelty. These changes include gene duplication and loss, horizontal gene transfer between distinct organisms, and the melding of two genomes through endosymbiosis. Over the past decade, new molecular capabilities have transformed evolutionary biology and enable us to trace the history of complexity at the whole-genome level. The studies below use comprehensive, genomic approaches to elucidate the forces that drive changes in genome architecture, and to link those genome changes with major evolutionary shifts. We will dissect the early and more recent genomic adaptations of endosymbiosis between bacteria and eukaryotes, resulting in close assimilation of two distinct genomes. This study will illuminate mechanisms by which genome changes contribute to major evolutionary transitions.

Descriptions of early life posit the progenote as a complex network of replicating entities engaged in fluid interactions. Close interactions and DNA exchange between genomes of early microbial forms yielded communities of ever increasing genetic and biochemical complexity. Genome-genome interactions remain an important evolutionary driver for microbial consortia. At a different scale but of equal importance, microbial symbionts that contributed to the formation of stable organelles have dictated the evolution of complex organisms. Endosymbiosis, in which one partner lives within the cells of another, represents the most intimate contact between organisms. Mitochondria and chloroplasts result from endosymbiotic events in which host cells acquired the ability to harness light energy and thrive in the presence of oxygen [250]. These innovations forever changed cellular structure, species diversity, and the range of acceptable habitats of Life.

Unlike single mutations, genome interactions can catalyze the acquisition of entirely new combinations of functions and drive major evolutionary transitions. Through studies of binary interactions between two species - i.e. a symbiont and its host- we can dissect the mechanisms of genome communication and coevolution. The Proteobacteria is especially important because many of its lineages have switched from free-living lifestyles to obligate genome interactions. For example, mitochondria evolved from an α-Proteobacterial endosymbiont. Other extant members of this diverse bacterial phylum live exclusively within eukaryotic host cells, but they represent incipient stages of cellular integration and organelle evolution. As such, they offer valuable insights into the mechanisms that drive genome interactions, including lateral gene transfer, gene loss, and modifications of gene expression. Through a comparative genomic approach, we will decipher the 'language' used to establish and maintain genome-genome interactions represented by stable and dynamic symbioses. Our ultimate objective is to understand how such interactions drive organismal integration and evolutionary novelty.

As model systems, we have selected two Proteobacterial endosymbionts that represent both stable and transient genome interactions. *Blochmannia* is a γ -Proteobacterial endosymbiont that has coevolved with ants for >40 MY [251-254]. Like mitochondria, *Blochmannia* benefits its host and experiences maternal transmission to host offspring. We study this endosymbiont as a model to understand coevolution in a stable genome interaction that may resemble early organelles. In contrast, the host switching of labile endosymbionts can drive lateral gene transfer and phenotypic plasticity. The endosymbiont *Wolbachia* is an excellent model to study evolutionary dynamics of unstable genome-genome interactions. Transfer of this intracellular bacterium among hosts explains its current infection of at least hundreds of thousands of invertebrate species and its status as the most abundant endosymbiotic bacteria on Earth [255]. The diverse lifestyles of *Wolbachia* span modification of host reproduction to enhance its own

spread (reproductive parasitism) to beneficial effects in its long-term association with nematodes (mutualism). The proposed *Wolbachia* projects extend upon the postdoctoral fellowship of Seth Bordenstein, an NAI/NRC Fellow in the Wernegreen lab.

Major research themes and experimental objectives: We aim to elucidate changes in gene content and expression patterns that catalyze the establishment and diversification of genome-genome interactions. The studies below examine invertebrate endosymbionts as model systems to address the following broader questions: How do genome interactions reshape genetic content and architecture? Do genetic differences drive phenotypic and ecological variation within a genome interaction? What specific biochemical functions mediate genome-genome interactions? Do changes in gene expression profiles catalyze the formation of genome interactions? How does plasticity in gene expression help to fine-tune coevolved interactions and drive diversity among them?

Remodeling of genetic architecture in genome-genome interactions. Genome interactions dramatically alter the genetic architecture and gene content of the organisms involved and partly explain the wide variation of genome sizes among taxa. Genome size changes are severe among bacterial endosymbionts, which include the smallest known prokaryotic genomes (as small as 450 kb, and typically < 1 Mb) [256, 257]. By comparing gene contents of endosymbionts and free-living relatives, we can better understand the process of genome reduction and the diverse strategies by which bacteria form intimate associations with eukaryotic cells. Genomes of the stable endosymbionts *Buchnera* (of aphids) [258, 259] and *Wigglesworthia* (of tsetse flies) [260] have shrunk by 80% but retain specific biosynthetic genes required by their hosts. Full genome sequences of two *Wolbachia* strains are now in progress [261, 262] and will allow us to interpret the observed variation in genome sizes of *Wolbachia* strains [263]. Despite the insights provided by current data, discerning general rules of genome remodeling will require a richer dataset across phylogenetically independent endosymbionts. In order to expand this comparative framework, we will explore patterns of gene loss and acquisition during the transition to both stable (*Blochmannia*) and transient (*Wolbachia*) genome interactions.

Genetic remodeling in stable genome interactions. *Blochmannia* is closely related to other endosymbionts and free-living bacterial species, yet acquired an intracellular lifestyle independently from *Buchnera* and many other endosymbionts. We are currently sequencing draft (5X) coverage of the small (810 kb; [264]) *Blochmannia* genome to explore unique opportunities for comparative genome analyses. We will compare the gene repertoires of *Blochmannia*, related insect symbionts (e.g. *Buchnera* and *Wigglesworthia*), and free-living bacteria such as *E. coli.* We will focus on the 'complementarity of gene repertoire' of host and symbiont metabolisms, as evidenced by pathways requiring gene products of both, and the retention of host-beneficial biosynthetic genes in tiny endosymbiont genomes. We will employ well-established approaches such as the program Clusters of Orthologous Groups of proteins (COG) [265] to identify shared orthologs among genomes. In addition, we will trace the process of genome reduction by determining size, genetic content, and timing of deletions events. This general approach has illustrated large deletion events in *Buchnera* that were probably deleterious [266]. By comparing multiple endosymbiont lineages including *Blochmannia,* we will distinguish whether genome integration typically occurs via large deletions that encompass many loci, or the gradual loss of individual genes that are dispensable for life within a host cell.

Endosymbionts of mealybugs represent the only documented example of a bacteria (i.e., γ•subdivision Proteobacteria) living within the cells of another bacterium (a larger β-subdivision Proteobacterial symbiont) [267]. This prokaryote-prokaryote binary association is a unique analog to the type of ancient genome interactions that led to the eukaryotic cell and equipped it with organelles. We will test for genome reduction as a consequence of this prokaryotic integration by sizing the genomes of both bacteria. A mixed bacterial sample will be prepared

from lab-reared insects through a homogenization and filtration technique [268] that we successfully applied to aphids, ants, *Drosophila*, and other insects [264, 269]. Bacterial samples will be embedded into agarose plugs, and DNA will be digested with I-*Ceu1*, a homing enzyme that cuts at a specific site within the bacterial 16S rRNA gene. Linear genomic fragments will be resolved by Pulsed Field Gel Electrophoresis (PFGE) using a Biorad CHEF Mapper XA in the Biology Department. Genomic fragments will be assigned to the γ• or•β•subdivision by amplifying the 16S rRNA gene from each fragment with group-specific PCR primers and determining the partial sequence of this gene [270]. Fragment sizes will be summed to calculate respective genome sizes. We will test for genome size of γ•subdivision symbiont compared to its close relative β•subdivision symbiont to its close relative *Burkholderia pseudomallei* (7.25 Mb) and by comparing the γ-subdivision with free-living bacterial relatives such as *E. coli* (genome size of 4.5-5.5 Mb; [271]).

Gene content and phenotypic plasticity of *Wolbachia*. *Wolbachia* exhibit the most extensive plasticity in genome size of any intracellular bacteria. The genome of the nematode *Wolbachia* (~1.0 Mb) is 30% smaller than that of arthropod *Wolbachia* (1.66 Mb) [263]. We aim to decode the evolutionary processes that shape this genome plasticity. We will first determine the direction of lifestyle transitions by resolving phylogenetic relationships among the six *Wolbachia* subgroups (A and B – arthropod parasites; C and D – nematode mutualists; E and F – arthropod, unknown lifestyle) and determining the root of this tree. To this end, we will sequence five genes from each of 30 lineages spanning representatives of the A-F *Wolbachia* subgroups. The root of the tree will be determined statistically by comparing the likelihood of phylogenies with alternative root positions.

Previous genome size differences among *Wolbachia* strains are based on just four isolates. We will size genomes of phylogenetically diverse *Wolbachia* using PFGE. DNA from *Wolbachia* will be isolated, digested, and analyzed by a purification technique that is previously published [263] and has been successfully used in our lab. Genome size changes will be mapped across the *Wolbachia* phylogeny to correlate gene loss or acquisition with switches in host taxa or interaction type. We predict that the beneficial and stable *Wolbachia* engaged in long-term associations with nematode hosts will have smaller genomes due to reduced opportunities for lateral transfer, small effective population sizes, and high mutation rates [272], while the parasitic and more transient *Wolbachia* will have larger genomes due to frequent recombination and lateral gene transfer [273]. We will then reconstruct the history of genome fluidity throughout the diversification of *Wolbachia* by mapping gene loss and acquisition events onto the *Wolbachia* phylogeny. Results will advance our understanding of the importance of genome fluidity in this unstable endosymbiosis and the timing of genome-level changes.

Identifying 'key symbiotic genes' that catalyze genome-genome interactions. Functions that mediate genome-genome interactions reflect the evolutionary duration and context of a particular association [274]. Insect endosymbionts rely on distinct evolutionary strategies that span the supply of host-beneficial compounds to horizontal infection and reproductive parasitism. Genomes engaged in such distinct strategies are likely to vary in the types of 'key symbiotic genes' they encode, defined here as functions that catalyze the establishment and/or maintenance of a host-symbiont interaction. We will identify candidates for key symbiotic genes in *Blochmannia* and *Wolbachia* based on the retention of loci in these small endosymbiont genomes, their distribution across strains, and inferred functions. As a second step, we will confirm the expression of these loci by analyzing bacterial mRNA isolated directly from natural host populations.

Key genes shaping stable genome interactions. The hosts of beneficial, stable endosymbionts may provide proteins or cell structures to facilitate symbiont transmission to new cells. In this case, functions of key symbiotic genes may not involve cell invasion, but might

benefit the host and thus provide an evolutionary 'incentive' for stable symbiont transmission [258-260]. We will identify genes that are specifically retained in the small *Blochmannia* genome and may function to maintain its long-term interaction with ants. We will target genes related to proposed functions of *Blochmannia*, such as nutrient biosynthesis, pathogen defense, or pheromone production. Our preliminary data for the *Blochmannia* genome (460 kb of this endosymbiont sequence) indicates a high frequency and diversity of amino acid biosynthetic genes and suggest this symbiont plays a nutritional role. Draft coverage of the *Blochmannia* genome will reveal many putative symbiotic genes, of which we will identify ~20 as top candidates. We will perform real-time RT-qPCR (reverse transcriptase quantitative Polymerase Chain Reaction) to quantify expression patterns of those 20 candidate symbiotic genes, relative to expression of a *Blochmannia* housekeeping gene as an internal standard to control for variation in the number of endosymbionts infecting a host. RT-qPCR allows extremely sensitive detection of relative mRNA levels in a mixed sample.

Even in stable associations, variation at key symbiotic genes may drive ecological diversification within a given host group. We will explore genome variation among *Blochmannia* strains as it contributes to the wide ecological diversity of its host genus, the ant *Camponotus*, a dominant player in many ecosystems [275]. We will use Suppressive Subtractive Hybridization (SSH) to compare gene contents of *Blochmannia* associated with three distinct ant species. SSH is a cost-effective alternative to full genome sequencing when one aims to identify genes that are present in one genome (the 'tester' DNA), but absent from another (the 'driver' DNA)[276]. Briefly, tester and driver genomes are fragmented and hybridized, the hybrid molecules are removed, and the remaining (unhybridized) fragments represent tester-specific DNA. Since relatively low quantities of bacterial DNA can be isolated directly from hosts, we will use a modified SSH in which test-specific DNA is amplified by PCR (Clontech PCR-Select Bacterial Genome Subtraction Kit). We will generate four libraries from two reciprocal crosses (*Blochmannia* of *C. pennsylvanicus*, versus each of *C. festinatus* and *C. castaneus*), and will sequence these libraries to identify genes that are unique to a given *Blochmannia* strain. We predict that *Blochmannia* genomes will differ primarily at key symbiotic genes that allow their respective hosts to exploit distinct niches.

Key symbiotic genes as drivers of *Wolbachia* diversity. Recent data reveal unusual levels of genome fluidity in *Wolbachia*, including large differences in genome sizes [263], presence of mobile genetic elements [277], lateral gene transfer, recombination, and symbiont transfer to new hosts [273]. We will compare gene contents using DNA microarrays, a technique that can circumvent the laborious and expensive sequencing projects of multiple related genomes [278]. We will investigate variability of all *Wolbachia* ORFs (including housekeeping genes and mobile elements) to quantify the extent of gene loss and acquisition at an extensive scale. One particular focus will be the identification of key symbiotic genes that mediate distinct *Wolbachia*-host interactions. Arrays will be constructed from PCR-amplified products of all coding genes in the 1.3 Mb genome of *Wolbachia* infecting *Drosophila melanogaster*, either within the Bay Paul Center NASA-funded microarray facility or through an active collaboration (J. Werren, Univ. Rochester, pers. comm.). Genomic DNA of 20-30 *Wolbachia* strains will be hybridized to arrays at the MBL.

Analyses of arrays will address several components of genome flux in *Wolbachia*. We will identify genes that are shared across the 20-30 *Wolbachia* lineages hybridized against the array, but absent in larger α-Proteobacterial genomes that are not engaged in obligate endosymbiosis (i.e., the 5.7 Mb genome of *Agrobacterium tumefaciens*, the 9.1 Mb genome of *Bradyrhizobium japonicum*), as candidates for functions required in the establishment of this genome-genome interaction, and as targets of further gene expression analysis. In addition, we will explore gene loss and acquisition throughout the evolutionary history of this endosymbiosis, by (i) comparing genome fluidity in harmful and beneficial *Wolbachia* strains (ii) determining

the evolutionary history of specific gene loss or acquisition events across the *Wolbachia* phylogeny (iii) deciphering the size of gene loss and acquisition events (i.e., 1kb or 100kb changes in genome size) and (iv) identifying genes that correlate with interaction type (reproductive parasitism versus mutualism) transmission mode (strict vertical transmission versus occasional horizontal transmission), and distinct host taxa. This will be the first study to compare gene contents across closely related endosymbiont strains engaged in diverse interaction types.

Genome stasis versus lateral transfer in genome-genome interactions. Darwinian evolutionary processes of mutation and selection have shaped all of biology; however, such processes may operate quite differently in microbes. For example, transposons, plasmids, bacteriophages and other GTAs (Gene Transfer Agents) drive frequent lateral gene transfer in many prokaryotes. Such transfer can move genes that are already refined by natural selection in the donor genome and thus allow rapid exploitation of new niches in the recipient [279]. Persistent gene transfer between intracellular prokaryotes or organelles and the eukaryotic nucleus plays a vital role in genome integration [280-282].

Genome stasis versus fluidity in stable associations. In contrast to frequent gene transfer in free-living bacteria and unstable symbionts, genomes of certain stable endosymbionts lack GTAs and show no evidence of lateral gene transfer. Without gene transfer, their genome reduction is irreversible and may impose severe constraints on evolutionary potential. For example, two *Buchnera* strains have undergone no gene rearrangements or acquisitions during the 50–70 MY since their divergence, in contrast to a 2,000-fold greater genome lability in the related enterobacteria [259]. This genome stasis may reflect a lack of molecular tools for transfer (e.g., GTAs, recombination functions, and repeated DNA sequences) and limited opportunities to recombine with genetically distinct bacteria. However, certain features of the *Blochmannia* genome suggest this stable endosymbiont may be prone to rare lateral transfer. The *Blochmannia* chromosome is about 25% larger than related endosymbionts (*Buchnera* or *Wigglesworthia*) and might contain repeated elements, phage, or recombination functions. *Blochmannia*-filled host cells line the midgut where other bacterial species may occur. Other intracellular endosymbionts, including *Wolbachia,* may coexist with *Blochmannia* in the ant ovary.

We will characterize genome fluidity in *Blochmannia* to ascertain if even rare gene transfer influences this stable symbiosis. Based on our full genome analyzes we will assess the potential for gene exchange by comparing recombination functions (*rec* genes) and mobile elements in *Blochmannia* and other small bacterial genomes. Candidates for acquired genes would include those unique to *Blochmannia* or those with unusual codon usage or base compositions [283]. We will also use a phylogenetic approach to test for transfer of key symbiotic genes among *Blochmannia* strains. Congruent phylogenies of *Blochmannia* housekeeping genes and host genes indicate that most bacterial loci are evolutionary stable and track host diversification [284]. However, gene transfer of key symbiotic loci might experience strong selection at the host level if it expands the host's ecological range. We will identify up to 10 symbiotic genes for PCR amplification and sequencing across symbionts of several host species to test for horizontal transfer using phylogenetic approaches.

***Wolbachia* bacteriophage and other GTAs.** In order to understand mechanisms of lateral gene transfer in unstable symbioses, we will identify GTAs that mediate large-scale genomic changes in *Wolbachia*. The bacteriophage 'WO' has been associated with *Wolbachia* for at least 60 MY and undergoes high levels of horizontal transfer [281]. However, we do not know if WO and other GTAs mobilize bacterial and/or host genes and drive evolutionary innovation. We will investigate these features of WO and uncharacterized GTAs (transposable elements, S.L. O'Neill pers. comm.) by comparing the distribution of these mobile elements

across *Wolbachia* lineages with varied lifestyles. We will first determine the abundance and distribution of GTAs across *Wolbachia* genomes by screening 30 *Wolbachia* lineages with PCR. Primers will be designed from mobile elements identified in fully sequenced *Wolbachia* genomes. Two sequenced genomes (of *Wolbachia* group A and D) are currently available via BLAST servers (http://tigrblast.tigr.org/ufmg/index.cgi?database=wolbachia, http://tools.neb.com/wolbachia/search.html) and annotated versions will be publicly available in mid-2003 (S. O'Neill, pers. comm.). We will sequence two ORFs per GTA across all strains. We will then compare phylogenies of GTAs and chromosomal genes to evaluate the frequency of lateral transfer of each GTA. We will assess recombination within GTAs by comparing the gene phylogenies of the two ORFs sampled for each. Furthermore, if these mobile elements undergo lateral transfer between *Wolbachia* genomes, we expect them to be transcriptionally active (i.e., to encode mRNA products). We will use quantitative PCR approaches to determine the gene expression levels and copy numbers of GTAs across six *Wolbachia* subgroups relative to a single-copy housekeeping gene. Expression data will be correlated with distribution data to link GTA expression to its ability to spread throughout *Wolbachia*. We will compare *Wolbachia* strains with respect to GTA copy numbers and the fraction of the genome devoted them.

Lateral gene transfer can also move genes from the endosymbiont to the host nucleus, as exemplified by the transfer of mitochondrial and chloroplast genes to the eukaryotic nuclear genome [285]. If such transfer characterizes intracellular associations, we expect to find signals of similar events in younger endosymbioses. *Wolbachia* is a particularly promising system to investigate this phenomenon given its abundance, the presence of genetic machinery for lateral transfer in the genome, and genetic evidence of a recent lateral transfer event between *Wolbachia* and a beetle host [280]. We will screen host DNA for *Wolbachia* genes in order to test for endosymbiont gene transfer to the eukaryotic nucleus. We have identified 10-15 *Wolbachia*-infected host systems in which eliminating *Wolbachia* does not adversely affect the host. We will eliminate *Wolbachia* from these hosts, isolate host genomic DNA from uninfected individuals, and hybridize this host DNA against the *Wolbachia* microarray to test for endosymbiont gene transfer to the host. *Caveat:* Only recent cases of transfer will be detected, since a *Wolbachia* gene transferred to the host nucleus in the distant evolutionary past might have a different base composition that would prevent its detection through microarray hybridization.

Deciphering the language of genome-genome communication: Modulation of gene expression in endosymbionts. As endosymbiont genomes continuously streamline, changes in gene expression may become increasingly important relative to genome content differences. Currently, we do not understand how such changes in expression are linked to physiological or other changes in the host, nor do we know whether changes in gene copy numbers or regulatory functions typically account for expression changes. To understand the relevance of expression plasticity in the evolution of complex life, we will link symbiont expression patterns to natural environmental variation that the host experiences.

Plasticity of gene expression in stable genome interactions. Stable genome interactions can trigger the over-expression of chaperonins (*groEL*) as a mechanism to stabilize endosymbiont proteins [286], and up-regulation of biosynthetic or other genes according to the particular ecological requirements of the host [287]. We will explore links between *Blochmannia* gene expression and ecological and evolutionary novelty of their ant hosts. Specifically, we will quantify bacterial expression across several levels: distinct host species, host developmental stages, and different castes (queens, workers, etc). Ant castes represent the most extreme morphological, biochemical, and behavioral variation ever documented among genetically identical organisms and result from differential gene expression during larval stages [288]. *Blochmannia* might influence this process in ants, given its close involvement with larval development [289]. We will perform RT-qPCR of up to five candidate symbiotic genes (identified above). For any gene showing differential expression among treatments, we will

quantify the copy number of that locus using qPCR, in order to attribute expression changes to either gene duplication or regulatory changes.

Linking gene expression to variation of interaction types in unstable associations. We will perform the first study to identify gene expression profiles that underlie distinct interaction types among closely related endosymbionts. From the microarray analysis of *Wolbachia*, we will identify genes that are shared among diverse *Wolbachia* subgroups for further expression analysis. Although we will not exclude other promising candidates identified in the microarray analysis, we will focus primarily on genes encoding bacterial Outer Membrane Proteins (OMPs), which experience constant exposure to the eukaryotic host cellular environment and might mediate key host-symbiont interactions. Expression levels of several OMPs from up to 30 *Wolbachia* lineages from the major subgroups will be quantified using RT-qPCR, relative to a housekeeping gene as an internal control. Expression levels will be mapped onto gene phylogenies to determine when expression differences evolved and if changes associate with switches among host taxa or interaction types. Finally, we will examine DNA sequence variation at OMPs that show expression differences to test whether changes in gene regulation correspond with episodes of natural selection, such as positive selection resulting from an "arms race" between *Wolbachia* and the host.

5.5 SAMPLE PROCESSING, DNA SEQUENCING AND PHYLOGENETIC METHODOLOGIES

The projects described under research themes 5.1a, 5.1b and 5.2b require the collection of samples from field sites and isolation of cultures and/or extraction of DNA for molecular analyses. All three of these projects will share access to collected samples, DNA preparations and will work together to build an integrated database. We have established a DNA extraction laboratory and high throughput genomics capability that allows expanded experimental design well beyond what might be achieved in a more traditional laboratory setting. With high-throughput robotics and efficient DNA sequencing capabilities, molecular-based analyses of microbial populations can process 1000s of samples per day rather than 100s of samples analyzed over weeks if not months. With the technology available in the Bay Paul Center an investigator can afford to economically increase resolution and achieve better statistics (through inclusion of a larger number of sequence reads) in sequence-based studies of metabolic and microbial diversity. The very same technology allows for cost-effective genome level analyses of the sort described in themes 5.2a, and 5.4. The direct reagent cost for DNA sequencing in our environment is well below $1/sample and with anticipated improvements to our pipeline it would not be unreasonable to expect those costs to fall below $0.50/sample. One highly skilled technician oversees the operation of the facility but the workflow is performed by technicians associated with individual research projects. This allows for improved throughput without the cost of maintaining a large technical staff.

The acquisition of DNA sequence data is rapidly becoming a minor cost of molecular analyses. There has been an ever-increasing demand for computational capabilities for the purpose of converting DNA sequences into database entries and downstream processing in the form of database searches, annotation, sequence alignment and phylogenetic inferences. These tasks are common to all but one of the projects described in this proposal for continued membership in NASA's Astrobiology Institute. To address these requirements we have established a common bioinformatics "pipeline" that incorporates tools for sequence assembly using ARACHNE, programs such as CRITICA and GLIMMER for finding open reading frames, and scripts for controlling search algorithms that use FASTA, BLAST and other software tools to search both local and remote databases for annotating sequences. We perform

computationally expensive procedures such as the alignment of large datasets with CLUSTALX on a large Beowulf cluster or on a large memory, 8 gigabyte high performance Alpha processor. Similarly, we use a variety of phylogenetic packages including PAUP, MEGA, MrBayes, PUZZLE, etc. to infer phylogenetic histories for the sequences under study. Because different questions in molecular evolution can only be resolved by the most appropriate phylogenetic tool, it is well beyond the scope of this proposal or any other to summary all contingencies that might be encountered in analysis of molecular data.

5.6 MANAGEMENT PLAN.

This Astrobiology program involves four institutions including The Marine Biological Laboratory at Woods Hole, Woods Hole Oceanographic Institution, Brown University and the University of North Carolina. The project will be managed by a core group of investigators who represent each of the four institutions and major research themes. This management structure will include PI M.L. Sogin (PI MBL), Lorraine Olendzenski (EPO coordinator at MBL), and Co-Is from the collaborating institutions; Andreas Teske (UNC), Katrina Edwards (WHOI), and John Mustard (Brown University). All of the participating institutions have convenient access to video conferencing facilities capable of communicating over internet2. The management group will hold bi-monthly video-conferences. Dr. Sogin will serve as Chair and Dr. Andreas Teske will be the Vice-Chair. The management group and invited guests will assess progress and priorities regarding all Woods Hole Astrobiology research projects, make decisions regarding allocation of available funds and monitor budgeted spending**.** The committee will carefully monitor use of Astrobiology funds relative to time and effort for personnel supported by this interdisciplinary project. Other responsibilities of the management committee will include the pursuit of additional funding for new projects that are relevant to Astrobiology, making decisions about including new members or research groups within this Astrobiology program, and coordinating attendance and participation of investigators associated with the Marine Biology Laboratory Astrobiology team at NASA sponsored and national meetings.

On a bi-annual basis, the management committee will meet with all participants in the Woods Hole Astrobiology initiative. During those meetings, research funded by the Astrobiology program will be presented and openly discussed. Each summer, an Astrobiology retreat will be held in the Woods Hole area for all participating research groups and their associated personnel. The objective of this retreat will be to stimulate discussion on all Astrobiology research topics, giving researchers an opportunity to present their results and receive feedback, and nurture emerging opportunities for collaborative research in Astrobiology.

The PI will oversee administrative aspects of the Astrobiology program including management of the high-throughput DNA sequencing facility. Dr. Sogin will direct the workshop **Advances in Genome Sciences and Bioinformatics.** Administrative assistants in the Bay Paul Center provide secretarial and purchasing support for team members.

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7. STRENGTHENING OF THE ASTROBIOLOGY COMMUNITY

The efforts of our interdisciplinary group of research scientists, EPO, and IT specialists, contribute to the overall enhancement of the science and education initiatives of NAI. By maintaining a vigorous education program at the K-14, advanced undergraduate and graduate level, and providing unique content-rich web resources for education and public outreach, we bring understanding to those outside our research program, and train the next generation of scientists. Our facilities for high-throughput DNA sequencing and spectral analysis offer support to research initiatives across NAI. Many of our PI and CoIs have formed collaborations within the broader scope of the NAI and they bring an astrobiology perspective to other endeavors of the Space Science Community. Through the implementation of innovative IT solutions for collaboration, we deepen the relationships among members of our team and foster communication with other members of the virtual institute. Elements of our program are:

7.1 EDUCATION AND PUBLIC OUTREACH (L. Olendzenski, D. Patterson, M. L. Sogin).

Our research themes **EARTH'S EARLY BIOSPHERE; TERRESTRIAL ANALOGUES FOR EARLY MARS; BIOSIGNATURES AND LIFE DETECTION** and **EVOLUTION OF GENOME ARCHITECTURE IN PROKARYOTES** address the goals and objectives of NASA's Office of Space Science (OSS) to probe the origin and evolution of life on Earth and to determine if life exists elsewhere in our solar system. Our EPO program and plan for strengthening the Astrobiology community extend the OSS education goals (to enhance the quality of science, math and technology education at the pre-college level) by integrating our scientific expertise in microbiology, molecular evolution, and planetary geology into intensive workshops for both teachers (Gr. 6-14) and research scientists. The NAI team at MBL offers the workshops *Living in the Microbial World, Life and Living in Space*, and *Astrobiology Mini-Workshops* for teachers. Two MBL courses, the *Workshop in Molecular Evolution* and *Advances in Genome Technology and Bioinformatics,* provide advanced training for graduate students, Postdoctoral fellows and Principle Investigators. We will continue to provide web-based resources for educators and we will expand our web-site **micro*scope** to provide integrated access to data and results from our research projects.

Teacher Enhancement at the MBL. During our initial membership in the Astrobiology Institute, we developed three opportunities for teacher enhancement at the MBL. Middle, high school and community college teachers receive first-hand exposure to current astrobiology topics presented through lecture and discussions with active research scientists and spend significant time in the laboratory gaining hands-on experience with essential lab and teaching techniques, available classroom curricula and inquiry learning. Our program fulfills the following recommendation of the National Science Education Standards for Professional Development for Teachers of Science that:

> "…prospective and practicing teachers must take science courses in which they learn science through inquiry, having the same opportunities as their students will have to develop understanding", and that providers of professional development "must design courses that are heavily based on investigations, where current and future teachers have direct contact with phenomena…. and are involved in groups working on real, open-ended questions."

By partnering with expert teacher Barbara Dorritie of the Cambridge, MA Public School system (see letter of support), we incorporated appropriate models of inquiry into our workshop (*Living in the Microbial World*). We will adopt similar strategies for all of our workshops. Time is devoted to formulation of testable questions, pursuit of individual and group investigations, and to a thorough understanding of the scientific process. We also provide methods for classroom implementation and for the sharing of teacher's experiences while doing inquiry.

Our workshop series includes "*Living in the Microbial World*", a one-week intensive summer workshop for middle and high school students, focusing on microbial diversity and evolution. "*Life and Living in Space*", a four-day workshop for high school and community college teachers, convenes in the fall and is offered in conjunction with the Center for Advanced Studies of Space Life Sciences (CASSLS) at the MBL. This workshop combines NASA Life Science topics and areas of Astrobiology content including planetary protection and life detection. Astrobiology Mini-Workshops, offered during the spring, focus on a single topic and set of activities that change each year. To date these one and a half day workshops have included: *Mapping Time and Space with Forams*, *Life on Earth and Elsewhere*, and *Inquiry into Extremophiles*. *Mapping Time and Space with Forams* focuses on a series of activities from the Living Sands curriculum (www.neosci.com). "*Life on Earth and Elsewhere*" makes use of the activities in the Astrobiology Institute Educators Resource Guide, supplemented by lectures on life in extreme environments and evidence for water on other planets. In "*Inquiry into Extremophiles*", we use a format that encourages teachers to directly observe results from experiments on thermophiles and hyperthermophiles, halophiles, acidophilic eukaryotes and UV-resistant microorganisms. The participants then design and carry out their own investigations.

As part of our continuing EPO program we will improve the curricula through enhanced participation of science team members from MBL, WHOI and Brown. Our Astrobiology Mini-Workshop offerings will include a new workshop that capitalizes on the geologic expertise offered by the Brown node; one or more workshops will employ the SETI Institute's "Voyages Through Time" curriculum pieces; and a third workshop will focus on the **micro*scope** web-based resource in microbial diversity and astrobiology (see below).

Dissemination and expected impact. *Living in the Microbial World* and *Life and Living in Space* collectively accommodate 40 teachers yearly. Participants in either of these workshops have the opportunity to attend *Astrobiology Mini-Workshops* in the spring. We preferentially recruit educator teams that are made up of at least one classroom science teacher and another science teacher, administrator, science coordinator, support staff or math teacher from the same school or school district. We ask teachers to share content and activities with their colleagues through a short workshop or meeting upon return to their school. These mechanisms help to insure that workshop elements will be propagated and incorporated into existing classroom curricula by other teachers in the same school or district. We recruit our teachers from Cape Cod and surrounding Massachusetts areas, through presentations at national meetings (e.g. NABT, NSTA) and through Astrobiology Institute contacts. The MBL Education Office participates annually in the Society for Advancement of Chicano and Native Americans in Science (SACNAS) national conference to promote our education programs. Working in partnership with Marge Anderson, the SMART! Education Program Coordinator of Pfizer Inc., we directly recruit teachers from school districts associated with Pfizer Research Centers and support their travel to our workshops in Woods Hole. In partnership with Pfizer, we will target minority teachers and educators from underserved communities including New York City and New Jersey, Puerto Rico, San Diego/La Jolla, and the Midwest (see letter of support). We have established contacts with Cambridge and Boston, Massachusetts's school district science coordinators to attract teachers from these urban centers. To engage Hispanic minority teachers and students, we will incorporate spanish language astrobiology resources into our programs as they become available. Two or our CoIs (Morrison and Amaral-Zettler) are Spanish speaking Hispanic minorities and postdoctoral researcher Carmen Palacios is a citizen of Spain.

Assessment. We use mid-course feedback and comprehensive end-of-course questionnaires to assess the content, activities and pedagogy of our workshops. Through these mechanisms we can make immediate improvements in workshop structure and content. Teachers who return to subsequent workshops report orally on their success in incorporating astrobiology and microbial diversity related activities and content into their curricula. As part of our ongoing efforts to

improve the effectiveness of our EPO program, we will comprehensively survey all teachers who have participated in our programs to determine what content has been incorporated into their curricula and which activities and modifications they have found most successful. The survey will include written questionnaires, follow-up oral interviews, and continuing interaction on web-based bulletin boards served from MBL. We will publish resultant summaries and assessments on the education pages of the web site **micro*scope** (see below) to assist others who seek to implement astrobiology content in the classroom.

Traveling Collaborative Workshop Team. The Collaborative Workshop Team integrates EPO leads, educators and researchers from five Astrobiology Lead Teams. The proposed team of Catherine Tsairides, (EPO lead, NASA Ames Research Center), Lorraine Olendzenski, (EPO lead, MBL), Lisa Brown and Angela Phelps (EPO leads, Pennsylvania State University), Jackie Allen and Kay Tobola (EPO leads, NASA Johnson Space Center), has developed effective professional development centered around existing astrobiology educational products. Team members have expertise as researchers, principals, university instructors, publishers and classroom educators, and they offer a mix for professional development that includes utilizing members of the research team as speakers, panel members, and hands-on presenters. Education specialists provide expertise in understanding the needs of teachers, classroom management, pedagogy and modeling of activities. The Team consistently models science as inquiry and connects educators to the world of scientific research through the presentation of various programs that directly involve astrobiologists in professional improvement nationwide. We will provide hands-on science experiences and activities through presentations, workshops and short courses, at venues including, but not limited to NSTA, NABT, Astrobiology meetings, and meetings organized by the American Society for Microbiology, Geological Society of America, and other relevant professional societies. These workshops will provide another distribution mechanism for upcoming astrobiology products while continuing to promote the Life on Earth and Elsewhere Educator Resource Guide, the **micro*scope** web based resource in microbial diversity, Johnson Space Center lessons and materials associated with the Yellowstone National Park Astrobiology Initiative.

7.2. WEB RESOURCES FOR ASTROBIOLOGY EDUCATION AND PUBLIC OUTREACH (L. OLENDZENSKI, D. PATTERSON, M. L. SOGIN, B. OLSSON).

The **micro*scope** web site (http://www.mbl.edu/microscope) is an innovative resource for education and public outreach. **Micro*scope** is an image-rich resource providing descriptions and pictures of all categories of microorganisms, currently containing over 4500 downloadable high-resolution images. The images and accompanying information can be accessed by a number of methods, including by habitat, cell shape, alphabetically by genus name and by hierarchical taxonomic classification. Surveyed habitats include marine environments, saltwater marshes, fresh water ponds, and others including the extreme environments, Yellowstone National Park, Río Tinto, and Lake Toolik in Arctic Alaska.

Currently, we are developing **micro*scope** into a central educational repository for students and teachers interested in astrobiology and microbial diversity. Educational resources under development for the site include an Astrobiology Gallery - a page that highlights organisms of interest to Astrobiology researchers, and a collection of hands on-activities related to microbes accessible by grade level and content area. The site provides information on how each activity ties into the National Science Content Standards, links to other useful educational sites in microbiology and astrobiology, glossary elements, and instructions on using **micro*scope**'s Lucid and X-ID matrix taxonomic identification guides. Over the course of this program, we will continuously add to these elements, and include new activities developed through our teacher workshops, presentations and collaborative teaching efforts with the MIT/Harvard team. We will develop resources in both English and Spanish about the microbial diversity of Río Tinto that will be contained in or linked to **micro*scope**, providing a valuable tool for minority education.

7.3 EPO PROGRAM MANAGEMENT. Lorraine Olendzenski is a research scientist in microbiology and molecular evolution and participates as a member of our science team and will serve as our EPO lead. She will report directly to the PI and will oversee and carry out all aspects of our on-site teacher enhancement programs, including implementation of the three annual workshops, and acquisition of additional operating funds. She will be responsible for coordinating and implementing the comprehensive program assessment during year three of the Woods Hole Astrobiology team. She will participate in the traveling Collaborative Workshop Team. She will continually liaise between Astrobiology Co-Is, EPO leads from other sites and NAI Central to facilitate the provision of content and other expertise in the review and development of curriculum materials as necessary. Co-I David Patterson, a senior research professor with expertise in protist taxonomy, diversity, and ecology, will oversee all aspects of the development of the **micro*scope** database structure, including expansion of its content areas and the development of generic software tools which expands its capabilities (described in more detail in a separate section, below). As part of her duties, Lorraine Olendzenski will expand the educational portions of micro*scope including activities and supporting materials.

7.4 TRAINING THE NEXT GENERATION OF ASTROBIOLOGY RESEARCHERS: COURSES FOR GRADUATES STUDENTS, POST DOCS AND RESEARCHERS. (M. SOGIN, L. OLENDZENSKI, H. MORRISON, A. TESKE, K. EDWARDS, S. SIEVERT, J. MUSTARD, J. HEAD).

Increasingly, comparative molecular and genomic approaches are being used to address questions of phylogeny, ecology and early evolution related to Astrobiology research goals. The MBL offers intensive laboratory-training courses designed for advanced researchers and graduate students who want expert instruction in molecular biology and bioinformatics techniques. These courses combine presentations from distinguished faculty on current research topics in molecular phylogenetic analyses and genomics with hands-on instruction using the newest equipment, algorithms, software and techniques. Our Astrobiology program participates in two of these:

Advances in Genome Technology and Bioinformatics is a comprehensive, four-week course in genome science that integrates bioinformatics with the latest laboratory techniques for genome sequencing, genome analysis, and high throughput gene expression (DNA microarrays). A distinguished faculty from major universities, bioinformatics centers, The Institute for Genomic Research (TIGR) and the MBL provide instruction that integrates lectures with laboratory exercises both at the computer and in a high technology, high throughput facility. Astrobiology PI Mitchell Sogin is one of the course co-directors, and Co-Is from the MBL Astrobiology team participate as faculty. The major laboratory modules include 1) Genome Sequencing (vector development, library construction, high throughput sequencing technologies, principles of automation using advanced robotic liquid handlers, genome assembly algorithms and closure strategies); 2) Bioinformatics (Gene prediction algorithms, annotation, database construction and searching, phylogenetics and molecular evolution); and 3) Functional Genomics (DNA microarrays, data analysis). Symposia focusing on Environmental and Evolutionary Genomics, Eukaryotic Microbial Genome Projects, Organelle Evolution and other topics are also part of the program. The course takes place during the month of October and is open to twenty-four students.

The Workshop on Molecular Evolution, in its sixteenth year, is recognized as the finest course of its type in the world. The Workshop includes a series of lectures, demonstrations and computer labs that span the field of molecular evolution. A distinguishing feature of the course is a state-of-the-art computer laboratory furnished with Linux and Unix servers and workstations for comparative analysis of molecular data. Software authors and experts in the use of packages such as Clustal W and Clustal X, COMPARE, FASTA, GCG, LAMARC, PAML, PAUP*, and PHYLIP provide demonstration and consultation. This two-week program is open to 60 participants with strong interests in molecular evolution, systematics, and population genetics. A one week extended topics session allows 15 selected students to spend time analyzing their

research data sets. Topics covered in the course include: databases and sequence matching, phylogenetic analysis including Bayesian analysis and maximum likelihood theory, molecular evolution at organismal and higher levels, molecular evolution and development, gene duplication and divergence, gene family organization, evolution of large multigene families, molecular evolution in bioinformatics and comparative genomics.

University Education. Through new courses offered on the graduate and undergraduate level at University of North Carolina, Brown University, and WHOI, our Co-Is are raising the profile of Astrobiology in related disciplines. A new joint MBL/Brown graduate program will allow implementation of additional courses in September, 2004. Ongoing and proposed University courses include:

Extremophilic Microbiology. A. Teske (UNC Chapel Hill) teaches a comprehensive marine microbiology course every fall semester that emphasizes extreme microbial physiologies and ecosystems (hydrothermal vents, the deep marine subsurface) as model systems for astrobiology. He also advises two Ph.D. students and two PostDoctoral students who are working on key gene analyses of deep subsurface sediments from a wide range of geochemical settings, as outlined in our project description on evolution and environmental extremes of sulfur and methane cycles. In this way, we are beginning to train a new generation of Ph.D. students and PostDoctoral students who are growing up with astrobiology as an integral part of their research agenda.

Planetary Geoscience. Graduate students in the Brown University Department of Geological Sciences are trained in planetary geosciences. Students that will be supported under this effort will be further trained in astrobiological concepts related to planetary geosciences, with their Ph.D. research focusing on investigations of theRío Tinto region. They will also participate in the analysis of Mars mission data through efforts lead by Co-Is Mustard and Head and thus be able to relate the astrobiological research directly to observations of Mars.

Geomicrobiology. WHOI offers a Joint Graduate Program with MIT that confers Master's Degrees and Doctorates in the areas of Biological, Physical, Chemical and Applied Oceanography, as well as Marine Geology and Geophysics. WHOI Co-Is Stephan Seivert and Katrina Edwards will offer a course "Geomicrobiology on Earth and in the Universe" to Joint Program students. This course will focus on roles of microorganisms in the formation of minerals and signatures, impact of microbes on Earth's geological record, biogeochemical cycling, geology of habitable planets, and potential mineralogical biosignatures for detection of life beyond Earth.

Graduate Courses and Training and at the MBL. Starting in the fall of 2004, the MBL will initiate a Joint Graduate Program with Brown University. Members of our science team currently include researchers from the Brown University Department of Planetary Geology. As part of the proposed graduate program, we will develop upper level survey courses for graduate students focusing on early evolution, planetary geology processes, microbial ecology and evolution and astrobiology. These will be seminar format courses that take place at Brown University campus or the MBL, organized by the Astrobiology PI and CoIs from both Brown University and MBL. The MBL/Brown Graduate Program will accept its first students in September, 2004 and will support a steady-state population of ~40 graduate students whose interests will span basic molecular and cell biology to astrobiology and environmental sciences. Our proposed team will be in a position to explore the creation of an Astrobiology Certificate Program through the University.

Minority Undergraduate Fellowship Program at the Woods Hole Oceanographic Institution. The Woods Hole Oceanographic Institution offers special educational opportunities in oceanography for minority undergraduates enrolled in U.S. colleges or universities. This Minority Fellowship program provides students from minority groups with a first-hand introduction to scientific research. Fellowships are awarded for a ten- to twelve-week period in the summer or for a semester during the academic year, to allow students to pursue an independent research project under the guidance of a member of the WHOI research staff. Each Fellow works with their lab

advisor to design a project that can yield meaningful results in the time allotted, prepares a research paper describing their project and gives an oral presentation of their results. CoIs Stephan Sievert and Katrina Edwards will each accept one qualified fellow per year through this program Fellows will interact with our entire MBL Astrobiology group through the attendance of lab meetings, journal clubs and seminars.

7.5 DEVELOPMENT OF THE MICRO*SCOPE WEB-BASED RESOURCE IN MICROBIAL DIVERSITY AND ASTROBIOLOGY INTO A RESEARCH AND TEACHING TOOL FOR THE ASTROBIOLOGY COMMUNITY (David Patterson).

Tools for gathering information about microbes are few in comparison to those available for larger organisms, yet there are many instances in which this information is crucial for advancing research and improving classroom teaching. The **micro*scope** website fills the gap in available resources and provides students, teachers and researchers with easy access to high quality digital micrographs and information about diverse microbes, both prokaryotic and eukaryotic. A major innovation of the site is the use of uBio software, developed in conjunction with the MBL-WHOI Library, which uses a registry of names as part of the navigational structure and as a filing system upon which to attach information about organisms or other entities. The software allows the recognition of synonyms of names in remote databases and resources. **Micro*scope** is designed to bring together information distributed at other authoritative sites. It uses software to track the taxonomic location of the user and to initiate searches into remotely located databases or other sites generally available on the web, based on the genus being examined. The list of databases and remote sites to be searched can be customized to return results from only certain selected databases.

As an internet-based resource capable of linking distributed knowledge, **micro*scope** has many attributes appropriate to its growth as a knowledge network. Although the content contained in micro*scope focuses on microbes, the underlying organizational structure of micro*scope is flexible and can be adapted to the needs of researchers from a variety of disciplines. The hierarchical classification structure will be expanded so it can be used as an organizing and navigational structure for non-biological data sets. As this system is based on the TNS (taxonomic name server) of uBio, this retains the capacity to reconcile alternative names for the same entity, thereby linking together data about the same thing even if logged under different names. The data model allows for data to be bundled within hierarchical clusters to increase the flexibility of access to data. Linkouts allow access to remotely held data. The result is not a database even though it incorporates databases. The result is a knowledge network capable of integrating data held locally and remotely. It is powerful and flexible, simple to populate, and with relatively little effort can be molded to provide its power to other suites of data.

Our goal is to maintain **micro*scope** as a resource to assist those interested in gathering information about microbial taxa and in understanding better the diversity, complexity and evolution of the microbial world. Through our involvement in the Institute, we will expand its use for archiving image rich data from habitats currently being studied by astrobiologists, including Río Tinto, Guerrero Negro, Baja California, Mexico, and Yellowstone. We will also make the generic structure of **micro*scope** available to interested groups for the creation of similar data management resources to accommodate the diverse different types of data obtained by researchers across the Astrobiology Institute.

7.6 RESEARCH FACILITIES FOR THE GENERAL ASTROBIOLOGY COMMUNITY.

High Throughput DNA Sequencing Facilities at the MBL: DNA sequence data allows astrobiology investigators to survey microbial diversity in the environment, make inferences about the evolution of proteins and physiologies, and reconstruct phylogenies of organisms. Vast quantities of data, including extensive environmental surveys, the sequencing of whole microbial genomes and profiling of protein expression patterns can be obtained quickly using high-throughput sequencing and DNA microarray technology. The Josephine Bay Paul Center at the

MBL maintains and operates an 1800 square-foot laboratory dedicated to state of the art automated DNA sequencing, DNA microarraying, and high-throughput robotics (see **Facilities and Equipment** for details).

Our DNA sequencing capability represents an investment of approximately $1.5 Million in equipment, largely to support our astrobiology research efforts. We designed this facility to include all of the advanced technology currently available within DNA sequencing factories, but at a smaller scale. The operating philosophy is to provide biologists access to the powerful tools of genome science in a cost efficient manner. This capability has radically modified the scale of projects pursued by the Woods Hole astrobiology team and we have carried out DNA based projects for astrobiologists and exobiologists at the University of Washington in Seattle, the University of Colorado, the University of Rhode Island, and the University of Connecticut. We provide access to this facility for NASA supported research studies at our institutional costs for reagents and technician time. The capital equipment acquisition and maintenance costs are not charged back to the users. Our current costs for DNA sequencing are remarkably low at a direct cost of less than $1/reaction. Should demand exceed our capacity, we will seek funds to lease additional equipment for use in astrobiology research projects that would benefit from acquisition of extensive environmental, genomic or other DNA sequence data. The Bay Paul Center also hosts and trains visiting researchers interested in using the equipment for their own projects.

A high-throughput data pipeline connects the sequencing facilities with a well-equipped facility for bioinformatics and computational biology. The core of the center consists of a 104-processor Beowulf cluster, a farm of large-memory 64-bit Alpha servers and several terabytes of fast-access data storage (see **Facilities and Equipment** for details). We maintain the latest versions of both commercial and open source software packages for analysis of genetic sequences and inference of phylogenetic trees. The computer facilities can be accessed by other NAI Teams over fast Internet2 connections, and for visiting researchers we also offer personal workstations and full-time staff support.

The Reflectance Experiment Laboratory (RELAB) at Brown University. The composition of inaccessible planetary surfaces can be derived from remotely obtained reflectance spectra interpreted with the help of spectroscopic laboratory data. The RELAB is supported by NASA as a multi-user spectroscopy facility, and laboratory time is available at no charge to investigators who are in NASA-funded programs. This facility is designed to acquire high precision and accuracy reflectance spectra of materials from 0.3 to 25 μm under variable incidence and emergence geometries and variable temperature and pressure regimes. Data acquired through RELAB form a foundation upon which many remote sensing algorithms have been developed and tested. The primary instrument is a high precision bidirectional reflectance spectrogoniometer that permits the measurement of samples under conditions similar to those for aircraft and space-based systems. The RELAB facility also has a Nicolet Nexus 870 Fourier Transform Infrared (FTIR) spectrometer acquired with funds from the Paduano foundation. This instrument measures biconical reflectance from 1.0 to 200 μm. Microscopic samples can be measured with a recently acquired FTIR microscope.

7.7 Information Technology and the Virtual Astrobiology Community (B. Olsson).

The MBL Astrobiology team believes that interdisciplinary collaboration is an integral part of astrobiological research, and that successful team efforts require clear communication, and a good collective knowledge of goals, priorities, and timeframes. We consider a solid information technology infrastructure to be a necessary, albeit not sufficient, prerequisite for building a virtual institute.

We will continue to be an active participant in NAI information technology pilots and projects, and intensify the use of tools already procured by the NAI collaborative research support

team. The WebEx conferencing system will be promoted in projects where it can improve the efficiency and quality of the scientific work, and we are preparing science projects for the incorporation of knowledge management systems planned for NAI pilots in the near future. The Polycom videoconference system was for a time underutilized due to ISDN connectivity problems, and because of internet bandwidth restrictions we were unable to find feasible alternatives. This situation changed dramatically when the Marine Biological Laboratory joined a partnership of local scientific institutions sharing multiple high speed connections to the Internet. The members of this partnership are the Woods Hole Oceanographic Institution (WHOI), the NOAA Northeast Fisheries Science Center, and the USGS Field Center for Coastal and Marine Geology, along with the MBL and a variety of smaller institutions.

The Partnership's Internet services include an OC3 running at 155 Mb/s for Internet2 services and various commercial T1's. The OC3 connects into the Internet2 POP at Northern Crossroads (NOX) in Boston, Massachusetts, which facilitates high performance academic networking in New England. NOX connects to Abilene, the Internet2 backbone, with an OC12 interface running at 622 Mb/s. The speed of this and the commercial T1's, combined, provide a basis for high performance networking and use of advanced network applications at the MBL, including the Polycom system, which has now been permanently switched over to IP-based communication. This has drastically reduced the videoconferencing costs while increasing the bandwidth, and has made our virtual meetings significantly more frequent and productive. A second improvement was last year's addition of new research space to the Bay Paul Center including the construction of a new conference room. We designed this facility to meet the environmental requirements for use of the Polycom by as many as 30 participants. As a result of the improved performance and the availability of a high quality environment, we have begun to meet our original expectations about use of the Polycom system for communication with members of our team at remote locations e.g. A. Teske at the University of North Carolina, colleagues at other NAI sites, etc. In a similar manner, this improved capability will have a major impact on our ongoing collaborative research efforts with the Centro de Astrobiologia. We also will take advantage of the increased connectivity by including desktop video conferencing in our teaching and research activities. Our collaboration with the Brown University Planetary Geology Group is especially exciting in this context, since they pioneered the use of international videoconferencing beginning with broadcasts and interactions with the Soviet Union already in the 1980's.

Technology in and by itself does not create a fully efficient virtual organization: Active and productive use of the technology requires demonstrations and training, and group efforts require a trust among the participants that can only be built during personal interactions in a non-technical context. In light of these cultural facts, we plan to place an emphasis on problem-oriented personnel development and regular face-to-face meetings where we can collect feedback and user metrics. Any identified problems will be solved in close collaboration between scientists and technology maintainers within our group. This blended approach will improve both the scientific and the information technology work, strengthen the sense of community, and create a more dynamic and innovative work environment.

While the formal incorporation of information technology has been successful at most NAI lead team sites, we believe there is room for improvement in establishing the *informal* features of our virtual community. This is clearly a concern from a cultural point of view, since most tacit organizational knowledge is gained through informal channels like hallway conversations, mentoring, etc. It is hard to envision a thriving multidisciplinary virtual institute lacking such casual communication. We will proactively address this problem by developing a web communication portal containing user-friendly tools for frequent and spontaneous online communication, both synchronous and asynchronous, to supplement the NAI tools already used for preplanned communications like seminars and focus group meetings. Some requirements for the web communication portal have been given especially high priority:

- Local serving to simplify maintenance and development/testing of customized functions for teams or individual researchers.
- Integration with customizable desktop audio and video "spur-of-the-moment" communication tools.
- Interactive discussion forums for all types of astrobiology dialogues.
- High availability over all bandwidths from all locations.
- Online streaming of stored videos from seminars and workshops.
- Consolidated dynamic and fixed storage of all types of project information (e.g., scientific data, EPO multimedia, software documentation, and streaming seminar videos).
- Well-organized and customizable user interface, to ensure active and productive use.
- Tools for collecting metrics and user feedback, to allow rapid realignment to current needs and fast identification of desired customized functions.
- High user- and group level security, efficient hacker defense, and reliable backup tools.
- Other planned functions include interfaces for distributed computing using XML-RPC, wireless access, and various Java tools for scientific data analysis and visualization.

To ensure cost-effectiveness and an active developer community the web communication portal framework will be built from the open source system PostNuke (http://www.postnuke.com). This content management system uses the freely available MySQL database for content and parameter storage, and provides functionality through modules written in PHP and JavaScript. Functions that cannot be found in open source PHP modules will be written by our own programmers. The communication tools will be designed from Flash Communication MX server modules, which in preliminary tests gave acceptable quality even over modem connections.

The construction of the web communication portal is an ambitious undertaking. However, the bioinformatics and database work routinely performed at the MBL makes us an ideal team for this kind of task, as illustrated by the **micro*scope** web site. A working prototype of the web communication portal containing a subset of the functions mentioned above was demonstrated at the 2003 NAI General Meeting in Tempe, Arizona. We are confident of our ability to create a product that will augment NAI information technology to the same degree that the **micro*scope** site has enriched the institute-wide EPO work.

The introduction of new information tools requires a very good rapport between researchers and technical staff. For that reason, we intend to create an Astrobiology Information Technology tiger team consisting of both IT personnel and researchers at MBL and Centro de Astrobiologia, Spain. This team will tightly integrate information technology and scientific work, and be responsible for the design, implementation, and testing of the web communication portal. The team will also do much exploration of procured NAI information technology in the laboratory and during field work atRío Tinto, Spain, described elsewhere in this proposal. Examples of planned activities include field data collection using wireless devices, frequent desktop video interaction and heavy use of knowledge management tools.

7.8 NAI COMMUNITY PARTICIPATION.

Videoconferencing and Director's Seminar Series. The MBL Astrobiology node regularly attends video presentations made possible through the Polycom system, broadcast from both NAI Central and other sites, and looks forward to continued participation in these opportunities to share scientific results. Team member Linda Amaral Zettler will participate by presenting an upcoming Director's Seminar Series lecture entitled "Life at pH Extremes". Lorraine Olendzenski has

presented the activities and teaching model used in the *Living in the Microbial World* to Institute members via videocon.

Participation in the NAI Focus Groups. Members of the MBL Astrobiology team participate in the EcoGenomics focus group and have been an integral part of a collaborative study on the microbial mat community of Guerrero Negro, Baja California Sud. Our research efforts on expression profiling of the cyanobacterium *Microcoleus chthonoplastes* from Sippewissett Salt Marsh under different light regimes, have served to work out a reliable protocol for further investigations on organisms from Guerrero Negro. This work forms the basis of future comparative projects on organisms from these complex communities under different environmental conditions, including salt concentration and sulfate concentration. We intend to continue our active participation in this Focus Group to obtain data delineated to be of interest to the group as a whole. Jim Head and Graduate Research Assistant G. Wesley Patterson are both active members of the NAI Europa Focus Group.

Collaborations with URI Deep Biosphere group. The URI Astrobiology group focuses on biogeochemical and microbiological analyses of the deep subsurface biosphere. Here we are in an excellent position to collaborate on the microbiology of extreme deep subsurface microbiota, facilitated by proximity and personal ties (A. Teske is also a member of the URI team). Through research cruises on deep subsurface drill ship JOIDES Resolution, the URI group directly probes the deep marine subsurface biosphere, which is out of reach by any other means. So far, extensive sample sets have been collected from hydrothermally active accretionary margins (Nankai Trough, ODP leg 190), from inverted methane-sulfate gradients in the equatorial Pacific and the Peru Margin (leg 201), from methane-hydrate-dominated sediments (Hydrate Ridge offshore Oregon, Leg 204), and deeply buried black shales (Demarara Rise, leg 207). Initial 16S rRNA analyses of Nankai Trough sediments have revealed highly unusual archaeal and bacterial assemblages. In particular, our prokaryotic projects on diversity and evolution of anaerobic and autotrophic metabolisms and their key genes, will benefit from this collaboration.

Collaborations with the Centro de Astrobiología (CAB) in Spain. Our proposed work on the Rio Tinto will strengthen existing collaborative research that is ongoing between the MBL and the CAB. We continue to rely on the laboratory of Dr. Ricardo Amils as a base for processing samples from field studies. This includes chemical analyses of water samples collected as part of our population biology studies, as well as for molecular biology protocols that requires immediate processing. Dr. Amils has also agreed to prescreen Rio Tinto fungal isolates that his lab maintains in a culture collection as part of our investigation into iron oxidation metabolism in eukaryotes. As part of our population biology studies we will coordinate our sampling efforts with those of our Spanish colleagues and freely share data. We will take full advantage of the IT resources available to us to foster good communication and exchange of information. In the past, we have hosted scientists from the CAB in our lab to be trained in phylogenetic methods and inference and intend to continue to serve as a resource to members of the CAB.

Other Collaborations. The Astrobiology community also shares diverse and rare sample materials from extreme environments and microbial ecosystems, such as hydrothermal vent and deep subsurface sediments and water samples. These can be shared between Astrobiology partners on a flexible basis for projects of mutual interest, as exemplified by our successful collaboration with David A. Stahl (UW), on molecular analysis for deeply-branching sulfate reducers in Guaymas Basin hydrothermal sediments.

Relationships to the Space Science Community. PI Mitchell L. Sogin is a member of the Space Sciences Board Executive Committee of the National Research Council. The Space Sciences Board conducts advisory studies and program assessments, facilitates international research coordination, and promotes communications on space science and science policy between the research community, the federal government, and the interested public. He is also a member of the science definition team of the Jupiter Icy Moons Orbiter mission (JIMO). Dr. Norman

Wainwright has been an active participant in Planetary Protection Workshops sponsored by NASA. EPO Lead Lorraine Olendzenski currently collaborates with a team under the direction of NASA Planetary Protection Officer John Rummel to design and deliver training workshops in Planetary Protection for managers and technicians involved in meeting NASA requirements for spacecraft.

Joint Workshops, Seminar Series and Courses with the Center for the Advanced Study of Space Life Sciences (CASSLS) at the MBL. Over the last five years, we have collaborated on a number of research, education and outreach efforts with the Center for the Advanced Study of Space Life Sciences located at the MBL (see letter of support), including workshops and seminar series. Funded through a cooperative agreement between the MBL and the Life Sciences Division of NASA, CASSLS acts as an interface between NASA and the basic sciences community, promoting interactions and discussion in areas of mutual interest. As a direct result of input from PI Mitchell Sogin and our Astrobiology group, CASSLS sponsored the workshop *Outcomes of Genome- Genome Interactions*. This meeting brought together a diverse group of investigators comprised of microbiologists, biogeochemists, ecosystem experts, molecular phylogeneticists and molecular ecologists to foster discussions about how to link biogeochemical measurements with metabolic processes and microbial population structures in natural settings. This type of cross-disciplinary interaction is necessary to advance experimental design and subsequent understanding of how microbes function in consortia and about the responses of structured microbial communities to cyclic and transient environmental cycles. The proceedings of this meeting will be published in The Biological Bulletin in April 2003. Additionally, we work with CASSLS personnel to offer the *Life and Living in Space* Teacher Workshop. NASA Life Sciences also provides support to the *Advances in Genome Technology and Bioinformatics* workshop.

Flight Missions. Jack Mustard is an official collaborator with the French-built OMEGA experiment on ESA's Mars Express mission, which will launch in May 2003. He is also a co-investigator on the Compact Resolution Imaging Spectrometer for Mars (CRISM), which is scheduled to fly on the Mars Reconnaissance Orbiter (MRO) in 2005 and collect high spatial resolution spectroscopic data through 2009. He participated in the planning for the instrumentation on the Mars Odyssey and MRO, and recently participated in the Mars Pathways steering group. He is participating in several efforts to develop flight hardware for upcoming missions.

Jim Head is a co-investigator on the Mars Global Surveyor Mars Orbiter Laser Altimeter experiment, and a co-investigator on the Galileo Mission Solid-State Imaging System, being responsible for planning one half the Europa encounters. He is also a co-investigator on the Mars Scout ARES mission, proposed to be the first airplane flight over the surface at low altitudes. He is a co-investigator on the Mars Express High Resolution Stereo Camera (HRSC) and on the Discovery MESSENGER to Mercury. In the past he has participated in planning and execution of Apollo, Viking Lander, Magellan, Soviet Venera 15/16 and Phobos missions.

Links to Other Agencies. Jim Head participates in the National Science Foundation Antarctic Research Program in the Dry Valleys, investigating Mars analogs. He recently completed a six-week deployment and is presently preparing the results for publications.

8.0 FACILITIES

The Marine Biological Laboratory at Woods Hole MA

The Josephine Bay Paul Center at the MBL has approximately 10,000 square feet of recently renovated laboratory space for molecular biology. This space includes rooms for microscopy, tissue culturing, cold rooms, dark rooms, and a climate controlled room for housing larger computer systems. We have a dedicated 1800 square-foot laboratory for automated DNA sequencing, DNA microarraying, and high-throughput robotics. A separate computer room houses our Hewlet Packard and Dell File servers.

Computational capabilities: The Josephine Bay Paul Center maintains extensive high-performance computing capabilities for genomic, phylogenetic and bioinformatic analyses as well as web server presence. We have nine Alpha systems including a Compaq EV-6 DS10 server with over a terabyte of memory storage, a dual Compaq EV-6 DS20 server with 4Gb RAM, and a newly acquired large memory (8 gigabytes) Hewlett-Packard EV-68 ES-45 server. For high performance parallel processing we operate a Beowulf Linux cluster containing 108 AMD Athlon XP and Intel P4 processors. Scattered around the laboratory we have dozens of Intel P4 servers and workstations running various versions of Linux and Windows, Macintosh G4 workstations running Apple OSX, and Sun SPARC workstations running Solaris 9. The centralized data storage consists of a 2.5 terabyte Apple Xserve RAID connected over fiber-channel to a dual-processor 1.33GHz G4 Apple Xserve that provides connectivity to Linux, Unix, Windows and Apple systems over a 100BaseT switched network. Data integrity is ensured by a dedicated Intel P4 backup server controlling a 1.6 terabyte RAID and a 100Gb Ultrium LTO tape-drive. All systems handling large data sets will soon be connected directly to the RAID device, to form a fiber-web SAN that will further increase the analysis throughput. High-availability web presence is ensured by four dedicated Intel P4 systems running Microsoft IIS 5.0 under Windows 2000 and Apache under Linux, and which serves mySQL and Filemaker databases, dynamic PHP sites like micro*scope and a web communication portal, Flash Communication MX servers, and various interactive Perl scripts.

We actively maintain locally installed genomics and bioinformatics packages such as Phred, Phrap, SEALS, DARWIN, Accelrys GCG and the Arachne whole-genome shotgun assembler. We also maintain local Genbank, Swissprot and Pfam databases, phylogenetic analysis programs including PAUP, PHYLIP, PUZZLE, MEGA, etc. and a host of supporting software for examination, visualization and presentation of data. A full time administrator with special expertise in astrobiological research and scientific programming, and a database specialist highly knowledgeable in PHP, SQL and design of interactive database-driven web sites manage the systems and network.

Office: Dr. Sogin has an ~150 square foot office and our administrative assistants have 160 square feet of office. The institution provides purchasing, personnel, and grants administration services. The Josephine Bay Paul Center provides office and laboratory space for seven other senior scientific investigators. Our offices and laboratories lie adjacent to the MBL library, which subscribes to a wide variety of journals and provides electronic access to many databases.

Other: General equipment in our laboratories include a very well accessorized Zeiss Axioskop 2 microscope equipped for fluorescence microscopy and digital imaging, modern preparative and table-top ultracentrifuges plus rotors, three high speed Sorvall centrifuges plus rotors, a Beckman DU 70 UV spectrophotometer, SpeedVac concentrators a Virtis freeze dryer/vacuum pump system, centrifuges plus rotors for microplates, multiple bench top water baths, gyratory shaking

water baths, heating blocks, analytical balances, a pH meter, several incubators and microplate shakers for 96-well cultures, six large -80°C freezers, several -20°C freezers and refrigerators, fraction collectors, column monitoring equipment, film processor, and cell disruption equipment. Equipment for recombinant DNA work and molecular biology include an Eppendorf electroporation apparatus, ten gradient thermocyclers, a rotary hybridization oven, a dozen high and low voltage power supplies, miscellaneous horizontal gel boxes, trans-illuminators, an Ultra-Lum gel documentation system, and a variety of manual and electric pipetters, an Amersham-Pharmacia FPLC and a Storm 860 Phosphorimager/Fluorimager.

Major Equipment: For high-throughput sequencing, the Bay Paul Center operates an Applied Biosystems 3730 XL capillary sequencing system, a Beckman CEQ2000 capillary system, and seven LI-COR Long ReadIR, two-channel DNA sequencing systems. We have a GeneMachines RevPrepOrbit II for automated, production of ~3600 sequencing DNA templates/day, a Tecan Miniprep75 robot for the preparation of DNA sequencing reactions, a GeneMachines Mantis colony picker, an OmniGrid DNA arrayer, an Axon array reader, and a BioRad CHEF-Mapper pulsed-field gel electrophoresis system for separation of chromosomes and high-molecular weight DNA. For cycle-sequencing, we have several 96-well and 384-well dual-block AppliedBiosystems 9700 thermal cyclers. All of this equipment is located in the **W.M. Keck Ecological and Genetics Facility**, which is housed and managed by the Josephine Bay Paul Center at the MBL.

Microscopy: The MBL maintains a complete facility for state of the art electron microscopy and new higher performance confocal microscopy. Equipment includes a Zeiss 10CA transmission electron microscope and a JEOL 840 scanning electron microscope (with cryo stage and EDS), supported by preparative and ancillary equipment such as ultramicrotomes, sputter coaters, etc. and a Balzers freeze-etch apparatus

Conference Facilities: The MBL has a large modern dormitory (the Swope Center) in the village of Woods Hole, with close proximity to banking, shopping, restaurants, and bus lines connecting to major airports in Providence, RI and Boston, MA. The Swope Center also provides access to computers, online email, the WHOI/MBL library, plus catered dinners. MBL also operates the J. Erik Jonsson Center of the National Academy of Sciences (http://www.mbl.edu/housing/jonsson/) with facilities for meetings and conferences, located just outside of the village of Woods Hole.

Woods Hole Oceanographic Institution

The Laboratories of Katrina J. Edwards consist of three rooms, ~1400 ft^2 total in the Department of Marine Chemistry and Geochemistry. Facilities and equipment exist for standard microbiological, molecular biological, aqueous geochemical, and light, fluorescence, and atomic force microscopy and imaging. Within Edwards' department, facilities exist for a variety of chemical and geochemical analysis of both solid material and aqueous solutions (organic & inorganic).

Laboratory Equipment: Laminar flow bio-hood & chemical fume hood, Barnstead Water distillation & purification (UV/UF), Shimatzu UV-1601 Spectrophotometer & printer, Autoclave, Beckman High-speed refrigerated centrifuge , Controlled temperature & benchtop shakers, Benchtop microfuges, Benchtop pH meter, Mettler Analytical balance, Unisense clark-type microelectrodes (for O_2, H_2S, pH), PA2000 picoammeter, MM33 micromanipulator (apparatus also suitable for field usage), Stirring hot plates, -20 & -80°C freezers, & 4°C refrigerators, Fisher Water bath & dry-block incubators, Fisher & Hibid Hybridization ovens, Sonicator, Vortexes, Perkin-Elmer PCR machines, Biorad iCycler PCR machine, Transilluminator, photodocumentation, & electrophoresis power supplies, Invitrogen E-gel mini-

electrophoresis apparatus, Pressure-filtration equipment, Bead beaters, Pipetters, Multiple computers, printers, software

Microscopic analysis*:* ZEISS Axiovert ST100 TV research grade microscope, Digital Instruments Atomic Force Bioscope (AFM). This microscope can be used in conjunction with the inverted ZEISS, or independently as a free-standing AFM, 2 Vibration isolation tables, HBO100 mercury-arc lamp, Filters for UV, Rhodamine, FITC, OG, CY3, and A-Pol (for rDIC) fluorescence, HAMAMATSU Orca CCD video camera (B/W) & Polaroid color camera, Nikon D1 Digital camera (for microscope use or with independent lens), OPENLAB software modules for the Macintosh computer for image capture and analysis, Nikon, Photoshop, NIH-image, and Digital Micrograph software for image capture and analysis, 10X, 20X, 40X (+ rDIC prism), & 63X (+ rDIC prism; oil) objectives

Isotope Ratio Analysis (MC-ICP-MS): The Woods Hole Oceanographic Institution Inductively Coupled Plasma Facility is an analytical service lab that provides trace element and isotopic analyses for a variety of clients within and outside WHOI. The instrumentation is based on the argon inductively coupled plasma. Currently the ICP Facility operates a high resolution single collector ICP-MS, the Element1, and a high resolution multicollector ICP-MS, the Thermo Finnigan Neptune MC-ICP-MS. The argon plasma source provides a high ionization potential and the ability to atomize aqueous as well as gas sample sources. This makes available approximately 75% of the elements in the periodic table for analysis. The instrumentation housed in the ICP Facility is the best of this type of instrument currently available. The Element1 is a general purpose ICPMS that can sequentially scan masses from 4 to 240 atomic mass units. This instrument is generally used for trace element concentration measurements with detection limits ranging into single parts per quadrillion (10^{-15} or 0.001 parts per trillion) to parts per billion (10^{-9}) range. The Element1 is also used for isotope ratio analyses. The Neptune is expressly designed to make high precision isotope ratio analyses for many different isotope systems. Due to its ion optic design the Neptune can operate in high-resolution mode eliminating common interferences for elements such as sulfur and iron. The facility is staffed with highly qualified technical assistants who are aware of the proposed astrobiology project and are eager to assist with the analytical and methodological development described in the text.

The laboratories of Eric A. Webb in the WHOI Department of Biology are well equipped, modern laboratories of approximately 1500 ft^2 that are set up for microbiology and molecular biology. These ancillary lab facilities will be used for some biochemical analysis (see letter). They include three separate laboratories; one specialized for culture and physiological studies, one equipped for molecular biology and one for the use of radioisotopes. Available equipment includes: Biorad electrophoresis equipment, Perkin Elmer PCR machine, Biorad iCycler quantitative PCR machine, Eppendorf gradient Master cycler and thermomixer R, column chromatography equipment, Shimadzu 1601 UV Spectrophotometer, Beckman and Packard Liquid Scintillation Counters, Beckman High Speed Refrigerated Centrifuges, Beckman Preparative Ultracentrifuge, Pecival incubators

Dr. Stephan Sievert maintains a fully equipped, 980 sq. ft. microbiology and molecular biology laboratory in the Department of Biology, Redfield Laboratories of WHOI, with special capability in the culture of anaerobic prokaryotes. Available equipment includes spectrophotometers, autoclaves, hybridzation oven, scintillation counters, high speed centrifuge and microfuges, a Zeiss Axioplan 2 epifluorescence research microscope with digital imaging capabilities, an inverted microscope with micromanipulator, pH meters, incubators, Coy anaerobic hood, culture rooms, and Hungate apparatus for anaerobic culture. Equipment for PCR (PE GeneAmp PCR 9700 thermocycler), quantitative PCR (Biorad iCycler), and Denaturing Gradient Gel Electorphoresis (DGGE) (Biorad Dcode) is available as well as capability for Fluorescence In Situ Hybridization (FISH) and DNA sequencing. For initial preparation of clones for high

throughput sequencing, an automated Mantis colony picker is housed within the WHOI Department of Biology. Sequencing is accomplished using the facilities at the Josephine Bay Paul Center, MBL.

Brown University

Earth and Planetary Image Processing System: A network of computers to support image processing and data analysis is housed in the laboratories of the Planetary Geology group at Brown University. These include a network of Sun workstations (1 Blade 2000, 5 Blade 150's, 5 Ultra 10 Creator #D, 1 UltraSparc 30 Creator 3D) and a Sun V880 server with 8 processors, 32 Gbytes of RAM and over 1.5 terrabytes of storage. Mission data analysis is accomplished using the VICAR and ISIS software developed by JPL, EASI/PACE software package, ENVI for hyperspectral analyses, and the full ARC/INFO software package including ArcView. Additional software has been and is continuing to be developed at Brown to implement new analysis techniques. A number of Apple and Microsoft operating system microcomputers are also available.

RELAB: The Reflectance Experiment Laboratory (RELAB) is a multiuser facility housed at Brown University for the acquiring high precision and accuracy reflectance properties of materials from 0.3 to 25 µm. Funded under a joint agreement between NASA and Brown, RELAB accepts samples from NASA funded investigators. Data acquired through RELAB form a foundation upon which many remote sensing algorithms have been developed and tested. The primary instrument is a high precision bidirectional reflectance spectrometergoniometer that permits the measurement of samples under conditions similar to those for aircraft and space-based systems. Measurements can be made under variable incidence and emergence geometries and variable temperature and pressure regimes. Details of the instrument capabilities are contained in the user's manual available on request. The RELAB facility also has a Nicolet Nexus 870 FTIR spectrometer acquired with funds from a much appreciated grant from the Paduano foundation. This instrument measures biconical reflectance from 1.0 to 200 µm. Recent acquisition of FTIR microscope allows the measurement of microscopic samples.

Field Spectrometers: Two Analytical Spectral Devices field spectrometers (a PSII and a FieldSpec FR) are available for use. The PSII has a 512channel detector that covers the wavelength range from 0.3 to 1.05 µm, while the FieldSpec FR covers the wavelength range from 0.35 to 2.5 µm. Both instruments are portable and simple to use, and acquire high quality data. In addition, we have an Oriel 100 watt quartzhalogen radiometric lamp and power supply for laboratory measurements.

General Facilities: Facilities to determine sample composition are available at the Department of Geology at Brown which include: a microprobe, X-ray diffraction, X-ray florescence, and a wet chemistry laboratory to determine Fe^{2+}/Fe^{3+} ratios, equipment for sedimentological analysis (grain size and geophysical properties), and uniaxial stress analysis. The geochemistry laboratories are well equipped for the application of chemical and particularly isotopic techniques. Equipment includes: automated mass spectrometers capable of analyzing both light and heavy elements, including O, C, Rb-Sr, REE and U-Th-Pb; a clean laboratory for the preparation of samples for isotopic analysis, an XRF spectrometer for major and minor element analysis; all the necessary chemical and vacuum equipment to support mass spectrometer operations; and the ancillary equipment used in mineral separation, radiochemical storage, etc., a Cameca electron microprobe analyzer.

University of North Carolina, Chapel Hill

Dr. Andreas Teske's laboratory (started in Sept. 2002) currently contains molecular biology equipment for PCR, Denaturing gradient Gel Electrophoresis (DGGE), and digital gel image analysis and documentation. Spectrophotometers, electroporators, and incubation

chambers for transformed cells are available and updates to real-time PCR are planned. A new deionizing/UV water purification system and -80°C freezer dedicated to sample storage are in place. Current computers(Macintosh G4 and G3) allow medium-volume sequence alignment and phylogenetic analysis; the computer facilities will be updated with a Linux workstation in order to run the high-throughput phylogeny package ARB. Automated 96-well plate high throughput sequencing facilities are available in the sequencing center of the University, although the laboratory generally plans to collaborate with MBL on sequencing. The Department of Marine Sciences provides a permanently installed Polycom Viewstation SP videoconferencing system (with 50-inch plasma display screen).The molecular biology equipment will be complemented with the setup for anaerobic microbial cultivations, including anaerobic hood and Hungate gassing station for anaerobic culture during the year 2003.

The current laboratory space (ca. 700 square feet) and office space (ca. 200 square feet) accommodates at present two Ph.D. students and two Postdoctoral. The University of North Carolina at Chapel Hill has started an ambitious building program for a new Science Complex, and will provide new, personally customized laboratory and office space in for the Teske group in winter 2004/2005 (Phase I of the Science complex).

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Evolution of a Habitable Planet

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THE EVOLUTION OF A HABITABLE PLANET

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EXECUTIVE SUMMARY

The Evolution of a Habitable Planet

Achievements

The Penn State Astrobiology Research Center (PSARC), created five years ago as part of the NASA Astrobiology Institute, was composed of 16 (Co)-PIs and their research teams from The Pennsylvania State University (13), The University of Pittsburgh (2), and SUNY Stony Brook (1). The investigators represent a wide range of disciplines: geochemistry (7), paleontology (1), atmospheric chemistry (2), geomicrobiology (2), evolutionary genomics (2), and biochemistry and microbiology (2). The proximity of all members has enabled close interaction and a variety of collaborative research, teaching, and public outreach programs. PSARC has supported all or part of the research/education/PO activities carried out by 142 persons (16 (Co-)PIs, 22 research associates and postdoctoral fellows, five research assistants, two technicians, 49 graduate students, 37 undergraduate students, and five staff in administration/IT/EPO). This proposal requests funds from NASA to continue our research activities and to promote education and outreach programs in Astrobiology during the next five years.

Our primary research goal over the past five years (under the theme *Coevolution of the Earth and Life)* has been to increase understanding of the connections between the rise of major life forms during the early history of Earth (between ~3.8 and 0.5 billion years ago) and the evolution of the environment (especially atmospheric O_2, CO_2, and CH_4). This goal has involved investigations of: (1) pre-biotic atmospheric chemistry; (2) hydrothermal and photochemical reactions involving organic compounds and sulfide minerals; (3) evolutionary genomics; (4) biogeochemistry of methanogens and psychrophiles; (5) microbe-mineral interactions; (6) carbon isotope characteristics of organisms in laboratory and natural ecosystems; and (7) biogeochemical investigations of Precambrian soils and sedimentary rocks. Important accomplishments from our research include, but are not restricted to: (i) recognition of the importance of photochemical reactions in controlling atmospheric and oceanic chemistry prior to ~2.3 billion years ago (Ga); (ii) development of a theory for a methane-rich atmosphere and sulfate-poor oceans in the Precambrian; (iii) introduction of several new hypotheses for the rise of atmospheric O_2 and glaciation on early Earth; (iv) discovery of the oldest (2.6 Ga) remnant of microbial mats on land; (v) recognition from genomics of early (~1.2 Ga) diversification of plants, animals, and fungi; (vi) novel insights into microbial phylogenetics and into the biogeochemistry of methanogens and psychrophiles; (vii) increased understanding of microbe-mineral interactions; and (viii) recognition of the important roles played by anaerobic organisms, often living in consortia, in the evolution of the environment and life on Earth.

New Directions in Science and Research

For the next five years, our research to be conducted by 17 (Co)-PIs will build on these achievements while turning our focus toward critical issues concerning *planetary habitability*. Some of the important new frontiers include:

1. The impact of early Earth microorganisms: methanogens, sulfate-reducers, cyanobacteria, S-reducers, fermenters, sulfide-oxidizers, and eukaryotes. When did they arise and how did environmental changes (e.g., the rise of O_2) influence their evolution? How did these organisms influence the environment (atmospheric contents of CH_4, CO_2, and O_2; ocean chemistry; climate) of early Earth? How did they influence the global cycles of C, S, nutrients (N and P) and various

redox-sensitive metals (e.g., Fe and Mo) and the evolution of other organisms? What was the impact of these organisms on CH_4 and kerogen recycling during different stages of our planet's evolution, and how effective was such recycling?

2. The roles of photochemical reactions, especially of sulfur species in the atmosphere S and of S and Fe species in waters. How did various photochemical reactions influence Earth's early biosphere? What specific reactions and conditions led to the creation of mass independent fractionation (MIF) of S isotopes before ~2.0 Ga? How much sulfate in the early oceans was created by atmospheric photochemical reaction of volcanic SO_2, and how much by photochemical reactions involving pyrite in shallow water?

3. Detection of biosignatures on other planets. What are the likely biogenic gases on other planets, and how will they be found? What other astronomical methods are useful in the search for habitable planets?

Our approaches to answer these questions are multi dimensional. For the same questions, we seek clues from: (i) biogeochemical investigations on Precambrian rocks and modern ecosystems, (ii) laboratory (bio)chemical experiments in simulated environments, (iii) analytical investigations of genomic sequences, and (iv) numerical modeling. These approaches are summarized as follows:

Section I. Geochemical Record of the Early Earth's Biosphere. Research will focus on "fresh" drill core samples of sedimentary rocks (3.5 – 2.7 Ga in age) from the Pilbara district in Australia. Drilling will be completed under the Archean Biosphere Drilling Project, a new initiative by the NAI. By applying a variety of geochemical methods (e.g., molecular biomarkers, stable and radiogenic isotopes) on these and other drill core samples from various Precambrian districts, we will determine whether the organic matter preserved in Archean sedimentary rocks is biogenic or abiotic, and if biogenic, we aim to identify the organisms responsible. We will also determine temporal and spatial variations in the chemistry of these ancient sedimentary rocks, including MIF of S isotopes; C, N, and Fe isotopes; and the abundance ratios of bioessential metals.

Section II. Photochemical Reactions of Sulfur and Iron in the Early Earth. To understand the mechanism(s) for the creation of MIF of S isotopes and to aid in the reconstruction of the Precambrian S cycle using the isotope record in rocks, we will conduct a series of photochemical reactions of SO_2 by utilizing a reflectron time-of-flight mass spectrometer (RETOF-MS) and a femtosecond laser system coupled with the pump-robe technique. We will also conduct photochemical experiments on Fe-bearing compounds to aid in understanding the formational mechanisms of banded iron formations and seawater sulfate. Modeling of atmospheric reactions and isotopic analyses of the experimental products will also aid in interpretation of the isotope record.

Section III. Genomic Record of the Earth's Early Biosphere. We will continue analyses of protein and DNA sequences of key organisms, especially geologically significant microbial lineages, to better understand their relationships, times of origin, and history of lateral gene transfers. We will also strive to improve methods of analysis and integration of genomic data with the fossil record, biomarkers, and other aspects of biosphere history.

Section IV. Laboratory Microbial Simulations: Astrobiological Signatures. In order to relate the biogeochemical signatures in Precambrian rocks to specific organisms and environments and to understand the response of organisms to their environment, we will investigate the biological, biochemical, isotopic, and genetic response of pure cultures and consortia of important microbes

(e.g., methanogens, sulfate-reducers, and cyanobacteria) under simulated Precambrian environments. Special focus will be placed on the chemical and isotopic signatures of inorganic substrates, biogenic gases, and microbial lipids, and on the responses of anaerobes to oxygen and aerobes to methane and sulfide.

Section V. Modern Analogues of Precambrian Microbial Ecosystems. We will conduct a comprehensive multidisciplinary investigation of Fayetteville Green Lake, New York, a modern analogue of the Proterozoic marine biosphere. This provides a unique opportunity to link the physical and chemical properties of a water body to biological, biogeochemical, and genetic characteristics of both the planktonic and benthic ecosystems. Additionally, the biochemistry and genetics of microbial communities in polar ice will help us understand how organisms survived through the Snowball Earth during the Precambrian and how to detect life on the Europa. Investigations of extreme soil environments will help constrain the Precambrian terrestrial environment.

Section VI. Planetary Habitability and Life Detection. We seek to (i) investigate the chances of finding habitable planets around different types of stars; (ii) aid in the search for life on habitable planets of our Solar System and of other stars; and (iii) integrate studies of Earth's atmospheric evolution with astronomical observations. This will be approached by a combination of theoretical, observational, and laboratory-based research.

Section (VI) is an entirely new component of PSARC involving two astrophysicists and an atmospheric scientist to extend the knowledge acquired from studies on the Earth system to the search for life on other planets, and also to develop more complete educational and public outreach programs in Astrobiology. The goals of our proposed research are closely related to Astrobiology Roadmap Goals 1, 2, 4, 5, and 7.

Education and Public Outreach Programs

Public Outreach: A one-week Astrobiology workshop for high school teachers (~25 each year), conducted for the past four years, will continue. Also, in collaboration with WPSX, we have produced two 15-minute TV segments on Astrobiology for PBS' "What's in the News" program. These programs were viewed by more than five million K-12 students, and we plan to produce two more segments in the next five years. The College of Earth and Mineral Sciences at PSU plans to open a new museum by Fall 2003, where ~ 1400 sq. feet of space will be devoted to Astrobiology exhibits. We will also conduct a "Traveling Astrobiology Museum" program in which astrobiology exhibits will tour public schools (K-12 grades) in Pennsylvania and neighboring states.

Undergraduate Minor in Astrobiology: An inter-college undergraduate program in Astrobiology was established effective Fall 2000 semester at PSU. Approximately ten students from the Departments of Geosciences, Astronomy, Microbiology, Biology, and Mathematics have already received this B.S. minor. Student enrollment will grow substantially in the future.

Dual-Title Ph.D. Degree Program in Astrobiology: The proposal to create a dual-titled Ph.D. degree program in Astrobiology at PSU is currently under Faculty Senate review. Approximately ten graduate students from Geosciences, Biology, Chemistry, and Astronomy and Astrophysics departments are expected to enroll in the dual-title program effective Fall 2003 semester.

University Commitment: PSU's strong commitment to support NAI/PSARC programs is demonstrated by substantial support, which includes the creation of *two additional tenure-track faculty positions in Astrobiology*, pending funding of this grant proposal.

The Evolution of a Habitable Planet

INTEGRATED RESEARCH AND MANAGEMENT PLAN

Introduction and Overview

The research proposed here will be carried out during the next five years by the PI (Ohmoto) and 16 Co-PIs of the Penn State Astrobiology Research Center (PSARC) and their research staff and students. The 17 (Co-)PIs represent a wide range of expertise in Astrobiology: Atmospheric Chemistry (J. Kasting), Experimental Chemistry and Physics (A.W. Castleman), Geochemistry (M. Arthur, S. Brantley, R. Capo, L. Kump, H. Ohmoto, M. Schoonen, and B. Stewart), Paleontology (M. Patzkowsky), Microbial Biogeochemistry (K. Freeman and C. House), Evolutionary Genomics (B. Hedges), Biochemistry and Microbiology (J. Brenchley and J. Ferry), and Astronomy and Astrophysics (S. Sigurdsson and A. Wolszczan); Castleman, Sigurdsson, and Wolszczan have replaced the retiring R. Minard (Organic Chemistry) and M. Nei (Evolutionary Genomics).

Much of the early history of Earth's surface environment involves changes in the redox state of the atmosphere, oceans, and sediments. The chemical milieu that existed during the origin and early evolution of life has been viewed as strongly reducing (Miller & Urey, 1974) or oxidizing (Towe, 1994). This issue is inextricably intertwined with the Faint Young Sun Paradox, which has been addressed by invoking an atmosphere rich in greenhouse gases, either methane and ammonia (Sagan & Mullen, 1972; Sagan & Chyba, 1997; Pavlov et al., 2000a, 2001) or carbon dioxide (Kasting 1987, 1993). The establishment of an oxygen-rich atmosphere and ocean allowed for the evolution of energetic new life forms and perhaps extinction of some O_2-intolerant organisms. For these reasons, *the major research focus of PSARC has been directed toward our understanding of the connections between the evolution of the environment (especially atmospheric pO_2, pCO_2, and pCH_4) and organisms (both anaerobes and aerobes) and ecosystems during the period of ~3.8 Ga to 0.5 Ga.*

We have pursued the above goal using a variety of approaches, including: (a) modeling of photochemical reactions in the pre-biotic atmosphere; (b) laboratory experiments on hydrothermal and photochemical reactions involving organic compounds and sulfide minerals; (c) evolutionary genomics; (d) analyses of the biogeochemistry and genomics of methanogens and psychrophiles; (e) experimental microbe-mineral interactions; (f) carbon-isotope characteristics of individual organisms and consortia in laboratory and modern natural ecosystems; (g) search for biogeochemical signatures (molecular biomarkers; kerogen C isotopes; pyrite S isotopes); and (h) search for geochemical signatures for the redox states of the atmosphere and oceans in sedimentary rocks and soils of Precambrian ages. Results of these investigations have been published in 159 papers (including those submitted and in review but excluding abstracts of papers presented at national and international meetings) in refereed journals and books, including 23 published papers in *Science, Nature*, and *Proceedings of National Academy of Science* (see List of Publications, Volume II). Important accomplishments include, but are not restricted to: (i) recognition of the importance of photochemical reactions in controlling the atmospheric and oceanic chemistry prior to ~2.3 Ga (Pavlov et al., 2001a, b; Pavlov & Kasting, 2002); (ii) development of a theory of a methane-rich atmosphere and sulfate-

poor oceans in the Precambrian (Pavlov et al., 2001a, b); (iii) formulation of several new hypotheses for the causes of rise of atmospheric O_2 and glaciation on the early Earth (Kump et al., 2001; Pavlov et al., 2000a; Pavlov & Kasting, 2002; Lasaga & Ohmoto, 2002); (iv) discovery of the oldest (2.6 Ga) remnant of microbial mats (probably of cyanobacteria) on land (Watanabe et al., 2000); (v) recognition from genomics of early (~1.2 Ga) diversification of plants, animals, and fungi (Wang et al., 1999; Heckman et al., 2001); (vi) novel insights into microbial phylogenetics (Hedges et al., 2001; House & Fitz-Gibbon, 2002; Sheridan et al., in press) and into biogeochemistry of methanogens and psychrophiles (Ferry, 1999; Sheridan & Brenchley, 2000); (vii) increased understanding of microbe-mineral interactions (Brantley et al., 2001); and (viii) recognition of the important roles played by ecosystems of anaerobic organisms (e.g., methanogens and sulfate-reducers) in the evolution of environment and life on Earth (Kasting et al., 2001; Orphan et al., 2001, 2002).

Although we have made considerable progress toward achieving the initial goal of our research, many important questions still remain unresolved. For example, the sequence of oxygenation is unclear: an oxidized surface ocean may have been sandwiched between anoxic deep waters and atmosphere for some time before complete oxygenation (Kasting, 1993), initial oxidation may have been modest (Canfield, 1998), or the spread of O_2 may have been abrupt, and complete (Ohmoto, 1996). The most elusive element has been an explanation for this change in redox state. Was it simply the advent and proliferation of oxygenic photosynthesis (Hedges et al., 2001), or might a gradual rise in the oxygen fugacity of volcanic gases finally have ceased to serve as an effective sink for cyanobacterial O_2 (Kasting et al., 1993; Kump et al., 2001; Catling et al., 2001; Holland, 2002)? In either case, atmospheric oxygenation undoubtedly had climatic consequences, perhaps creating an interval of time during which the greenhouse effect was insufficient to prevent global glaciation (Pavlov et al., 2000**).** Biomarkers of cyanobacteria and eukaryotes have been found in ~2.7 Ga black shales from Western Australia by Brocks et al. (1999) and by our group (Eigenbrode et al., 2001). However, questions remain as to whether or not the pre-2.7 Ga sedimentary rocks contain biomarkers of any anaerobic and/or aerobic organisms. A recent controversy over the origin of organic matter in the 3.46 Ga Apex chert from Western Australia, whether it represents fossil material (Schopf, 1993) or a remnant of abiogenic hydrocarbons in a submarine hydrothermal vent (Brasier et al., 2002), illustrates how little we know about the origins, distribution, and evolution of organisms on the early Earth.

Major Questions

Our research during the next five years will address the remaining key issues and explore new frontiers in "the evolution of a habitable planet." The important new questions for our future research are summarized in the following three groups:

(1) **Questions related to the impact of important microorganisms on the early Earth, methanogens, sulfate-reducers, cyanobacteria, S-reducers, fermenters, sulfide-oxidizers, and eukaryotes**: When did they evolve? How did they influence the environment (the atmospheric contents of CH_4, CO_2, and O_2; ocean chemistry; climate) of the early Earth? How did they influence the cycles of nutrients (P, N) and bioessential elements (Fe, Mo, Zn, Cu, etc)? How did they influence the evolution of other organisms? How did the changes in environment (e.g., rise of O_2) influence their evolution? How was CH_4 recycled during different stages of our planet's evolution, by sulfate-dependent methanotrophy, aerobic methanotrophy, or through abiotic mechanisms (e.g., high-temperature reactions with ferric-bearing rocks)? During the

Phanerozoic era, the long-term global carbon cycle is characterized by the burial of atmospheric CO_2 as organic C (kerogen) and carbonate in sedimentary rocks and by the complete recycling of both kerogen and carbonate in sedimentary rocks to CO_2 (and HCO_3^-) during weathering. In the absence of atmospheric O_2 in the Archean, how did the global C cycle operate? What were the recycling mechanisms for kerogen? The global sulfur cycle in the Phanerozoic era is characterized by sulfate-rich oceans, an abundant formation of biogenic pyrite by sulfate-reducers in the oceans, and the complete conversion of pyrite S to SO_4^{2-} during weathering of rocks under an O_2-rich atmosphere. The S cycle is closely linked to the geochemical cycles of O, C, Fe, and many other redox-sensitive elements, and the evolution of a variety of organisms. How did the global S cycle operate in the Precambrian? Were Archean oceans sulfate-poor? Why was there relatively little variability in the carbon isotopic composition of the ocean during the Mesoproterozoic (1600-1000 Ma)? Was its deep ocean sulfidic, and if so, what effect did this have on the biogeochemical cycling of essential nutrients including trace metals? What were the fluxes of extraterrestrial materials (meteorites and cosmic dust) and their effects on the early biosphere?

(2) **Questions concerning the roles of photochemical reactions, especially of sulfur species in the atmosphere and of sulfur and iron species in waters**: What are the specific reactions and conditions that led to the creation of mass independent fractionation (MIF) of sulfur isotopes in rocks older than ~2.0 Ga (Fig. 1)? Is the MIF of S isotopes the "smoking gun" for the rise of atmospheric O_2? Why are there serious discrepancies between the MIF record in rocks and the MIF characteristics found by the previous photochemical experiments (Farquhar et al., 2000)? How much sulfate in the early oceans was created by atmospheric photochemical reactions of volcanic SO_2, and how much by photochemical reactions involving pyrite (FeS_2) in shallow water? How did various photochemical reactions influence the early Earth's biosphere?

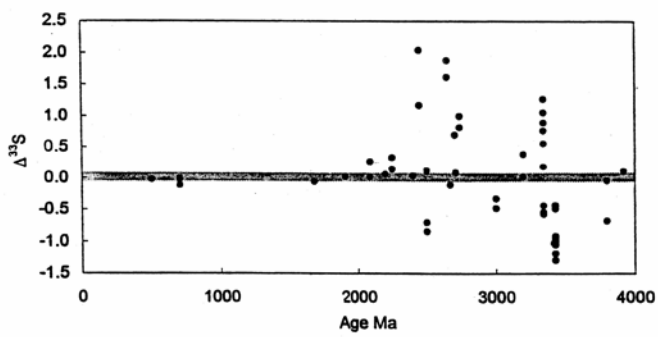

Fig. I. MIF factors of S isotopes ($\Delta^{\square\square}$Ṣvalues) in sedimentary rocks vs. their age (Farquhar et al., 2000). The magnitudes of deviation in the $^{34}S/^{32}S$ and $^{33}S/^{32}S$ ratios of a sample (*i*) from the corresponding ratios in Canyon Diablo troilite are expressed by $\delta^{\square\square}S_i$ and $\delta^{\square\square}S_i$ values, respectively. The magnitude of fractionation among the isotopes of the same element during normal (bio)chemical reactions is roughly proportional to the difference in their masses, leading to the relationship of: $\delta^{\square\square}S_i = 0.514\delta^{\square\square}S_i$. The deviation from this relationship, $\Delta^{\square\square}S = \delta^{\square\square}S_i - 0.514\delta^{\square\square}S_i$., is defined as the MIF factor. When $\Delta^{\square\square}S > |\pm 0.1|$‰, the sulfur is "mass independently fractionated".

(3) **Questions concerning biosignatures on other planets**: What are the likely biogenic gases on other planets, and can we observe them spectroscopically? What other astronomical methods might be useful in the search for habitable planets?

These questions are related to many of the goals of the NASA Astrobiology Roadmap, especially to Goal 1 "Understand the nature and distribution of habitable environments in the Universe", Goal 2 "Explore for past or present habitable environments, prebiotic chemistry and

signs of life elsewhere in our Solar System", Goal 4 "Understand how past life on Earth interacted with its changing planetary and Solar System environment", Goal 5 "Understand the evolutionary mechanisms and environmental limits of life", and Goal 7 "Determine how to recognize signatures of life on other worlds and on early Earth".

Research and Management Plan

Our approaches to answer the above questions are multi-dimensional. For the same questions, we seek clues from: (i) biogeochemical investigations on Precambrian rocks and modern ecosystems, (ii) laboratory (bio)chemical experiments in simulated environments, (iii) analytical investigations of genomic sequences, and (iv) numerical modeling. In this proposal, we have grouped our approaches into six, closely linked "Sections". Each Section of this proposal has a Section Leader and a group of Co-PIs (Subsection Coordinators) who are responsible for coordinating research projects focused on specific scientific problems. The PI (Ohmoto) will coordinate efforts to integrate the activities of all the Sections. While it is necessary to subdivide the research this way, we consider all of the sections to be intimately related. Our approaches are summarized as follows:

Section I. Geochemical Record of the Earth's Early Biosphere (Ohmoto-Leader, Arthur, Capo, Freeman, House, Kasting, Kump, and Stewart). Research will focus on "fresh" drill core samples of sedimentary rocks (3.5 – 2.7 Ga in age) from the Pilbara district in Australia. Drilling will be completed under the Archean Biosphere Drilling Project, a new initiative by the NAI. By applying a variety of geochemical methods (e.g., molecular biomarkers, stable isotopes) to these and other drill core samples from various Precambrian districts, we will determine whether the organic matter preserved in Archean sedimentary rocks was biogenic or abiogenic and, if biogenic, we will identify the original organisms. We will also attempt to identify the existence of any systematics in the temporal and spatial variations in sedimentary rocks, specifically the MIF of S isotopes; C, N, and Fe isotopes; and the abundance ratios of bioessential metals.

Section II. Photochemical Reactions of Sulfur and Iron in the Early Earth (Kasting-Leader, Arthur, Castleman, and Schoonen). To understand the mechanism(s) for the creation of MIF of S isotopes and to aid in the reconstruction of the Precambrian S cycle using the S isotope record in rocks, we will conduct a series of photochemical reactions of SO_2 by utilizing an innovative experimental technique developed by Castleman: a reflectron time-of-flight mass spectrometer (RETOF-MS) and a femtosecond laser system coupled with the pump-robe technique. We will also conduct photochemical experiments on Fe-bearing compounds to aid in understanding the formational mechanisms of banded iron-formations and seawater sulfate. Modeling of atmospheric reactions and isotopic analyses of the experimental products will help focus interpretations of the S isotope record in rocks.

Section III. Genomic Record of Earth's Early Biosphere (Hedges-Leader, House, and Patzkowsky). We will continue analyses of protein and DNA sequences of key organisms, especially geologically significant microbial lineages, to better understand their relationships, times of origin, and history of lateral gene transfers. We will also strive to improve methods of analysis and integration of genomic data with the fossil record, biomarkers, and other aspects of biosphere history.

Section IV. Laboratory Microbial Simulations: Astrobiological Signatures (House-Leader, Arthur, Brantley, Ferry, Freeman, and Ohmoto). In order to relate the biogeochemical signatures in Precambrian rocks to specific organisms and environments and to understand the response of organisms to their environment, we will investigate the biological, biochemical, isotopic, and genetic response of pure cultures and consortia of important microbes (e.g., methanogens, sulfate-reducers, and cyanobacteria) under simulated Precambrian environments. Special focus will be placed on the chemical and isotopic signatures of inorganic substrates, biogenic gases, and microbial lipids, and on the responses of anaerobes to oxygen and aerobes to methane and sulfide.

Section V. Modern Analogues of Precambrian Microbial Ecosystems (Kump-Leader, Arthur, Brenchley, Capo, Freeman, House, and Stewart). A modern analogue of the Proterozoic marine biosphere is Fayetteville Green Lake, New York. We will conduct a comprehensive, multidisciplinary investigation of the ecosystems in this lake to understand the connections between: (a) the changes in the physical and chemical characteristics of the water body, and (b) the changes in biology, biochemistry, and genetic characteristics of both the planktonic and benthic ecosystems. We will also carry out investigations on the biochemistry and genetics of microbial communities in polar ice in order to increase our understanding of how organisms survived through the Snowball Earth during the Precambrian and to predict the nature of organisms that might exist on Europa. We will investigate the biogeochemical processes of the formation of modern soils in order to aid in deciphering the record in Precambrian paleosols.

Section VI. Planetary Habitability and Life Detection (Sigurdsson-Leader, Capo, Freeman, Kasting, Stewart, and Wolszczan). In this section, we seek to integrate studies of Earth's atmospheric evolution with astronomical observations. The research objectives here are: (i) to investigate the chances of finding habitable planets around different types of stars; and (ii) to aid in the search for life on habitable planets of our Solar System and of other stars. The objectives will be approached by a combination of theoretical, observational, and laboratory-based research.

Many of the problems addressed in our investigations are controversial: the timing of the establishment of an oxygen-rich atmosphere, the interpretation of variations in the isotopic compositions of Precambrian rocks, and the fidelity of the Archean geochemical and fossil record. Diverse views are held on all of these and other issues, and are expressed in this proposal. Indeed, our group is not of one mind on all these issues, and that is reflected in what follows. We consider this a strength of our group: it provokes lively internal debates and hones our arguments and sparks further investigations. We all agree that in the end the ideas most strongly supported by observations, modeling, and experiments will prevail.

I. GEOCHEMICAL RECORD OF THE EARTH'S EARLY BIOSPHERE

Ohmoto (Leader), Kump, Arthur, Freeman, House, Kasting, Capo, and Stewart

This section presents an integrated study of the redox and biological evolution of the Earth preserved in the Precambrian rock record. A major focus of our field-related biogeochemical investigations will be high quality core samples (3.5-2.7 Ga in age) recovered from the Pilbara district in Western Australia under the Archean Biosphere Drilling Project (ABDP), a major new initiative of the NASA Astrobiology Institute (Section 1.1). We will use a variety of geochemical approaches to unravel the nature of marine and terrestrial biospheres on the early Earth, using ABDP and other samples (Sections 1.2.1-1.2.5). The results of these studies will be integrated with laboratory and theoretical investigations of the evolution of the Earth's early atmosphere, oceans, and biosphere (Sections 1.3, 1.4, and 2).

1.1. THE ARCHEAN BIOSPHERE DRILLING PROJECT (ABDP)

1.1.1. Overview

The most serious problem for paleontologists and geochemists attempting to understand the evolution of organisms and their biogeochemical environments from the ancient rock record has been the difficulty in obtaining important sequences of "fresh" rocks, i.e., rocks that have not been severely altered by post-depositional processes. This problem has been highlighted during discussions among researchers of the Mission to Early Earth (MtEE) Focus Group of NAI. Precambrian rocks, especially those of Archean age (>2.5 Ga), have generally been subjected to high-grade metamorphism (T >300 °C and P > 2 kb), often hydrothermal alteration, and a history of recent subaerial weathering (sometimes >10 million years) that has destroyed or altered the original biosignatures and mineralogical characteristics. Road cuts and tunnels can provide relatively "fresh" exposures, but they only intersect the most interesting geologic formations by chance. Exploration drilling typically focuses on areas exhibiting strong hydrothermal alteration, making these samples less desirable for geochemical studies.

The Archean Biosphere Drilling Project (ABDP) is an international drilling project to be undertaken by the Astrobiology Drilling Program (ADP), a new initiative of NAI. The main objectives of the ABDP are to recover "fresh" continuous sections of several key geologic formations (mostly sedimentary rocks 3.5-2.7 Ga in age) from the Pilbara district, Western Australia and use them to conduct systematic paleontological and biogeochemical investigations of the early Earth. These investigations will address (a) the nature of the biosphere, including the types and distribution of organisms in the oceans, lakes, and on land; (b) paleoclimate; (c) the chemistry of the atmosphere and oceans; (d) cosmic fluxes on the early Earth; and (e) geochemical fluxes of O, C, S, Fe, N, and other bioessential elements in the atmosphere-ocean-land mass-mantle system.

After several years of field surveys, the ABDP group has selected seven drilling sites where the target formations have been least affected by metamorphism, hydrothermal processes, and/or subaerial weathering subsequent to their formation. Our strategy is to drill several relatively shallow holes below the current and ancient groundwater tables rather than one or two long holes, in part for financial reasons: drilling five 300 m-deep cores is less expensive than drilling one 1000 m-deep core. More importantly, due to post-Archean tectonic movements, no area

exists in the Pilbara district where one or two deep drill holes will intersect all the geologic formations of interest.

The planning and execution of the drilling, core logging, sample distribution, and research will be coordinated by the ADP, including Bruce Runnegar (Director of NAI), representatives of the MtEE Focus Group, and representatives of the following four research groups: Hiroshi Ohmoto (PSARC, representing NAI), Munetomo Nedachi (Kagoshima University and other Japanese universities; PSARC Associate Member), Arthur Hickman (Geological Survey of Western Australia (GSWA)), and Mark Barley (University of Western Australia (UWA); PSARC Associate). With major funding from the Japanese Ministry of Education to M. Nedachi's group and from the NAI to PSARC, drilling will commence June 2003 and the final drilling will be completed by October 2003. Under the coordination of Ohmoto, Nedachi, and the ADP Advisory Committee, more than 50 researchers will be involved in various investigations of the ABDP drill cores, including: ~40 from the Japanese group; ~5 from the UWA, ~5 from the GSWA, and ~15 from PSARC (Ohmoto, Arthur, Freeman, Kump, House, Capo, and Stewart and their Research Associates and graduate students).

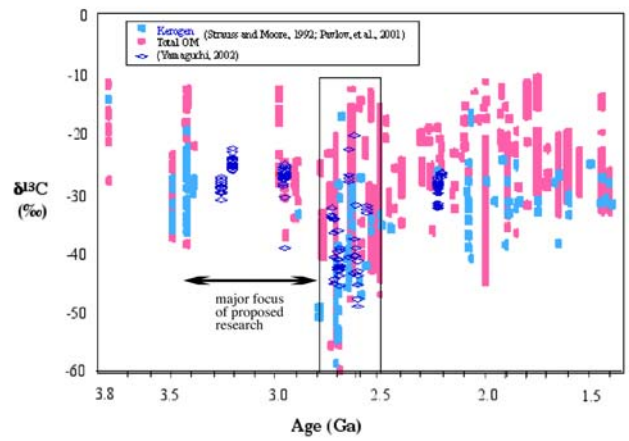

Fig 1.1 Carbon isotope record of sedimentary organic matter through Archean and early Proterozoic ages.

1.1.2. ABDP Drilling Targets

The ABDP will primarily focus on a variety of sedimentary rocks and submarine volcanic rocks in the Pilbara district of Western Australia that are older than 2.7 Ga (e.g., Buick et al., 1995, 2002). This district was selected primarily because (1) biogeochemical data (e.g., biomarkers and carbon isotope ratios) on rocks older than 2.7 Ga are very scarce (Fig. 1.1), and (2) Hickman and his GSWA colleagues have found that some areas of the Pilbara district were only subjected to very low-grade metamorphism (zeolites facies), and that many sedimentary units rich in organic carbon (black shales and black cherts) exist in these areas. Therefore, there is a high probability of discovering microfossils and a variety of molecular biomarkers of Archean age. The seven drilling sites (DDH 1 to 7) are located in a ca. 300 km x 200 km area (Fig. 1.2).

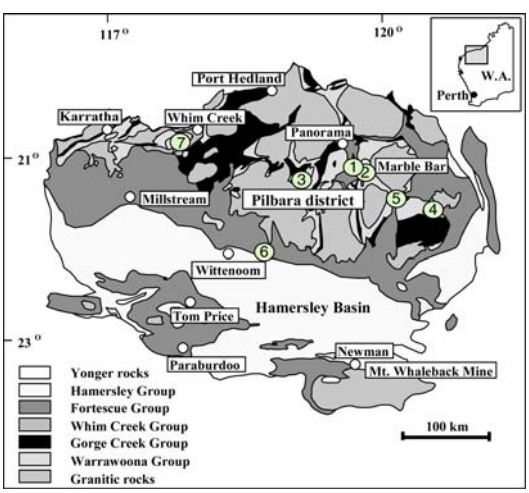

Fig 1.2 Sites of ABDP.

The Marble Bar chert (DDH 1) is one of the oldest chert formations (3.47 Ga). Black shales in DDH 2-4 are some of the oldest marine black shales (3.46-2.9 Ga), >100 m in thickness, and rich in organic carbon (>2 wt%) and pyrite (>0.2 wt%). DDH 5 and 6 offer some of the oldest (2.76-2.7 Ga) lacustrine shales, >100m in thickness. A ~20 m thick sericite-rich zone in the Mt. Roe Basalt (DDH-7; 2.7 Ga) has been cited by Rye and Holland (2000) as the best example of a paleosol formed under an anoxic

atmosphere, while Nedachi et al. (2002a, b) describe biomarkers of eukaryotes, cyanobacteria, and kerogens in this sequence and suggest that it could instead represent tuff layers deposited in a shallow marine environment that were altered by CH_4-rich hydrothermal fluids (T≈150°C).

1.1.3. Drill cores from other Precambrian Districts

During the past five years, the PSARC has collected a large number of drill cores of a variety of sedimentary and igneous rocks (~3.4 to ~1.8 Ga in age) from the Kaapvaal Craton in South Africa, the Hamersley district in Western Australia, the Abitibi, Wawa, and Wagigoon districts of Ontario, Canada, and the Outokump district in Finland. Because drilling of these was for mineral exploration, many of the samples were affected by hydrothermal fluids. Nevertheless, these drill cores have already yielded significant mineralogical and biogeochemical data (e.g., the contents of C, H, N, S, P, and major and trace elements, and $\delta^{13}C$ values of kerogen; Watanabe et al., 1997; Kakegawa et al., 1998; Yamaguchi, 2002; Watanabe, 2002). We will continue investigating these core samples as supplements to the ABDP cores.

1.2. RESEARCH AREAS: THE EARTH'S EARLY BIOSPHERE

1.2.1. Origins of Organic Matter in Archean Sedimentary Rocks

Major Questions: The recent controversy regarding the 3.46 Ga Apex chert, i.e., whether the organic matter (OM) is remnant of cyanobacteria-like organisms, and formed on a shallow continental shelf (Schopf, 1968; Schopf, 1993), or synthesized by the Fisher-Tropf reaction in a deep submarine hydrothermal vent (Brasier et al., 2002), illustrates how little we know about the genesis and environment of formation of OM in Archean rocks. Therefore, the first major question to be addressed in our investigations is:

(1). What were the origins and environments of OM in Archean cherts? Specifically, is the OM biogenic or abiotic? If biogenic, what type(s) of organisms produced it? What were the mechanism and environment for the formation of chert? (This last question will be addressed in Section 1.2.4). The Marble Bar chert (DDH 1) is an attractive drilling target because it is older than the Apex chert (3.47 vs. 3.46 Ga), but it has a very similar geologic environment (>10 m thick chert beds occur in a thick (> 2 km) succession of pillow basalt).

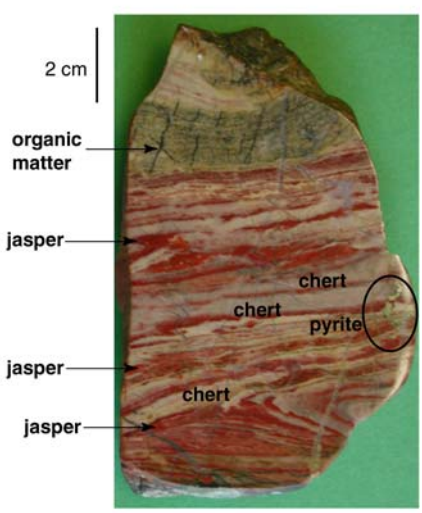

Fig 1.3. Marble Bar Chert/Jasper. Red color is due to an abundance of fine-grained hematite crystals.

Our preliminary investigation on outcrop samples has already revealed an abundance of organic matter in some chert layers (Fig. 1.3). The kerogen associated with pillow lavas is also an important research target because it may represent a remnant of microbial mats that developed on the seafloor during quiescent periods of submarine hydrothermal activity, while those in the chert/jasper units may represent microbial communities that developed during intensive periods of submarine hydrothermal activity.

Organic matter in shales from the ABDP target area is not a product of abiological hydrocarbon synthesis, because these thick formations (>100 m) extend over >100 km^2 of areas with uniformly high (>0.2 wt%) organic C contents. The organic matter in these shales is most certainly composed of various mixtures of the remnants of organisms that lived in the surface zone (planktonic organisms) of the oceans (or lakes), in a deeper water column, and on the sediment/water interface (benthic organisms), as well as possibly some "old" (detrital) kerogen. Considering the possible importance of methane in the Archean atmosphere, we add the following important questions for our investigations:

(2) Who were the most important primary producers (autotrophs) in the Archean? Were they methanogens that utilized the CO_2 and H_2 in the Archean atmosphere for organic synthesis (reaction 1-1), anoxygenic photoautotrophs, such as green sulfur bacteria (reaction 1-2), or oxygenic photoautotrophs, such as cyanobacteria (reaction 1-3)?

$$CO_2 + 2H_2 \Rightarrow CH_2O + H_2O \qquad (1\text{-}1)$$

$$CO_2 + 2H_2S \Rightarrow CH_2O + 2S^0 + H_2O \qquad (1\text{-}2)$$

$$CO_2 + H_2O \Rightarrow CH_2O + O_2 \qquad (1\text{-}3)$$

(3) What was the principal mechanism for the generation of CH_4 by methanogens? Was it by directly utilizing the CO_2 and H_2 in the atmosphere (reaction 1-4) or by the fermentation (reaction 1-5) of the OM that was generated by reaction (1-2) or (1-3)?

$$CO_2 + 4H_2 \Rightarrow CH_4 + 2H_2O \qquad (1\text{-}4)$$

$$2CH_2O \Rightarrow CO_2 + CH_4 \qquad (1\text{-}5)$$

(4) Who were the principal methane-oxidizing microbes? Were they methanotrophs that utilized dissolved O_2 (reaction 1-6) or anaerobic methanotrophs (Archaea and/or Bacteria) that utilized dissolved SO_4^{2-} (reaction 1-7)?

$$CH_4 + 2O_2 \Rightarrow CO_2 + 2H_2O \qquad (1\text{-}6)$$

$$CH_4 + SO_4^{2-} + 2H^+ \Rightarrow CO_2 + H_2S + 2H_2O \qquad (1\text{-}7)$$

Recycling of CH_4 is an important issue, because without the recycling, the conversion of crustal carbon to reduced forms, CH_4, "oil slicks" (Lasaga et al., 1971), and kerogen, might have been a substantial part of the carbon cycle influencing the habitability of particular microbial niches.

(5) What was the burial flux of organic matter considering primary production (e.g., photoautotrophy and chemautotrophy), and carbon remineralization through fermenters, methanogens, and sulfate reducers? What portion of this flux represents carbon produced from oxygenic photoautotrophy (reaction 1-3)? This information is critical in estimating global O_2 production fluxes (e.g., Holland, 1984; Lasaga & Ohmoto, 2002).

(6) Recycling of "old" kerogen to CO_2 by aerobic reactions (biological and abiological) is an essential element of the Phanerozoic carbon and oxygen cycles. This recycling has produced an essentially constant ratio (~1/4) of organic C to carbonate C and nearly constant $\delta^{13}C$ values (~-25‰ and ~0‰ for organic C and carbonate C, respectively) in Phanerozoic sedimentary rocks (e.g., Holland, 1984; Lasaga & Ohmoto, 2002). If kerogen is not expected to decompose during weathering under an anoxic atmosphere (Ohmoto, 2003), then what were the recycling mechanisms of "old" kerogen in the Archean? Did the ratio of detrital kerogen to the organic matter in (5) change with geologic history, and can it be related to the evolutionary history of atmospheric oxygen?

Kerogen with the most negative $\delta^{13}C$ values in Precambrian sedimentary rocks (~-60‰) was extracted from a few samples of the 2.7 Ga Tumbiana Formation in the Hamersley district, Western Australia (Strauss & Moore, 1992). These very negative $\delta^{13}C$ values suggest the existence of a microbial community in a 2.7 Ga lake that consisted of: (a) methanogens that

produced isotopically very light CH_4, (b) microbes that oxidized the CH_4 to isotopically very light CO_2, and (c) microbes that used the isotopically light CO_2 for organic synthesis. However, interpretations vary among researchers. For example, Hayes (1994) suggests that methanotrophs utilizing atmospheric CO_2 and O_2 provided the principal mechanism for methane recycling (reaction 1-6), and that ~2.7 Ga ago cyanobacteria and methanotrophs (aerobes) evolved and the oceans became stratified with an oxygenated surface zone and an underlying anoxic water body. Hinrichs et al. (2002) suggest that sulfate-reducing microbes (SRM) provided the principal mechanism for methane recycling (reaction 1-7) and that these microbes evolved around 2.7 Ga. Pavlov et al. (2001) suggest a linkage between the appearance of cyanobacteria and the formation of an organic haze-rich atmosphere at ~2.7 Ga. All of these interpretations have been based on a very limited set of data from weathered outcrops. Our multidisciplinary investigations, described below (1.2.1.1-1.2.1.3), will be carried out systematically on a much larger number of samples.

1.2.1.1. Geochemical analyses of kerogen-bearing rocks (Ohmoto)

Although we expect to find biomarkers in many of the drill core samples, nearly 100% of organic matter (OM) in Archean rocks occurs as kerogen. The previous studies carried out by Ohmoto's group on Precambrian shales (mostly 2.7-2.0 Ga in age) from South Africa, Canada, and Australia (e.g., Watanabe et al., 1997; Yamaguchi, 2002; Watanabe, 2002) have demonstrated the origins of kerogen and its depositional environment can be constrained from systematic investigations, including: (a) microscopic observations using optical and electron microscopes to determine the modes of occurrence (layers vs. disseminated), grain size, morphology, and textures of kerogen in rocks; (b) analyses of the abundance ratios of C, H, N, S, P, and major elements (especially Al, Ti, Si, and Fe) in bulk rocks and extracted kerogen; and (c) analyses of $\delta^{13}C$ and $\delta^{15}N$ values of kerogen and whole rocks. We will adopt the same approach to the studies on the drill core samples.

1.2.1.2. Biomolecular analyses (Freeman and House)

Biomarker compounds associated with cellular membranes preserved in Archean sedimentary rocks provide evidence for a wide diversity of microbial life on the early Earth. For example, published reports (Brocks et al., 1999; Summons et al., 1999) document abundant 2-methylhopanes in the Late Archean, suggesting the presences of cyanobacteria cells at that time. At PSU, Freeman and House's groups have been working to expand the investigation of Late Archean biomarkers with a goal of evaluating isotopic and molecular signatures in the context of geographic locations, stratigraphic position, and sample lithology. A key objective of this project is to evaluate authenticity of the biomarkers, and to identify possible contamination. Initial work has focused on kerogen and lipids preserved in core and outcrop samples of Late Archean (2.8-2.5 Ga) sedimentary rocks from across the Hamersley Province in Western Australia (Fig. 1.4).

Molecular analyses include both extracted compounds and those released by pyrolysis of the insoluble organic matrix (kerogen). We have employed a sequential strategy, whereby samples are treated to solvent extraction, mineral digestion (with HCl and HF), an additional solvent extraction, and pyrolysis in a H_2 atmosphere. Molecular structures provide supporting evidence for the authenticity of the extracted compounds. The ancient nature of the extracted biomarkers is supported by similarities in aromatic compounds, thermally mature molecular isomer data for the extracts, and the lack of any indicators for contamination or young age-specific biomarker

patterns in any fraction. This work is continuing with additional molecular and isotopic analyses. Molecular structures extracted from Late Archean samples provide evidence for all three domains of life: the Archaea (acyclic isoprenoids), the Bacteria (hopanes) and the Eukaryotes (diverse sterane structures). We have tentatively identified trace quantities of compounds associated with green sulfur bacteria (aryl isoprenoids); we have also found 3-methyl hopanes in low quantities, which signify inputs from either aerobic methane oxidizing bacteria or acetogenic organisms. We have also identified series of dimethyl and diethyl alkanes, which are associated with inputs from sulfide-oxidizing bacteria. In Figure 1.4, we highlight branches on a genome-based tree (Wolf et al., 2002; House et al, submitted) that reflect the broad taxonomic diversity of organisms recorded by Late Archean biomarkers.

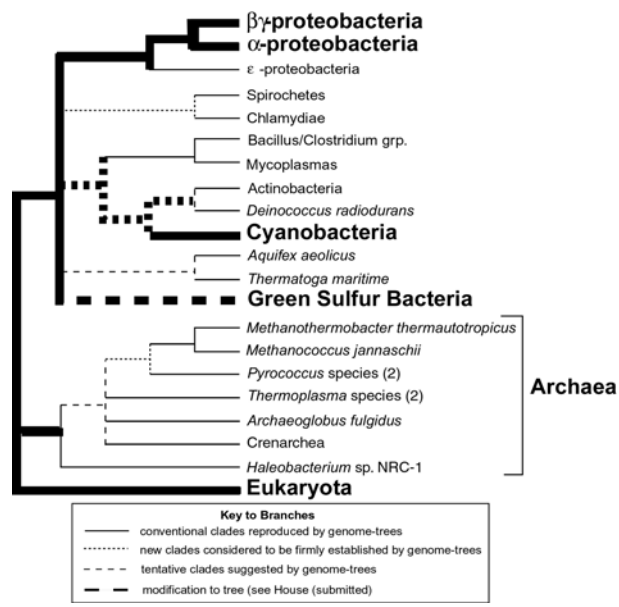

Figure 1.4. Genome-base phylogenetic tree modified from Wolf et al. (2002) and House, etal. (submitted). Highlighted lines indicate representation among late Archean biomarkers (Eigenbrode et al., 2003).

We will continue this line of inquiry by extending it to other samples of similar age and by employing older samples recovered by ABDP. We will initially conduct elemental and isotopic analyses of kerogen in order to evaluate thermal maturity and the overall association of $\delta^{13}C$ signatures with lithology and age. We will then follow a strategy that parallels our methods noted above, using a sequential process to isolate free compounds and compounds associated with the kerogen matrix released through mineral digestion and stepped H_2 pyrolysis. Work will target the Tumbiana Formation (2.7 Ga) and the Mosquito Creek Formation (2.9 Ga) in order to compare results with our current study (which inlcudes Tumbiana samples). We will also extend our findings further back in time with samples from the Sulfur Spring Formation (3.2 Ga), although we note that recovery of biomarker compounds become increasingly less probable in older units.

1.2.1.3. Microfossil isotopic analyses (House)

Geochemical studies have been extremely useful for identifying broad secular trends throughout the Precambrian and for suggesting the involvement of particular microorganisms in the carbon cycle at particular times during the past. Nonetheless, these analyses lack high spatial resolution, and therefore, are missing key information that might be useful in the discovery of biochemical characteristics of the earliest microbial ecosystems. Secondary ion mass spectrometry (SIMS) offers a powerful tool capable of isotopic analyses of 10 – 15 μm spots (Ireland, 1995).

Recently, several papers have shown the usefulness of SIMS for the carbon isotopic analyses of Precambrian graphite (Mojzsis et al., 1996; Ueno et al., 2002), of Precambrian microfossils (House et al., 2000; Kaufman & Xiao, 2001; Ueno et al., 2001), and of modern microbes from environmental samples (Orphan et al., 2001, 2002). In general, there are a number of

Precambrian paleobiological applications for carbon isotopic analyses by SIMS, including linking microfossils to metabolic pathways, constraining estimates of the partial pressure of carbon dioxide in the atmosphere at during the Precambrian, and ascertaining whether or not methane was an important part of an ancient microbial ecosystem. These investigations will be carried out in collaboration with the Center for Astrobiology at UCLA, and in conjunction with the studies in Section (*1.2.1.2)*.

1.2.2. Evolution of the Geochemical Cycle of Sulfur: Mass Independent Fractionation of S Isotopes, Sulfate-Reducing Microbes, and Sulfate in the Precambrian Oceans

Major Questions: In most previous investigations, evidence for sulfate-reducing microbes (SRM) in Archean oceans was sought in the variation of the $^{34}S/^{32}S$ abundance ratio ($\delta^{34}S$ value) of pyrite crystals in Archean sedimentary rocks, because until recently, the only known mechanism for $\delta^{34}S$ variations in surface environments was bacterial sulfate reduction. The SO_4^{2-} content of Archean oceans has also been estimated primarily from the difference in the $\delta^{34}S$ values between pyrite and barite ($BaSO_4$) crystals in Archean sedimentary rocks: $\Delta_{SO4\text{-}py} = \delta^{34}S_{SO4} - \delta^{34}S_{py}$. Such an approach has suggested that SRM evolved by ~3.4 Ga (Ohmoto et al., 1993; Shen et al., 2001). However, depending on the interpretation of the mechanisms controlling the $\Delta_{SO4\text{-}py}$ values, the suggested values for the SO_4^{2-} content of Archean oceans greatly vary from "the same as today" (Ohmoto & Felder 1987), to "less than 1/10 of today" (e.g., Lambert et al., 1978), to "less than 1/100 of today" (Habicht et al., 2002). An important question to investigate is the minimum level of seawater sulfate content required for the anaerobic recycling of methane (Reaction 1-7) to become important.

The discovery by Farquhar et al. (2000) of the MIF of S isotopes in some sedimentary rocks older than ~2.0 Ga (described in Section 1.1) has presented a serious problem in the conventional approach, which has assumed all (or most) pyrite crystals in sedimentary rocks formed by bacterial sulfate reduction, thus relating the $\Delta_{SO4\text{-}py}$ values to seawater SO_4^{2-} content. According to Farquhar et al. (2000), the observed $\delta^{34}S$ fractionation between pyrite and sulfate in Archean sedimentary rocks could have been created largely by atmospheric reactions, rather than by SRM. Some recent researchers (e.g., Pavlov & Kasting, 2002; Runnegar, 2001; Runnegar et al., 2002; Holland, 2002) have even suggested that all pyrite and barite crystals in Archean sedimentary rocks formed from S^0 and SO_4^{2-}, which were produced in an O_3- and O_2-free atmosphere by the UV radiation of volcanic SO_2. Therefore, an important objective of this research is to provide a link between the experimental and theoretical approaches (see Sections 2.1 and 2.2) and the geologic record of MIF of S isotopes, and in so doing, answer the following major questions:

(1) What are the causes of MIF in geologic samples? Related questions include: (a) What are the reason(s) for a serious discrepancy between the MIF values observed in natural samples and those observed in laboratory experiments (Fig. 1.5)? While most sulfide-bearing geologic samples with $^{33}\Delta$ values outside the 0±0.1 ‰ range are characterized by positive signs for both $\delta^{34}S$ and $\delta^{33}S$ values, S^0 produced by short-wave

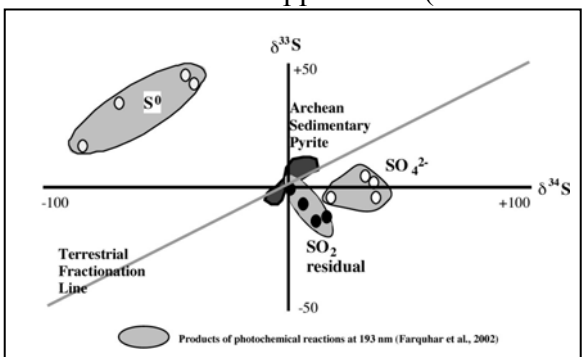

Fig 1.5. MIF of S isotopes, experiment vs. natural data

UV are characterized by very large and variable negative $\delta^{34}S$ and positive $\delta^{33}S$ values. (b) What important parameters determine the $\delta^{34}S$, $\delta^{33}S$, and $^{33}\Delta$ values of natural pyrites and sulfates? What mechanism(s) produce at least two well-defined MIF trends in natural samples (see Fig. 2.4 in Section 2.2)? Data obtained from sedimentary and igneous rocks (3.2-2.5 Ga in age) from the Pilbara-Hamersley district (Onoet al., submitted; Runnegar et al., 2002; Ohmoto et al., in prep.) indicate that most of the MIF values in these district fall around two lines: $^{33}\Delta \approx \delta^{34}S + 0.90$ or $^{33}\Delta \approx 1‰$ (see Fig. 2.4 in Section 2). (c) What are the connections between the MIF of S and volcanism? Recent discoveries of the existence of MIF ($^{33}\Delta$ = +0.6 to –0.4‰) in volcanic ash from the Mt. Pinatubo volcano (Savarino et al., 2002) and the correlation between $^{33}\Delta$ values and Zn contents in the 2.5 Ga McRae shales (Ohmoto, unpublished data) suggest the connection between the MIF and the atmospheric O_2 level could be more complicated than previously suggested.

(2) When did SRM evolve on Earth?

(3) How did the SO_4^{2-} content of normal oceans change through the Archean and Proterozoic eras? The sulfate content of Proterozoic oceans, as well as that of Archean oceans, is of great interest to us, because the disappearance of banded iron formations (BIFs), possible changes in the redox cycles of Fe, Mo, and other elements, and evolution of new organisms in the Proterozoic may have occurred due to the formation of H_2S-rich oceans, which required a high concentration of seawater sulfate (Canfield, 1998; Anbar & Knoll, 2002).

In order to answer the above questions, we will conduct the following investigations (1.2.2.1-1.2.2.2):

1.2.2.1. Multi-geochemical analyses of S-bearing sediments (Ohmoto)

Sulfur isotope analysis alone will not fully answer any of the above questions. Our investigations will be first directed toward identifying and quantifying pyrite crystals (and sulfate minerals) of different origin: (1) those of detrital origin (type-a), (2) those formed during the accumulation of host rocks (i.e., syngenetic and early diagenetic pyrite; type-b), and (3) those formed after the early diagenetic stage (typically by hydrothermal fluids; type-c). "Atmospheric pyrite" (i.e., pyrite formed by utilizing the S^0 produced by photochemical reactions in the atmosphere) and biogenic pyrite belong to type-a. Identification of different types of pyrite (and sulfate) crystals requires detailed petrographic, mineralogical, and geochemical investigations using a variety of microscopes (optical, electron, and X-ray) for the occurrences, morphologies, textures, and chemical compositions of individual pyrite crystals, as well as bulk-rock analyses of the abundance ratios of S, C, and major-, trace- and REE elements. For example, positive correlations can be expected between the contents of pyrite S and organic C if the pyrite crystals were mostly formed by SRM during the accumulation of sediments. This type of multi-geochemical investigations has been routinely carried out by Ohmoto's group (Watanabe et al., 1997; Naraoka & Ohmoto, submitted; Yamaguchi, 2002; Watanabe, 2002).

Following the above types of geochemical investigations, different phases of S-bearing compounds (pyrite, other sulfides, sulfate, and organic S) will be sequentially extracted, converted to Ag_2S, and analyzed for $\delta^{33}S$ and $\delta^{34}S$ values. We have already analyzed more than 30 samples of sedimentary and igneous rocks from the Pilbara district. Both the $\delta^{33}S$ and $\delta^{34}S$ values must be determined in order to quantitatively estimate: (a) the fractions of "atmospheric pyrite" and biogenic pyrite in sedimentary rocks, and (b) the true isotopic fractionation factors between the biogenic pyrite and the contemporaneous seawater sulfate. The $^{33}\Delta$ values should fall within 0±0.1‰ if the S atoms in pyrite (and other compounds) have only been involved in

normal biological and chemical processes during their entire history; $^{33}\Delta$values outside this range have been attributed to photochemical reactions (Farquhar et al., 2000, 2001). Since the amount of biogenic pyrite in a marine sediment depends on the sulfate content of the seawater, determination of the average contents of biogenic sulfides in Archean shales becomes critically important in constraining the sulfate content of the Archean seawater (Fig. 1.6).

The primary focus of our investigations will be the drill core samples from the ABDP. We will investigate sediments that were deposited under a variety of geologic environments, including: (a) lake sediments; (b) carbonates; (c) gray (organic C-poor) shales and sandstones that deposited on an open continental shelf; (d) black (organic C-rich) shales that deposited in marine anoxic basins; and (e) shales that accumulated in deep submarine hydrothermal environments. We will investigate lacustrine sediments because these rocks are expected to contain the highest proportion of "atmospheric pyrite". We will examine the relationships between the magnitude of MIF and the various geological and geochemical parameters (e.g., stratigraphy, lithology, paleogeography, and volcanic and hydrothermal inputs) in order to understand the causes of MIF of S isotopes and the significance of MIF records in geologic samples.

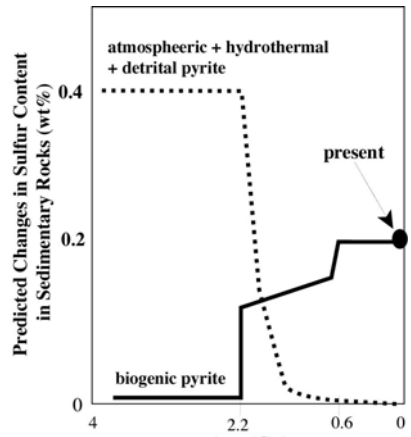

Fig. 1.6. The temporal changes in the contents of biogenic pyrite and of (atmospheric + hydrothermal + detrital) pyrite in sedimentary rocks as expected from the S-cycle models of Canfield et al. (1999, 2000), Habicht et al (2002), Runnegar (2002), and Holland (2002).

1.2.2.2. Sulfate analyses of carbonates (Arthur)

Sedimentary anhydrite ($CaSO_4$) is rare in Archean formations, possibly because it was lost during metamorphism and/or by reactions with groundwater. Therefore, previous researchers analyzed barites ($BaSO_4$), which may be the replacements of anhydrite through hydrothermal reactions, to estimate the S isotopic composition of Archean seawater sulfate. However, the SO_4^{2-} in Archean barites may be of hydrothermal origin, not normal seawater. Anhydrite beds are more common in Proterozoic formations, but are still limited in temporal and spatial distributions. In contrast, sedimentary carbonates are more widely distributed; most carbonates contain trace amounts of sulfate, which was probably incorporated into carbonates during precipitation from seawater (or lake water). At PSARC, Arthur's group has analyzed: (a) the content of trace sulfate in carbonate; (b) its $\delta^{34}S$ and $\delta^{18}O$ values; (c) the $\delta^{34}S$ value of trace pyrite in the carbonate; and (d) the $\delta^{13}C$ and $\delta^{18}O$ values of the host carbonate from ~600 Ma carbonate sequences in Namibia and Arizona, and reconstructed a detailed history of the content and isotopic composition of seawater sulfate during the period of the Snowball Earth (Hurtgen et al., 2002).

We will extend our investigations of sedimentary carbonates to those of the middle and early Proterozoic ages, and also of Archean age, in order to reconstruct the evolutionary history of sulfate in the oceans through geologic time.

1.2.3. The Nutrient Cycles (Arthur, Kump, and Ohmoto)

A combination of techniques, in part described above (Sections 1.2.1.1 and 1.2.2.1), will allow us to evaluate evolution of nutrient cycles in the Archean and Proterozoic oceans. These techniques include studies of the concentration and isotopic composition of nitrogen in preserved organic compounds, and an analysis of organic and inorganic phosphorus concentrations in sedimentary rocks of various lithologies, particularly black shales. A major question is how changes in the ocean-atmosphere redox state influenced cycling of nitrogen and phosphorus. Furthermore, how did evolutionary innovation, for example the origination and prominence of biological nitrogen fixation or the origination and proliferation of denitrifying bacteria, both of which impart strong isotopic signals on organic nitrogen, affect ecosystem productivity? What was the interdependence of environmental evolution, nutrient availability and productivity? For example, what was the balance of nitrogen fixation vs. denitrification following the onset of an oxygenated atmosphere? Another intriguing question is what led to a relatively low and flat carbon isotopic composition of carbonates through most of the Proterozoic, sandwiched between periods of apparently responsive carbon isotope variation in the early Paleoproterozoic and late Neoproterozoic? The solution to these problems may lie in changes in nutrient cycling as a response to redox variations, or, as proposed by Anbar and Knoll (2002), in limitations on nitrogen fixation imposed by certain redox-sensitive trace metals. This work will also test the validity of Canfield's (Bjerrum & Canfield, 2002) hypothesis that the productivity in the Archean oceans was much less than that in the Phanerozoic oceans because a higher proportion of phosphate in the Archean oceans was removed by ferric oxides in BIFs.

There have been several attempts to provide a temporal view of the nitrogen isotopic evolution of Precambrian organic matter. These studies have used either bulk nitrogen isotopic compositions of chert (Beaumont & Robert, 1999) or the isotopic composition of ammonia in low-grade metamorphic rocks (Boyd, 2001; Pinti et al., 2001). We propose to study the isotopic composition of nitrogenous compounds preserved in shales and carbonates. This requires an approach that sequentially examines organic N, absorbed N_2 and mineral-bound N in consideration of the possible effects of diagenesis; the similar approach was proven successful by Yamaguchi (2002) on shales from drill core samples (3.2-2.0 Ga in age) from the Kaapvaal Craton, South Africa. In an attempt to understand what the consequences of oceanic oxygenation might have been for nutrient cycling, we will also utilize a numerical biogeochemical cycle model for oceanic nitrogen and phosphorus that is used to test assumptions regarding the effects of widespread deepwater vs. whole-ocean (atmosphere) anoxia on nitrogen availability to plankton and nitrogen isotopic values for sedimented organic carbon.

In addition to the ABDP core samples, we will study suites of samples of black shale and interbedded lithologies from Labrador (Ramah Group, 1.9 Ga, Hayashi et al., 1997), Ontario Canada (Serpent Fm and Whitewater Group, 1.85 Ga, Fedo et al., 1997), Western Australia (Earaheedy Group, 1.7 Ga, Krapez and Martin, 1999; Bangemall Group, 1.6 Ga, Buick et al., 1995), Arctic Canada (Dismal Lakes Group, 1.3-1.27 Ga, Frank et al., in press) and NW Territories, Canada (Shaler Supergroup, 1.0 Ga, Rainbird et al., 1996), and Neoproterozoic samples from Australia, Great Basin, U.S., and Namibia from our earlier work (Hurtgen et al., 2002; Hurtgen & Arthur, in prep.). We propose further field work in Labrador and NW Territories and additional work in Western Australia in order to better sample units there. Suites of fresh samples are available to us through collaborative studies (T. Frank and T. Lyons: PSARC Associates) for the other sites.

1.2.4. Geochemical Cycles of Fe and Bioessential Metals

Major Questions: An important characteristic of Archean and early Proterozoic sedimentary rocks is the abundance of rocks comprising of mostly silica (quartz, SiO_2), iron oxides (hematite, Fe_2O_3; and magnetite, Fe_3O_4), and/or iron carbonates (siderite, $FeCO_3$). Depending on the relative abundance of these minerals, the rocks are classified as chert, ferruginous chert or jasper (see Fig. 1.3), oxide-type BIFs, or carbonate-type BIFs. Cherts are the most favorable for a search of microfossils due to their resistance to thermal and chemical modifications. Some important astrobiological questions concern: (1) the depositional environment of cherts, jaspers, and BIFs (shallow continental shelf or deep submarine hydrothermal fields); (2) the sources of SiO_2 (continental weathering or submarine hydrothermal fluids); (3) the source of Fe^{2+} (continental weathering, hydrothermal fluid from the mid-ocean ridges, or local submarine hydrothermal fluids); (4) the source of O_2 (locally produced or atmospheric); (5) the source of CO_2 and HCO_3^- (atmospheric and oceanic or hydrothermal); (6) the mechanisms for precipitation of the primary minerals (evaporation, photochemical reactions, microbial precipitation, or mixing of hydrothermal fluids and seawater); and (7) the biogeochemistry of heavy metals in the Archean oceans. These questions will be answered from the following two types of investigations (1.2.4.1-1.2.4.2).

1.2.4.1. Geochemical analyses of cherts and shales (Ohmoto)

We expect our mineralogical and geochemical investigations of the Marble Bar chert-jasper sequence (DDH 1) will help settle the controversies on the processes and environment of formation for cherts, Fe-oxides, and Fe-carbonates in the Archean oceans. Their geologic settings (e.g., close association with pillow lavas, absence of clastic materials) suggest they formed in deep (>1 km) submarine environments, where the precipitation mechanisms of these minerals are quite limited. Beginning with detailed petrographic and mineralogical investigations, we will conduct studies on fluid inclusion, Ge/Si ratios (e.g., Hamade et al. 2003), and $\delta^{18}O$ values of cherts (Karhu & Epstein, 1986), along with rare-earth element ratios (especially Eu and Ce anomalies: Kato et al., in press).

The solubilities of major host minerals in igneous and sedimentary rocks for Mo (molybdenite and pyrite: MoS_2, FeS_2), U (feldspars and uraninite: UO_2), W (sheelite: $CaWO_4$), and V (V-oxides) generally decrease with decreasing Eh, while those of host minerals for Fe, Ni, Cu, and Zn (ferrous silicates and sulfides) increase with decreasing Eh; most of these minerals increase their solubility with decreasing pH (Stumm & Morgan, 1996). We will investigate the contents of these bioessential metals in the Archean oceans and lakes by normalizing trace element abundances to Al and Ti (i.e., clastic components) in shales from DDH 2-5, and by comparing these data with those in Phanerozoic shales. Therefore, if the Archean atmosphere was anoxic and the oceanic pH was similar to today, we would expect the contents of Mo, W, U, and V in the Archean oceans to have been higher and those of Fe, Ni, Cu, and Zn to have been lower than those in the modern oceans. Such differences may have created Archean biota quite different from today because the types of metal requirements greatly vary among different microbes (Madigan et al., 2000).

1.2.4.2. Radiogenic and Fe isotope analyses (Stewart and Capo)

Recent advances in the measurement of Fe isotopes (Johnson & Beard, 1999; Mandernack et al., 1999; Anbar et al., 2000; Bullen et al., 2001) have opened a new avenue for studying this essential, redox-active element. The mass difference between isotope pairs is sufficient to allow for measurable mass fractionation during low-temperature (earth surface) geologic processes (Urey, 1947; Polyakov, 1997; Schauble et al., 2001; Anbar, 2001; Matthews et al., 2001). Samples to date show a δ^{56}Fe range of about –2 to +1‰, with a typical uncertainty of 0.1-0.2‰ (summarized by Anbar, 2001). Beard and Johnson (1999) suggested that biological processes should produce the strongest isotope fractionation effects. However, subsequent experiments have demonstrated biologically mediated reactions with no fractionation effects (Mandernack et al., 1999; Brantley et al., 2001), as well as the abiotic fractionation of Fe isotopes in laboratory experiments (Anbar et al., 2000; Matthews et al., 2001; Johnson et al., 2002; Skulan et al., 2002) and field studies (Bullen et al., 2001).

Currently, limited BIF data suggest small (<1‰), but systematic and significant positive shifts in δ^{56}Fe in certain BIF bands, which were interpreted as reflecting a biogenic origin (Beard et al., 1999). However, these shifts could also reflect abiotic fractionation processes (e.g., Schauble et al., 2001; Zhu et al., 2002), or Fe from different sources, such as hydrothermal fluids (Sharma et al., 2001) or continental weathering (e.g., Zhu et al., 2000). Iron isotopes have promise as a provenance indicator for Fe, especially when combined with other isotope systems such as Sm-Nd or Rb-Sr. For example, a Nd isotope investigation of the Hamersley Group carbonate demonstrates similar sources of REE for both shallow and deep water carbonates (Stewart et al., 2002). In our investigations of Precambrian BIF petrogenesis, we will apply Fe isotopes as a tracer for Fe provenance in conjunction with other radiogenic isotopes. Our studies will also include analysis of marine carbonates and cherts (see *1.2.1.1*) to obtain a more complete picture of variations in ocean Fe chemistry with depth.

1.2.5. Evolution of the Terrestrial Biosphere: Archean and Proterozoic Paleosols (Capo, Stewart, and Ohmoto)

Paleosols are a direct link between the lithosphere and atmosphere, and thus are essential for interpreting paleo-redox conditions on early Earth. They are also about the only geologic samples providing remnants of organisms and chemical signatures of life on the Precambrian land surface. Identification of geochemical signatures for the existence of organisms on the land surface (e.g., Fe^{3+}/Ti and Fe^{2+}/Ti ratios: Ohmoto, 1996; Beukes et al., 2002) will be extremely useful for the detection of life (past and/or present) on other planets, such as Mars. We have been conducting detailed investigations of organic matter, mineralogy, Fe chemistry, and REE chemistry of selected paleosols. Over the past five years, we have discovered: (a) the oldest (2.6 Ga) remnants of life on land (microbial mats composed mostly of cyanobacteria) at Schagen, South Africa (Watanabe et al., 2000), and (b) the oldest (2.3 Ga) lateritic soils in very large areas in the Kaapvaal Craton (Beukes et al., 2002). Because the formation of a laterite sequence requires abundant organic acids to leach Fe from rocks and abundant O_2 to fix the dissolved Fe as ferric oxides, our discovery suggests that most land surface was covered by microbial mats (probably of cyanobacteria) and the atmosphere was already oxygenated by 2.3 Ga.

We have recently demonstrated that Sm-Nd and Rb-Sr isotopic analyses can, in some cases, determine the absolute ages of soil formation and post-depositional metamorphism of Archean and Proterozoic paleosols (Stafford et al., 2000, 2002). Application of Fe isotopes to Precambrian paleosols from critical periods in Earth's history will provide a new tool to evaluate

paleo-redox conditions. This work will be closely integrated with studies of modern soils (Section 5), which will allow us to better understand Fe isotope behavior during weathering and pedogenesis and also to evaluate the effectiveness of usefulness of Fe isotopes in the search of present/past life on Mars.

We recently obtained preliminary REE data on paleosols from Kalkkloof, South Africa, which formed between 2.6 and 2.4 Ga ago. The REE data are exciting because they exhibit distinct Ce anomalies, which are excellent indicators that the soils formed under an oxygenated atmosphere. This paleosol profile may be the oldest soil section with unquestionable Ce anomalies. We have also obtained C isotope data on kerogen extracted from these paleosol samples. The data are also interesting because their $\delta^{13}C$ values (ca. -24‰) are quite different from those of the 2.6 Ga Schagen paleosols (ca. -15‰) and those of typical marine organisms of that time (i.e., ca. -28‰). We will continue detailed geochemical investigations of the Kalkkloof paleosols. Research will also continue on the following paleosol sections: (a) the 2.4 Ga paleolaterites at Hallavaara, Finland; (b) the 2.4 Ga paleosols at Pronto and Stanleigh mines, Ontario, Canada; (c) the 2.7 Ga "paleosols (?)" at Mt. Roe, Western Australia; (d) the 2.9-3.0 Ga paleosols at Steep Rock, Ontario, Canada; and (e) the 3.4 Ga Cooterunah Unconformity. We have already acquired a considerable amount of geochemical data on surface samples from (a), (b), (c), and (d). Our new initiatives will include deep drill core samples, which will be recovered from (c) in 2003 and from (e) in 2005 under the NAI drilling projects.

In addition to investigating the organic geochemistry, mineralogy, and bulk-rock inorganic geochemistry of the above paleosols, we will study the following new topics on paleosols: (a) weathering rates of pyrite crystals; and (b) the MIF of S isotopes. Because we have good kinetic laws for the dissolution of pyrite as a function of pH and pO_2 (e.g., Williamson & Rimstidt, 1994; Kamei & Ohmoto, 2000), we will be able to place constraints on the pO_2 and pCO_2 levels of the Archean and early Proterozoic atmosphere from the changes in the amount and grain size of the original pyrite crystals in paleosol profiles. If the uppermost soil zone was not eroded away, we could find "atmospheric pyrite" that formed from the S^0 generated by photochemical reactions of volcanic SO_2 in the atmosphere.

1.2.6. Extraterrestrial Influence on the Earth's Early Biosphere (Ohmoto and Kasting)

The scarcity of sedimentary rocks on Earth older than ~3.5 Ga has been attributed to the combination of plate tectonics and heavy bombardments by meteorites, which could have destroyed the early-formed proto-continents and oceanic islands (e.g., Windley, 1995a; Ward, 2000). The heavy bombardments could have also completely destroyed life on early Earth or at least hindered its early evolution (Maher & Stevenson, 1988; Sleep et al., 1989). However, the abundance of organic carbon in shales of the ~3.8 Ga Isua Formation in Greenland (Rosing, 1999) and in all sedimentary rocks in the Pilbara district (<3.56 Ga) (see Section 1.1.2) suggests the Earth may have experienced repeated histories of emergence, evolution, and extinction during the period of heavy bombardment (e.g., Windley, 1995b; Arrehenius et al., 1998; Nisbet & Sleep, 2001); post-3.6 Ga organisms may only represent those evolved from the last common ancestor (e.g., Nisbet & Sleep, 2001). The ~100 m *continuous section* of chert/jasper from the ABDP drill core (DDH 1) offers a unique opportunity to investigate variations in the flux of cosmic material (meteorites and cosmic dust) over time and its effects on marine life during the waning period of heavy bombardment; it is analogous to a >1 km deep polar ice core. The identification of layers enriched in volcanic ash, extraterrestrial materials, and/or organic matter

occurs in a background of mostly white-colored chemical precipitates (i.e., silica, goethite, and siderite precipitates from the ocean water ± hydrothermal fluids) is much easier than finding them in rocks dominated by clastic minerals (i.e., shales and sandstones). Using NAI and PSU funds, we have purchased an ideal instrument for our investigations, the Horiba XGT-5000 x-ray chemical microscope. This recently developed instrument is capable of chemically mapping ~30 elements simultaneously with a spatial resolution of 10 μm on a hand specimen-sized rock (or biological) sample under air and without a pre-treatment; chemical mapping of a 2"x3" rock chip can be performed in 0.5–2 hours, depending on the concentrations of elements of interest. In contrast, microchemical analyses by other instruments are typically performed on small, pre-treated (e.g., gold coated) chips placed in a vacuum chamber, and chemical mapping of the same area takes more than 100 times longer. Using this instrument, we will map the distributions of layers rich in cosmic materials by identifying enrichments in platinum group and siderophile elements (Fe, Ni, Co, Cr), and their relations to organic carbon-rich layers, in the ~100 m continuous section. An example of a Cr-rich layer in a 3.8 Ga Isua BIF sample is shown in Fig. 1.7. In order to identify whether a Cr-rich layer is composed of volcanic ash or extraterrestrial materials, other elements must also be analyzed. After identifying layers possibly enriched in extraterrestrial materials, we will search for enrichments of iridium using the neutron activation analysis. Organic-rich layers may be absent in the layers immediately following a Cr-rich layer but may become more abundant toward the next Cr-rich layer, if meteorite impacts affected the marine (and terrestrial) biosphere on early Earth.

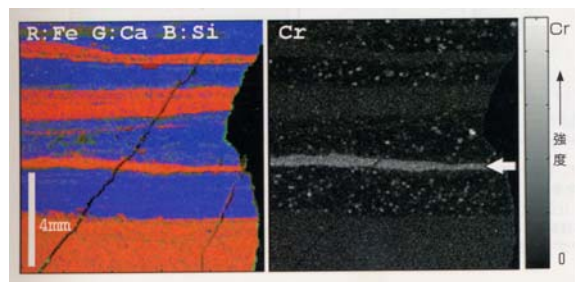

Fig. 1.7. Chemical maps of Fe, Ca, Si, and Cr on a 3.8 Ga Isua BIF sample, using the Horiba XGT-2800 X-ray chemical microscope (Takano, personal communication, 2002).

1.3. MODELING OF COUPLED S-C-O-FE GEOCHEMICAL CYCLES

(Kump and Arthur)

The experimental and analytical/observational approaches described above provide critical information on the evolution of Earth's surface environment and the intensity of biogeochemical cycling of the elements during 2.5 billion years of Earth history. We use box models to translate these observations into a quantitative assessment of the rates of biospheric processes, including weathering of the continents, biological productivity of the ocean (and land surface), burial of organic carbon and pyrite sulfur (and thus the fluxes of O_2 to the atmosphere), and make estimates of the atmospheric and oceanic composition for times in the distant geologic past **(**Kasting, 1993; Hotinski et al., 2000; Kump, 1991; Kump & Arthur, 1999; Beerling et al., 2002**).** More sophisticated models that provide spatial resolution (1-D atmospheric photochemical/radiative convective (e.g., Pavlov et al., 2001a) and oceanic diffusion models; 2-3D climate and ocean circulation models) are also used to address important questions concerning the coupling of atmospheric, oceanic, and biotic evolution. For example, such models must be used to deconvolve atmospheric evolution from geochemical records preserved in marine sediments (e.g., Hotinski et al., 2001): biological processes in the ocean can create

reduced (or oxidized) marine conditions that may not reflect the redox state of the atmosphere. Numerical models can be used to explore the conditions under which such decoupling occurs. Modeling focuses will be on the following two topics:

1.3.1. Deep Sea Anoxia

We will continue work begun under current funding to constrain the necessary and sufficient conditions for deep-sea anoxia. We are particularly interested in the interval from 1.8 Ga to the onset of Snowball Earth (0.75 Ga), when sulfidic waters may have existed in the deep sea (Canfield, 1998). Important issues include (1) the nutrient- (e.g., phosphate) and sulfate content of the ocean required to maintain euxinic conditions; and (2) the degree to which issue (1) depends (quantitatively) on the efficiency of the biological pump (see Section 2), atmospheric oxygen level, and climate-sensitive ocean circulation. Although we continue to develop improved models for paleo-applications, existing models (e.g., Hotinski et al., 2001) can be used to answer this question.

1.3.2. Carbon Isotopes of Paleoproterozoic Carbonates

What are the possible explanations for the very heavy $\delta^{13}C$ values of Paleoproterozoic (2.2-2.0 Ga) carbonates (Melezhik & Fallick, 1994; Melezhik et al., 1999; Karhu & Holland, 1996; Bekker et al., 2003)? The traditional explanation of isotopically heavy carbonates is that they reflect high rates of organic carbon burial, and thus rapid increases in atmospheric oxygen levels. However, the duration and magnitude of this event imply the establishment of O_2 levels many times the present. Kump (1989) has argued in the past that large positive $\delta^{13}C$ excursions occur at times when overall rates of carbon cycling are anomalously low. Such conditions would be realized during times of supercontinental stasis, when the degree of continentality is maximized, and thus rates of continental runoff (and weathering rates) are minimized. We propose to investigate this alternative hypothesis for the Paleoproterozoic event. The modeling we perform will be aided by new data collected in collaboration with Victor Melezhik of the Norwegian Geological Survey.

II. PHOTOCHEMICAL REACTIONS OF SULFUR AND IRON IN THE EARLY EARTH

Kasting (Leader), Castleman, Schoonen, and Arthur

The first part of research in this Section (2.1) deals experimental and theoretical investigations on mass independent fractionation of sulfur isotopes during photochemical reactions of SO_2 in order to provide the fundamental data necessary for geochemists to correctly interpret the sulfur isotope record in rocks (see Section 1.2.2). Specific focus of the experimental investigations (Section 2.1.1) will be placed on: (a) the details of gas-phase reactions that lead to mass independent fractionation of sulfur isotopes; and (b) to identify the specific sets of atmospheric conditions that match the sulfur isotope record in pre-2.3 Ga rocks. Section (2.1.2) presents modeling of atmospheric photochemical reactions that link the experimental results (Section 2.1.1) with the rock record from Section (1.2.2). Photochemical experiments on ferrous hydroxide mineral and pyrite are proposed in Section (2.2) in order to investigate the possible mechanisms to form ferric hydroxide minerals in banded iron-formation and aqueous sulfate in the early oceans. Sections 2.3 proposes to identify the oxygen and sulfur isotope signatures of SO_4^{2-} that was formed: (i) by photochemical reactions SO_2 in Section (2.1.1), (ii) by photochemical reactions of pyrite in Section (2.2), and (iii) in carbonate minerals during precipitation and diagenesis; these experimental data are necessary in order to relate the chemical and isotopic compositions of Precambrian carbonates (Section 1.2.2) to those of the Precambrian oceans.

2.1. MASS INDEPENDENT FRACTIONATION OF SULFUR ISOTOPES

2.1.1.Background

One of the most exciting developments in the past several years has been the discovery of mass-independently fractionated (MIF) sulfur isotopes in rocks of Archean and Early Proterozoic age, 2.3 Ga and older (Farquhar et al., 2000, 2001). (See Fig. I in "Introduction and Overview" of this proposal). Farquhar et al. (2000) interpreted the presence of MIF of S isotopes (Fig. I) as indicating that atmospheric O_2 concentrations were low enough prior to 2.3 Ga to allow SO_2 to be photolyzed. This gives an upper limit on pO_2 of ~0.01 PAL (times the Present Atmospheric Level). Following publication of their results, our PSARC group used an atmospheric photochemical model to place even tighter constraints on O_2 (Pavlov & Kasting, 2002). We showed that in order for MIF to be preserved in sediments, S must exit the atmosphere in a variety of different oxidation states. Today, virtually all of the S that leaves the atmosphere is oxidized to sulfate and combined in the oceanic sulfate reservoir. According to the model of Pavlov and Kasting (2002), this remains true down to O_2 concentrations of 10^{-5} PAL. Thus, Archean pO_2 must have been less than this value--probably significantly so. Theoretical considerations predict that pO_2 should be $<10^{-13}$ PAL as long as the volcanic reduced gas flux exceeds the photosynthetic source of O_2 (Walker, 1977; Kasting, 1993). This appears to have been the case prior to 2.3 Ga.

Although photochemical models have provided this important constraint, their further usefulness is limited by the lack of kinetic data on isotope fractionation within the atmosphere. When SO_2 is photolyzed at 190 nm, the following reactions are expected to occur (Yung & DeMore, 1999):

$$SO_2 + h\nu \rightarrow SO + O \quad (2\text{-}1)$$
$$SO + h\nu \rightarrow S + O \quad (2\text{-}2)$$
$$SO + SO \rightarrow SO_2 + S \quad (2\text{-}3)$$

Studies of MIF in oxygen isotopes during ozone formation (Gao & Marcus, 2001) suggest that symmetry is a key factor. We suspect that that symmetry may also be involved in MIF of S isotopes.

Farquhar et al. (2001) measured highly positive $\Delta^{33}S$ in elemental sulfur, S_8, which forms from S atoms via sequences such as: $S + S + M \rightarrow S_2 + M$, $S_2 + S_2 + M \rightarrow S_4 + M$, $S_4 + S_4 + M \rightarrow S_8 + M$. MIF could arise during sulfur chain formation by these or related reactions. A third possibility is that MIF occurs during the reaction of SO with itself (reaction 2-3). The reaction apparently proceeds by way of an unstable, excited dimer state, $(SO)_2^*$ (Herron & Huie, 1980) which splits apart if it is not stabilized by a collision with another molecule. Symmetry of this excited dimer species may be an important factor.

In light of the uncertainty about the fractionation mechanism, we propose to examine all of the key steps leading from SO_2 to elemental sulfur either experimentally or theoretically. This work is described in the next section.

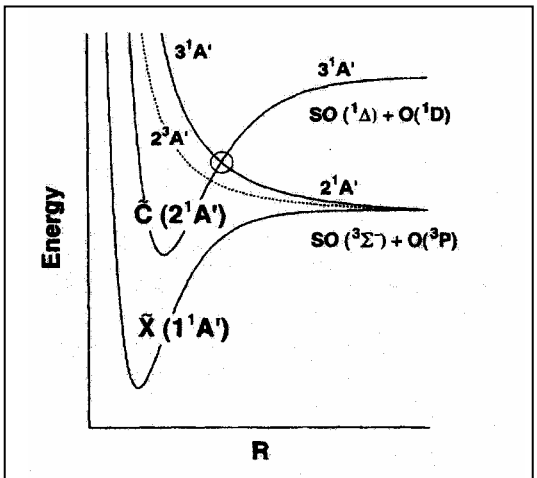

Fig. 2.1. Potential energy surface of SO_2 showing the C ($2^1A'$) state as well as other excited states. (From Katagiri et al., 1997).

2.1.2. Photochemical Experiments (Castleman)

2.1.2.1. SO_2 photolysis

We will begin with the photolysis of SO_2 because this is the initial step and is the most straightforward of the experiments. When SO_2 absorbs a photon at wavelengths near 190 nm it is raised to the excited C($2^1A'$) state (Fig. 2.1). Once in the excited state, the SO_2 molecule can do one of two things: 1) It can transition spontaneously to the unbound state, SO + O; or 2) it can collisionally deactivate to give ground state SO_2. Which of these two things happens depends on the lifetime of the excited state compared to the collision time. Collision times can be estimated from kinetic theory, given a known (or hypothesized) distribution of atmospheric pressure and temperature. Thus, the key measurement that is required is the lifetime of the excited state. We propose to measure this directly for ^{32}S, ^{33}S, and ^{34}S. How we will do

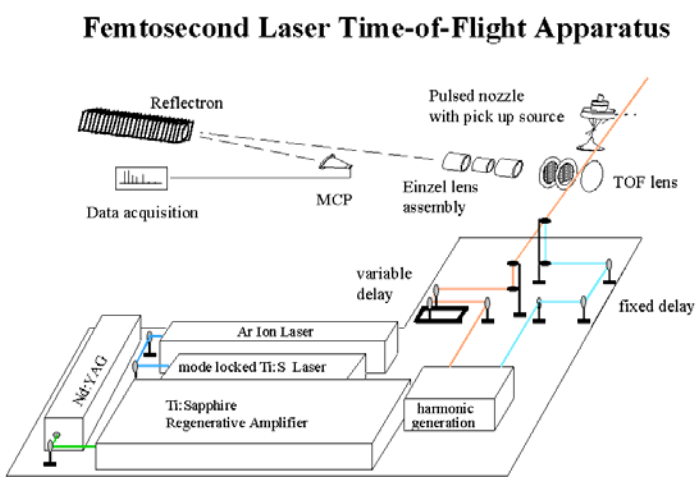

Fig. 2.2 Setup of the femtosecond pump-probe time of flight mass spectrometer.

this is described below.

A reflectron time-of-flight mass spectrometer (RETOF-MS) and femtosecond laser system coupled with the pump-probe technique will be utilized to study the dynamics of SO_2 and related sulfur species (Fig. 2.2). The laser system used in these experiments consists of a mode-locked Ti:Sapphire oscillator (Spectra Physics Tsunami) pumped by a 10W argon ion laser (Spectra Physics 2060) which generates an 82 MHz pulse train. Pulse amplification is carried out with a regenerative Ti:Sapphire amplifier pumped by the second harmonic of a 10Hz Nd:YAG laser (Spectra Physics GCR 150-10). Typical output from the amplifier is a beam with a wavelength centered around 795 nm, having a pulse-width of 100 fs and a pulse energy of about 3 mJ. The fundamental wavelength can be frequency doubled with a BBO crystal to generate the second harmonic. The third harmonic (~266 nm) of the fundamental wavelength can also be generated by frequency mixing of the fundamental and second harmonic with a BBO crystal.

The ^{33}S and ^{34}S isotopes of SO_2, seen in previous experiments by Hurley and Castleman, could be studied in a similar fashion. Figure 2.3 shows a mass spectrum of SO_2, SO, and their isotopes. Further experiments on the isotopes using the pump-probe could produce useful temporal information.

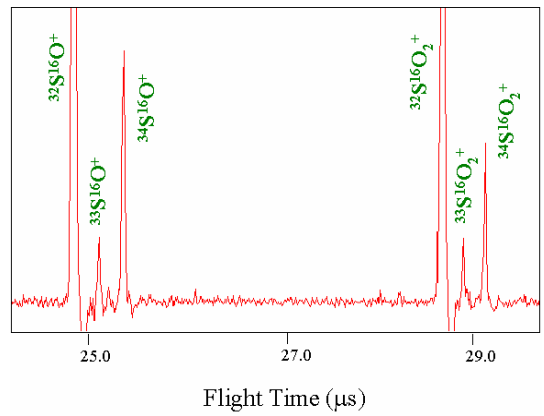

Fig. 2.3 Mass spectrum of SO_2, SO, and their isotopes.

A typical experiment will be performed as follows. The second harmonic will be used in a two-photon excitation to populate the C ($2^1A'$) state of SO_2. The third harmonic can also be used to excite SO_2 to the coupled $^1A_2$, $^1B_1$ state. Figure 2.4 shows a pump-probe trace of SO_2 with 2 photons of the second harmonic as the pump and 4 photons of the fundamental as the probe. Temporal data extracted from traces of the SO_2 isotopes (^{33}S, ^{34}S) will be useful in our model. With further modifications to the apparatus discussed above, pump-probe traces of the isotopes of SO_2 could be taken. These include a higher resolution detector with minimal ringing characteristics. Eventually, an OPA (optical parametric amplifier) will be required to select a wide range of wavelengths and, hence, molecular states.

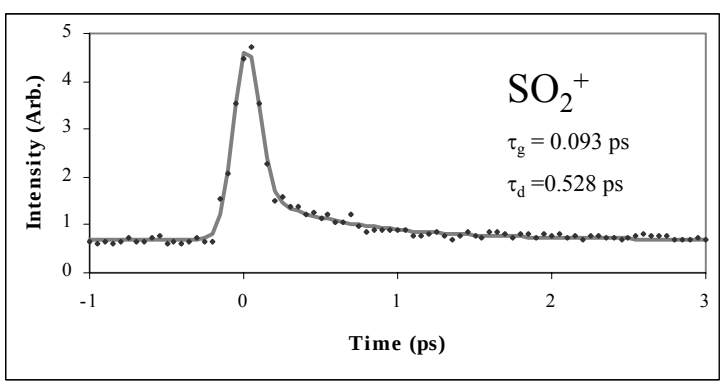

Fig. 2.4 Pump-probe trace of SO_2+, where τ_g represents a growth and τ_d represents the decay of the excited state.

2.1.2.2 Sulfur chain formation

The next set of reactions that we propose to study experimentally are those leading to sulfur chain formation. We are especially interested in the reactions:

$$S + S + M \rightarrow S_2 + M \qquad (2\text{-}4)$$

$$S + S_2 + M \rightarrow S_3 + M \qquad (2\text{-}5)$$

Reaction 2-4 is the simplest S recombination reaction. Symmetry is clearly involved because the S_2 molecule can either contain two identical S atoms or two different isotopes of S. Reaction 2-5 is analogous to the ozone formation reaction ($O + O_2 + M \rightarrow O_3 + M$). Hence, we can anticipate *a priori* that this reaction should involve MIF.

The sulfur clusters will be formed by the following procedure: Laser ablation of solid sulfur will produce gas phase sulfur atoms. These sulfur atoms, seeded in helium backing gas, will under go an expansion through a nozzle resulting in cooling and cluster formation. The isotopic composition of these clusters can then be studied using mass spectrometry and laser techniques.

2.1.2.3. $SO + SO \rightarrow SO_2 + S$ Reaction

Techniques exist for the generation of SO and S_2O_2 by thermal decomposition of sulfur-containing organic precursors. S_2O_2 and clusters of SO such as $(SO)_2$, which can be formed by supersonic expansion, can be studied by the photolysis technique described in the proposed work. As with SO_2, photolysis experiments will measure the time dynamics of the chemical process, in this case the formation of SO_2, and elucidate the influence of the sulfur isotopes on the time scale of the chemical process.

2.1.3. Photochemical Modeling of Sulfur Isotopic Fractionation (Kasting)

Once kinetic data become available for the primary process(es) causing MIF in sulfur isotopes, we can return to the photochemical model of Pavlov and Kasting (2002) and use it to derive more accurate estimates for the isotopic compositions of the various atmospheric sulfur gases and particles that can exit the atmosphere. When combined with new analytical data on the sulfur isotopic composition of Precambrian rocks, some of which will be collected here at Penn State (see Section 1.2.2), this should provide a powerful tool for constraining both the nature of the Archean/Early Proterozoic atmosphere and the processing of sulfur species that must have occurred within the marine ecosystem at that time. Several other NAI groups (Carnegie Inst., Harvard, UCLA) have also been collecting new multi-S-isotope data. We have been working with the first two of these groups, and these efforts have already resulted in one joint publication (Ono et al., submitted).

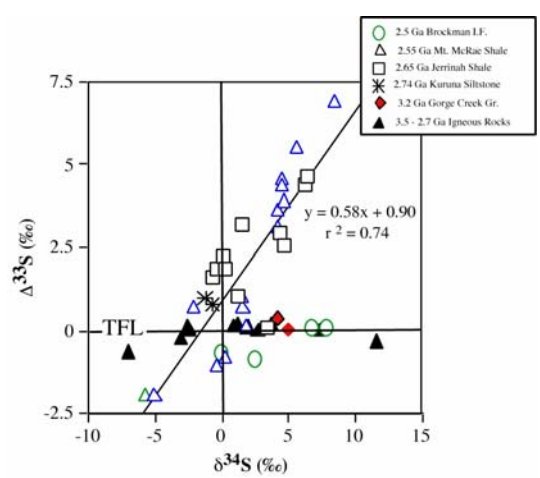

Fig. 2.5. S isotope data on sulfides from sedimentary and igneous rocks (3.2-2.5 Ga in age) from the Pilbara-Hamersley districts, W. Australia. Data from Farquhar et al. (2000), Onot et al. (submitted), and Ohmoto et al. (in prep.).

A brief description of what we hope to learn from this modeling follows: In the Ono et al. paper, we present data from the 2.7-Ga Jeerinah

formation and Lewin shale (Hamersley Basin, Australia) which show $\Delta^{33}S$ values of +4.7‰ and +8.2‰, respectively (see Fig. 2.5). These values may be compared with the maximum value of +2.2‰ in the original paper by Farquhar et al. The first question that comes to mind is: what is responsible for these very high $\Delta^{33}S$ values? Based on the measurements of Farquhar et al., we suspect that this signal is coming from elemental sulfur (S_8) particles created photochemically within the atmosphere. These particles are formed preferentially in the upper troposphere of our model and, hence, can in theory preserve the strong MIF signature created by the types of reactions described above. Gaseous sulfur species, by contrast, exchange their isotopes with other sulfur gases during their voyage through the lower atmosphere and are much less likely to have strong MIF signatures. We relate the $\Delta^{33}S$ values and deposition rates of S_8 particles to the CH_4 content of the atmosphere and the volcanic outgassing rate of sulfur. S_8 formation varies roughly as the 8th power of the atmospheric SO_2 concentration and, so, is a strong function of the sulfur outgassing rate.

Once in the ocean, we suggest that elemental sulfur was used as an electron acceptor by sulfur reducing bacteria. Reduction of elemental sulfur is thought by some researchers to have been among the oldest forms of metabolism (Canfield & Raiswell, 1999). We can potentially test this idea by looking as the sulfides that these organisms produced. We also see evidence in our data of mass-dependent S isotope fractionation, which we attribute to bacterial sulfate reduction. Three-isotope plots should eventually allow us to determine what types of sulfur-utilizing bacteria were present in the Archean ecosystem, as well as what types of atmospheric conditions prevailed at that time. None of these detailed questions can be answered, however, until the primary fractionation processes are understood. Thus, the measurements described in Section 1.2 are critical to making further progress with our model.

2.2. PHOTOCHEMICAL AND CHEMICAL PROCESSES INVOLVING IRON

(Schoonen)

Iron is the most abundant redox-active, metallic element on Earth, a major constituent on all other planetary bodies in our inner solar system, and is thought to be a major component on any Earth-like planet elsewhere. Hence, attempts to describe the evolution of the redox state of a planetary body such as the Earth must account for redox processes involving iron. The objective of this task is to investigate photochemical and thermal redox processes involving iron that may have been relevant in the evolution of the redox state of the oceans through time. Some of the processes that will be considered in this task may have also been relevant in prebiotic synthesis on the early Earth. The relative stability of Fe(III)-O-H and mixed Fe(II)-Fe(III)-O-H solid phases with respect to pure $Fe(OH)_2$ is the driving force for many of the processes that will be investigated here. Hence, $Fe(OH)_2$ in aqueous environments serves as an electron donor. In the early oceans, water, dinitrogen, carbon monoxide, and possibly carbon dioxide may have been the chief electron acceptors. Reduction of these compounds could have produced some of the precursors to the building blocks of life, such as ammonia and formaldehyde. Experimental studies of redox processes involving iron in aqueous environments may also provide constraints on the role of abiotic processes in the precipitation of iron-containing minerals that are a major component in the BIFs that formed up to 1800 Ma ago (with a reprise in the Neoproterozoic).

For the purpose of designing a research strategy it is convenient to consider the fate of dissolved ferrous iron (Fig. 2.6). Ferrous iron, predominantly derived from submarine hydrothermal exhalative systems (Kump et al., 2001), may be removed from the water column via redox and non-redox precipitation processes, assisted or unassisted by light. Out of the processes shown in Fig. 2.6 we have selected a few for further study. The selected processes have the potential to have been important in regulating the redox state of the early oceans.

Photo-oxidation of dissolved ferrous iron leads the formation to Fe(III)-OH or Fe(III)-Fe(II)-OH phases. A seminal experimental study by Braterman and collaborators (Braterman & Cairns-Smith, 1987; Braterman et al., 1983; Braterman et al., 1984) has shown that UV-irradiation of a near-neutral ferrous solution devoid of dissolved molecular oxygen leads to the precipitation of γ-FeOOH . In this process, protons act as electron acceptors producing molecular hydrogen. We propose to experiment over a wider range of solution compositions than deployed in the original work by Braterman et al. (1987.). In particular, we wish to establish if the quantum yield of the reaction is affected if the composition of the electrolyte solution is varied. This is of importance because hydrogen formed in this reaction will tend to escape Earth, leading to an overall oxidation of the ocean/atmosphere/crust. Better constraints on the quantum yield of the reaction will provide improved constraints on the hydrogen escape rate. In addition we wish to evaluate if CO and N_2 could act as electron acceptors in this process. Some of the experiments in the original work were conducted under nitrogen gas, but no attempt was made to analyze for ammonia. Formation of reduced N or C compounds would limit or eliminate the formation of hydrogen and the net oxidation state of the ocean would not be affected (cf. loss of H_2).

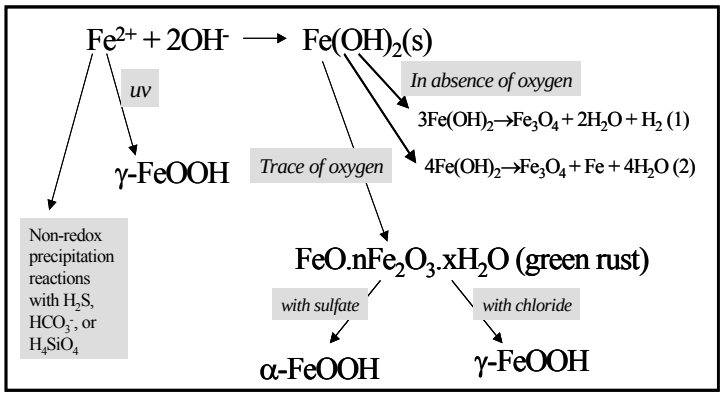

Fig. 2.6. Fate of Fe^{2+} in solution, including decomposition of $Fe(OH)_2$.

The second process of interest is the conversion of $Fe(OH)_2$. $Fe(OH)_2$ is a white solid that will undergo a spontaneous conversion to magnetite and metallic iron in systems devoid of oxygen, while it converts to mixed double-layer hydroxides if as little as a trace of oxygen is present (Blesa & Matijevic, 1989). The conversion under strictly anoxic conditions proceeds via two competing reactions, reactions 1 and 2 in Fig 2.6. As demonstrated by Schrautzer and Guth (1976) , metallic iron formed in reaction 2 is very reactive and capable of reducing water (Evans & Wanklyn, 1948), dinitrogen and carbon monoxide. Experiments with dissolved CO led to the formation of methane (Schrauzer & Guth, 1976). The state of the metallic iron produced in this process is of great interest. It appears to be in the form of small clusters, possibly nanoscale particles, dispersed in a magnetite matrix. The work by Schrauzer and Guth (1976) was conducted under conditions that are far from those that are relevant for the origin of life. We propose to explore the fate of $FeOH_2$ under strict anoxic conditions in NaCl solutions and vary the composition (including addition of CO, CO_2, N_2 and other plausible trace metals). The results will constrain the rate of H_2 production, methane formation, and formation of precursors to the building blocks of life via the processes associated with the conversion of $Fe(OH)_2$.

If a trace of oxygen, nitrate, or nitrite is present, $Fe(OH)_2$ convert readily to layered iron hydroxides (i.e. green rust). This class of materials contains structural anions that are derived from the solution (Brindley et al., 1976; Bessiere et al., 1999; Gehin et al., 2002; Hansen & Poulsen, 1999; Benali et al., 2001; Legrand et al., 2001)). While the mechanisms of the conversions are not fully understood (Blesa & Matijevic, 1989; Domingo et al., 1993; Hansen & Poulsen, 1999), green rust has been shown to be able to reduce nitrite, nitrate, selenate, chromate, and possibly dinitrogen (Denisov et al., 1991; Hansen et al., 1994; Myneni et al., 1997; Refait et al., 2000; Williams & Scherer, 2001). We propose to conduct experiments to explore if the conversion of green rust can reduce water, CO and dinitrogen. It is also noteworthy that mixed layer metal oxides may have been important in the prebiotic synthesis (Kuma et al., 1989).

While not the primary focus of research proposed in this task, it is logical to also study the nature of the Fe-O-H products formed as conditions are changed in these experiments. Not only is it of interest to study the structure of the products, but also their composition. For example, magnetite formed via the conversion of $Fe(OH)_2$ under strict anoxic conditions may contain metallic iron, while magnetite and hematite formed in the presence of a trace of oxygen may inherit a significant amount of sulfate or chloride as a result of the conversion of the intermediate green rust. If we can establish that there are indeed significant differences in chemical composition depending of the formation path, it is a logical next step to study natural magnetites from BIFs through time. While untested, the potential payoff, an independent constraint on seawater composition (Cl/SO_4 ratio) and a constraint on O_2 levels, is high. Changes in the nature of the Fe-O-H phases formed in the oceans as well as conversions from one form to another may have also affected the fate of phosphate and REE in the oceans as the surface chemistry (particularly the surface charge) of the various iron phases differs for a given ocean composition. The interaction of REE with the iron phases formed via the processes outlined here is poorly understood and will be addressed in a joint study by Kump and Schoonen.

The proposed experiments can be readily conducted in Schoonen's laboratory at Stony Brook. The lab is equipped with the necessary UV-photochemical equipment, GC and IC, a cryogenic Mossbauer spectrometer for the characterization of Fe phases, Raman, FTIR, and expertise and equipment to conduct research on systems devoid of molecular oxygen. Given that CO can be reduced by $Fe(OH)_2$ as it converts to magnetite and possibly also forms as green rust converts, the research proposed in this task may provide a constraint on the rate of abiotic methane production of methane.

2.3. SULFUR AND OXYGEN ISOTOPES OF SULFATE (Arthur)

2.3.1. Oxygen Isotope Signatures of Sulfate Produced by Photochemical Reactions

With the likely absence of large-scale oxidative weathering of sulfur on land during the Archean, sulfate-sulfur in seawater could have been derived from: i) the production of SO_4^{2-} from volcanic sulfur accompanying atmospheric photochemical reactions and disproportionation of SO_2 in surface seawater; ii) the SO_4^{2-} produced by photo-oxidation of pyrite utilizing H_2O_2 in shallow seas and on land; and iii) sulfate ion formed by bacterial sulfide oxidation in possible surface-water oxygen "oases." We are interested in distinguishing sulfate from each source, and suggest that oxygen isotopes might constitute a way to do so. Photochemical experiments

conducted in Schoonen's laboratory (see Section 2.2) indicate substantial peroxide formation from water in seawater under visible light illumination, using Fe^{+3}, and that this peroxide oxidizes pyrite. However, there are no experimental or theoretical data to evaluate the O isotopic composition of type-(ii) SO_4^{2-}. The objective of this study is, therefore, to experimentally determine the $\delta^{18}O$ characteristics of type-(ii) SO_4^{2-}. Such experimental data, together with the $\delta^{18}O$ values of trace SO_4^{2-} in Archean carbonates (see Section 2.3.2.3) may allow us to evaluate the relative importance of the two sources of SO_4^{2-} in the Archean oceans.

We will conduct a series of pyrite weathering experiments in an anoxic chamber, with visible (solar spectrum) illumination to examine the production rate of peroxide and the oxidation rate of pyrite in the absence of oxygen. Experiments will utilize different pyrite precursors, from fine-grained framboids to crystalline pyrite at a range of temperatures. We will examine the sulfur and oxygen isotopic signals of reactants and products in the experiment. In particular, we will analyze the oxygen isotopic composition of peroxide and the $\delta^{18}O_{sulfate}$ values in order to document oxygen isotopic discrimination resulting from peroxide oxidation and resulting dissolved sulfate production. Experiments conducted in Schoonen's laboratory indicate substantial peroxide formation from water in seawater under visible light illumination, using Fe^{+3}, and that this peroxide oxidizes pyrite (Borda et al. 2001; Borda 2003; Borda, et al. 2003). This process could conceivably have been important in terrestrial environments even under an anaerobic atmosphere. Such conditions probably prevailed prior to 2.7 Ga, and may have existed intermittently through the Proterozoic.

2.3.2. Sulfate Partitioning during Precipitation and Diagenesis of Carbonates

It is well known that the original isotopic compositions of Precambrian carbonates have often been modified by later alteration as the result of long-term exposure, prolonged contact with groundwater, or exchange with fluids during deep burial. Since groundwater typically contains much less sulfate than seawater and the oxygen isotope exchange rates between SO_4^{2-} and H_2O are extremely slow (Lloyd, 1968), it is likely that the $\delta^{34}S$ and $\delta^{18}O$ values of the trace sulfate in the Precambrian carbonates may have retained the values of the Precambrian seawater; however, we are concerned that sulfate contents of the carbonates might have been altered during diagenesis in pore waters that have experienced extensive bacterial sulfate reduction. If the sulfate content of carbonate is not readily changeable during reactions with groundwater, it may be used to estimate the sulfate content of Precambrian oceans. Therefore, the main objective of this research is to investigate the chemical and isotopic partitioning between trace sulfate in carbonate and sulfate in solutions (a) during precipitation of carbonates (calcite and aragonite) and (b) during recrystallization of carbonates in the presence of aqueous solutions.

2.3.2.1. Experimental precipitation of aragonite and calcite

There are no published data that allow quantification of the relationship between seawater sulfate concentration and concentrations in carbonate minerals. We will investigate sulfate incorporation in carbonates at range of known sulfate/carbonate ion ratios over a range of temperatures in both low-Mg calcite and aragonite. Because sulfate is strongly complexed by magnesium in seawater, we will also utilize Mg-free artificial seawater solutions in some experiments. In addition, we will conduct precipitation-rate experiments in order to gauge the extent to which supersaturation with respect to carbonate minerals and their rate of precipitation affects the distribution coefficient for sulfate.

2.3.2.2. Experimental diagenesis

We will study sulfate gain or loss and accompanying isotopic changes during mineralogic inversion (conversion of aragonite to calcite) in hydrothermal solutions at low temperature. We will also examine the effects of fluid interaction at a range of temperature in hydrothermal vessel experiments; we will add ^{34}S-enriched sulfate to some solutions in order to detect isotopic exchange of sulfate, independent of concentration changes, during diagenesis.

2.3.2.3. Analysis program

$\delta^{34}S$ analyses will be performed on SO_4^{2-} in solution and in carbonates, and $\delta^{18}O$ analyses on (a) SO_4^{2-} and H_2O in the experimental solutions and (b) SO_4^{2-} and $CaCO_3$ in the precipitates. In addition, we will use FTIR (FTIR microscopy) to examine spatial distributions of sulfate incorporation in carbonate minerals, for example as a function of precipitation rate. Raman spectroscopy (and/or Raman microscopy) will be used to explore patterns of bonding and inclusion of sulfate in interlattice sites or fluid inclusions. XAFS (non-routine) will be conducted on well-characterized samples to examine the potential lattice effects (distortions, etc.) of sulfate incorporation in order to understand how this might impact carbonate mineral solubility and mobility of sulfur under post-depositional conditions. XAS and possibly micro-XAS, available at Brookhaven National Laboratory, will be conducted on well-characterized samples to determine the mode of incorporation on the basis of the local structure around sulfate while incorporated into the carbonate host. Carbonate mineral solubility and mobility of sulfur under post-depositional conditions may depend on the mode of incorporation.

III. GENOMIC RECORD OF THE EARTH'S EARLY BIOSPHERE

Hedges (Leader), House, and Patzkowsky

3.1. Introduction

Typical prokaryote genomes contain between 1,000-6,000 genes and approximately 1-6 million nucleotides, which represent a great resource for astrobiological studies. Here, we will conduct research to improve analytical tools of genomic analysis and apply them to evolutionary questions concerning organisms that may have impacted the biosphere of the Archean and Proterozoic. For example, when did certain microbes evolve that were responsible for producing or consuming important gases (e.g., oxygen, methane) and minerals (e.g., sulfur, iron) in the early Earth environment? What are the evolutionary relationships among geologically significant microbial lineages (e.g., S-reducers, fermenters, acetogens, methanogens, sulfate-reducers, sulfide oxidizers, and oxygenic microbes)? What was the extent of lateral gene transfer in early Earth history and how did the transfer of genes affect the organisms and their environments? Clarifying the relationships and times of origin of such organisms will help to constrain models for the evolution of Earth's atmosphere, temperature, and biosphere in general.

We will use genomic data to construct evolutionary trees, estimate times of divergence, and determine whether genes and their functions were inherited vertically (e.g., speciation) or horizontally through genetic transfers between species. We will use sequence data from complete genomes and transcriptomes available in the public databases. The analytical tools have themselves evolved as data sets have grown; therefore existing tools will be evaluated through computer simulations, and new tools will be developed where applicable. The synthesis of evolutionary and environmental information will be facilitated by the development of a web database documenting the history of the biosphere. It will integrate evolutionary trees and divergence times with chronologically arranged panels containing geological, geochemical, atmospheric, and astronomical data.

3.2. Evolutionary Genomic Methods

Genomic data analysis continues to increase in complexity as sequence databases grow and tools are developed and refined. In general, protein sequences are desirable for the most ancient events because DNA sequences are usually too divergent in those cases to make accurate estimates on the basis of patterns of nucleotide substitutions, with the exception of some very conserved genes (e.g., rRNA). All phylogenetic methods minimize sequence change, but this can be accomplished in different ways, including maximum likelihood, minimum evolution, parsimony, and Bayesian methods (Li, 1997; Nei & Kumar, 2000). When sequences have not changed greatly over time, most methods produce the same results. However, the sequences that will be examined here are considerably divergent and therefore the methods of analysis take on greater importance. We will continue to use a diversity of methods, refine existing tools and develop new ones, especially through automation (where feasible), and conduct computer simulations to test the efficiency of different methods. This includes methods for detection of lateral gene transfer (Brochier et al., 2002; Brown et al., 2001).

Initial assembly of sequences is accomplished with the use of BLAST tools (Altschul et al., 1997). We use a local 48-cpu (24 gigabytes memory) dedicated parallel BLAST server housing

the latest versions of public sequence data (from GENBANK). To further increase efficiency, we have designed tools to automate the sequence assembly procedure, including BLAST analysis, sequence alignment, and initial tree construction. However, the automation only serves to speed the initial assembly of data and not to evaluate and select groups of orthologous sequences (those reflecting organismal rather than gene evolution). As the public databases grow and taxon sampling improves, orthology determination will become more easily automated. Also, we use a tool (Hedges et al., 2001; Shigenobu et al., 2000) that combines global (Thompson et al., 1994) and local alignment algorithms to better ensure alignment of homologous sites, which can be a problem with highly divergent sequences (Hansmann & Martin, 2000).

The most complex models of molecular evolution available for phylogenetic analysis, such as those that incorporate amino acid composition and rate variation among sites, do not account for all of the substitutional biases encountered in real data (Fitch & Markowitz, 1970; Nei & Kumar, 2000). For example, it appears that eukaryotes evolve faster than prokaryotes on average, across many genes (Hedges et al., 2001; Kollman & Doolittle, 2000). We will conduct simulations to determine how differences in parameters such as rate variation among sites affect phylogenetic inference to help improve methods. With complete genomes available, it is also possible to use the presence and absence of genes (genome content) to build phylogenies, and this general approach has proven useful (Fitz-Gibbon & House, 1999; Snel et al., 1999; Tekaia et al., 1999; Wolf et al., 2002; Wolf et al., 2001). We will continue to refine methods of gene content analysis and apply them to evolutionary questions.

Methods of molecular time estimation are important for understanding the early evolution of life because the Precambrian fossil record is relatively sparse. In all methods, the rate of sequence change inferred with reference to splitting events of known time (calibrations) is used to estimate the time of other divergences. The diverse array of sophisticated methods that exist today can be grouped into four categories by the way the data are handled (genes considered separately or combined in a supergene) and the allocation of rate variation among the lineages (global versus local clock) (Hedges & Kumar, 2003; Wray, 2001). Individual divergence times have been estimated with large numbers of genes (Feng et al., 1997; Gu, 1998; Heckman et al., 2001; Hedges et al., 2001; Kumar & Hedges, 1998; Wang et al., 1999; Wray et al., 1996) and such studies have indicated that the reliability of single gene estimates is low, emphasizing the need for multigene analyses (regardless of the specific method used). Fortunately, large data sets are appearing more frequently and permit greater precision in time estimation.

Global clock methods use genes and lineages that are not rejected in rate tests. Multigene time distributions are usually symmetric and have a strong central tendency (Kumar & Hedges, 1998). The supergene implementation involves concatenating all relevant genes (or gene segments) of a species to form a single alignment for time estimation (distances also can be averaged) (Nei & Glazko, 2002; Nei et al., 2001). This approach avoids the reduced statistical power of small data sets and potential statistical artifacts of the multigene method (Nei et al., 2001; Rodriguez-Trelles et al., 2002). Local clock methods, including the Bayesian and Likelihood smoothing methods, allow rate to vary in "local" parts of the tree and can be used with multigene and supergene approaches (Aris-Brosou & Yang, 2002; Hasegawa et al., 1989; Sanderson, 2002; Schubart et al., 1998; Thorne & Kishino, 2002). They offer the advantage that all genes and taxa can be used, and not just those that pass tests for rate constancy. Also, time constraints can be included in these analyses. However, the various methods differ considerably in their approaches, and it is not yet clear which method is optimal. Therefore, we will conduct computer simulations designed to reveal these differences so that users of the different methods

will be better informed, and to provide a guide for our own analyses. Publications, supplementary data, and tools will be made available on the NAI Evogenomics Focus Group web site (www.evogenomics.org).

3.3. Evolutionary Genomic Applications

Oxygen and methane are two components of the atmosphere that can be produced by microbes and which figure prominently in models describing the evolution of the atmosphere. To obtain better constraints on these models, we will use genomic data from the diversity of prokaryotes and eukaryotes in the public databases to estimate phylogenies and divergence times for lineages and groups relevant to the generation of those gases in particular, and to other biogeochemical reactions (e.g., sulfate reduction, sulfide oxidation). The origin of oxygenic photosynthesis can be constrained biologically on the early end by the time of divergence of cyanobacteria from other Bacteria and on the later end by the earliest divergence among living, oxygen-producing, organisms (cyanobacteria and plastid-bearing eukaryotes). Such time constraints can be refined by considering, in addition, the geologic record of the rise of oxygen (Holland, 2002) and biomarker evidence (Summons et al., 1999). Similarly, the origin of biological methane production can be constrained on the early end by the time of divergence of methanogens from other Archaea and on the later end by the earliest divergence among living methanogens (members of Euryarchaeota). In both cases, it must be assumed that oxygenic and methanogenic species now unavailable (e.g., extinct) would not substantially change the results. For cyanobacteria, this is probably a valid assumption because they form a monophyletic group, are relatively derived phylogenetically among Bacteria (Hedges, 2002; Wolf et al., 2002), and they appear to have a derived biochemical system for producing oxygen (Raymond et al., 2002; Xiong et al., 2000). It is also a reasonable assumption for methanogens, even though they are not monophyletic, because they are nonetheless restricted to one subgroup of Archaea (Perry et al., 2002; Wolf et al., 2002) and not scattered among prokaryotes. Also, additional genome sequences of methanogens are in progress (public projects) and this increased taxonomic sampling will better define the reference points constraining the origin of methanogenesis.

Our work will involve evolutionary analyses, using methods as described in the previous section, of all prokaryotic and eukaryotic genomes (>100 publicly available, with many more to be released over the next few years). Rate calibrations will come from the fossil record of eukaryotes (Butterfield, 2000; Hedges, 2002; Knoll & Carroll, 1999; Kumar & Hedges, 1998). Time estimation methods, as described in the previous section, are themselves complex, and rate differences between eukaryotes and prokaryotes, and among prokaryotes, bring additional complexity. Initial evaluation of methods with the use of computer simulations will help determine their usefulness (and weaknesses), suggest refinements, and provide a guide for their application. Lateral gene transfers will be identified using available methods (Brochier et al., 2002; Brown et al., 2001), and the function of genes transferred will potentially provide information for reconstructing ancient environments and communities.

3.4. Database of Biosphere History

Recently, we began the design and development of a public database of molecular clock and fossil divergence times, supported by the National Science Foundation (DBI 0112670; Hedges, PI). Dr. Sudhir Kumar (Department of Biology, Arizona State University) is a PSARC associate

member and collaborator on this project. The database will compute and visualize evolutionary trees scaled to time (timetrees) using data from published molecular clock studies and the fossil record. The timetrees will be hyperlinked in the sense that nodes and branches will be linked to additional trees (scaled up or down) and other information. For example, an initial tree might show the basic structure of the tree of life, whereby clicking on the Archaea branch will show the evolutionary history of that group, with greater taxonomic resolution. Clicking on a node in the tree will reveal error of the molecular time estimate, the number of genes or proteins, number of nucleotides or amino acids, PubMed publication link, and information on the earliest fossils pertaining to that node. Our initial funding has supported research and design of the database structure, and initial entry of fossil and molecular divergence time data from tetrapod vertebrates (for release to public later in 2003).

We propose here to extend the taxonomic scope of the database to microbes and early evolutionary events (Precambrian) and to incorporate geological, geochemical, atmospheric, and astronomical data in this database. The goal will be to visually integrate the biological (evolutionary) and non-biological histories of the planet. Most molecular phylogeneticists would have difficulty obtaining data on carbon and sulfur isotopes, and most geochemists would have difficulty in locating evolutionary trees. A database that integrates these diverse data in a common thread (time) will assist astrobiologists in better understanding how the biosphere as a whole evolved.

The non-biological data will be presented in the form of horizontal panels, scaled to time, appearing above or below the timetree. Each panel will show the temporal history of a different measurement (see figure). For example, one will present carbon isotope data while others will show sulfur isotope data, solar luminosity, continental breakup patterns, and extraterrestrial impact events. The database user would have control over the number and type of panels shown at any one time. Retrievable literature references will be linked to the displayed data (evolutionary and geological). Each panel will represent a separate database in itself, derived from the published literature and consultation with members of the astrobiological community, at Penn State and elsewhere. Information from existing, related databases (e.g., Tree of Life, Paleobiology) will be incorporated, and in some cases, linked. Penn State Co-I Patzkowsky has been a major contributor to the Paleobiology database (http://www.paleodb.org) (Alroy et al., 2001) and will assist with the assembly of the paleontological data. The most complex aspect of the overall database will be in accounting for differences of opinion (in all fields involved), when they exist. Our goal is to have a data-driven rather than authority-driven database that includes alternative viewpoints.

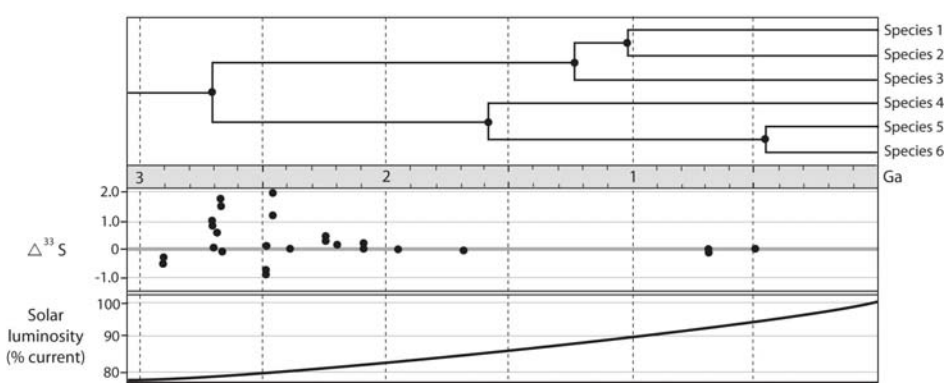

Figure 3.1. One possible presentation of the biosphere database illustrating a timetree of species relationships and divergence times (hypothetical) with panels showing deviation of delta 33-S from the normal mass fractionation line (middle) (Farquhar et al., 2000) and solar luminosity (lower) (Gough, 1981).

IV. LABORATORY MICROBIAL SIMULATIONS: ASTROBIOLOGICAL SIGNATURES

House (Leader), Arthur, Freeman, Ferry, Brantley, and Ohmoto

4.1. INTRODUCTION AND OVERVIEW

In order to understand the evolution of a habitable planet, it is essential that research be directed toward determining biosignatures and also toward understanding the interplay between microorganisms and their environment. We propose to investigate microbial biosignatures and to investigate microbe-environment interaction using a series of different culturing techniques coupled with an array of geochemical, biochemical, and genetic approaches.

First, we propose to use laboratory microbial culturing techniques, genetic analysis, and geochemical approaches to explore biosignatures produced by diverse microorganisms in pure culture and in mixed ecosystems. In particular, we will study the production of biogenic gases, the synthesis of lipids, the signatures recorded by carbon and sulfur isotopic fractionation, and the trace metal content of cells. The results will provide new data on how the geochemical and biochemical impact of microorganisms can be used to detect traces of life in the geological past, on Mars or Europa, and in the atmospheres of extrasolar planets.

Second, we propose to use laboratory microbial culturing to investigate microbe-environment interaction. A significant effort will be directed at investigating the response of anaerobic organisms (including sulfate-reducers and methanogens) to O_2. The goal of this research is to document the relative sensitivity of geologically significant anaerobes to O_2, and identify proteins essential for adaptation to low levels of O_2 by ancient anaerobic lineages. The project is expected to provide data on O_2 sensitivities, reveal responses to O_2 stress including biomarkers for microaerobic conditions, and possibly reveal novel facultative aerobic metabolisms in species traditionally thought of as strict anaerobes. Understanding these innovations will provide important insight into how Precambrian microbial ecosystems responded to the rise of O_2 during the evolution of our habitable planet. In addition to studying the response of microorganisms to O_2, we also propose to use microbial microcosms to study how O_2-producing microorganisms deal with high methane levels and/or sulfidic conditions, and how microbes respond to changes in nutrients, temperature, salinity, pH**,** and varying sulfate concentrations.

Approaches to the problems

In order to advance our understanding of microbial innovation, and the response of microorganisms to environmental impacts, we will draw upon all of the expertise in PSARC in order to apply a myriad of different techniques. This interdisciplinary work will entail genomic and genetic analyses, gas analyses, isotope geochemistry, lipid analyses, gene expression studies (using microarrays and 2D-protein gels), and DNA sequencing. While the integration of these various types of data will be an important challenge, the results will be a comprehensive new view of the interaction of microbes with their environment, and when integrated with other PSARC studies will help create a new understanding of the evolution of our habitable planet.

4.2. LABORATORY SYSTEMS

Specific experiments require different laboratory growth systems ranging in size from small

serum bottles to large-scale microbial microcosms. Currently, PSARC is set up for routine anaerobic microbial culturing in serum bottles of up to 0.5 L volume at temperatures of 0°C to 110°C with continuous orbital shaking, and from 10°C to 60°C with continuous orbital shaking and programmable (i.e., day/night) illumination. PSARC also has a 60-L anaerobic fermentor that can be used at temperatures up to 80°C. This project will use these microbial culturing systems for the cultivation of a wide range of microorganisms in pure culture or in simple artificial and natural consortia. Additionally, we propose to build a set of 0.5-L bench top chemostats and four identical large-scale mesophilic bioreactors to allow for pure-culture, simple consortia, or complex microcosm-style experiments with multiple experimental controls. Each proposed bioreactor will be constructed using a vertical 6-inch diameter, 5 foot long clear plastic pipe surrounded by a removable aluminum pipe to block ambient light (but allow for the viewing of any stratified microbial ecosystem). With illumination from above, these vertical bioreactors will allow for the simulation of some water column microbial ecosystems in a relatively inexpensive and reproducible design. Along the side of the structure, there will be a series of input/output ports for injection or withdrawal of gases and solutions and for sensors of Eh, pH, DO, and T. While we recognize that it will be difficult to control all conditions in such a bioreactor, we are confident that short term (up to a month) microbial culturing experiments will prove valuable for understanding modern microbial ecosystems (such as that of Fayetteville Green Lake), as well as for simulating conditions not presently found of on Earth (such as a low sulfate permanently stratified marine water column).

4.3. RESEARCH ON LABORATORY MICROBIAL COMMUNITIES

4.3.1. Chemical and Isotopic Signatures of the Laboratory Ecosystems

Here, we propose to advance several important areas of astrobiology by using microbial culturing to search for chemical and isotopic biosignatures. The work will answer the following key questions: (1) What were the biogenic gases in the Precambrian atmospheres? (2) What are the carbon isotope biosignatures of lipids and kerogen components of individual organisms and consortia growing under different environments? (3) What are the sulfur isotope biosignatures of pyrite, organic S, and sulfate during sulfate reduction in different ecosystems? (4) How do microbes extract essential metals from minerals? (5) Can cellular metals be used as a biosignature?

4.3.1.1. Biogenic gases (Ohmoto)

An important method for the detection of life (present or past) has been (and will be) to search for the presence of biogenic gases in planetary atmospheres. One approach is to search for a non-equilibrium assemblage of methane (CH_4) and ozone (O_3) (Schindler & Kasting, 2000) in which the CH_4 may have been produced by methanogenic microbes and the O_3 by photochemical reactions of the O_2 molecules generated by oxygenic photosynthetic organisms (e.g., cyanobacteria, eukaryotic algaes, and plants). However, a planet with anaerobic microorganisms, but without a large population of O_2-producing organisms (a condition that persisted on the early Earth) would fail this CH_4/O_3 detection test. Therefore, for the detection of life on such planets, we must identify the characteristics of gases generated by anaerobes (± minor aerobes) under dominantly anoxic conditions where entirely (or mostly) anoxic oceans are overlain by an anoxic atmosphere.

From a large number of analyses made by previous researchers on gases (and aqueous species) in anoxic environments (e.g., within sediments, euxinic seas, marshes, and ponds) (e.g., Hunt, 1974; Whelan et al., 1980; Oremland et al., 1980; Claypool and Kvenvolden, 1983; Ueda et al., 2000), it has become apparent that microbes generate the following products: CO_2 (and HCO_3^-), methane (CH_4), ethane (C_2H_6), propane (C_3H_8), isobutene and n-butane (C_4H_{10}), ethylene (C_2H_4), N_2O, H_2S (and HS^-), and H_2. Typical concentration ratios of these gases in pore waters of anoxic sediments are $CO_2/CH_4 \approx 0.5 - 1$ at the methane maximum, $CH_4/(C_2H_6 + C_3H_8) \approx 100 - 5000$, $(CO_2 + HCO_3^-)/(H_2S + HS^-) \approx 2$. The concentration ratios of these gases, however, vary greatly in the overlying anoxic and oxic water bodies because of variable productions/consumptions of these gases by anaerobes and aerobes in the water bodies. The ratios of these gases that escaped the atmosphere also differ greatly from those in the water bodies because of reactions with O_2 and the differences in Henry's law constants.

Although the information of biogenic gases in modern anoxic environments is useful in predicting the types of gases we could expect in an entirely (or mostly) anoxic world, the ratios of these gases in the Archean oceans (and atmosphere) may have been quite different. In particular, modern anoxic environments are typically overlain by oxygenated water bodies with abundant aerobes. Compared with modern anoxic settings, ancient ecosystems were potentially exposed to different organic substrates and decreased availability of SO_4^{2-} (Habicht et al., 2002) and other oxidized electron acceptors.

In order to predict the nature of biogenic gases in the Archean world (and also other planets with an early biologic history), we propose to create similar conditions in the laboratory and systematically investigate the relationships among the compositions of the atmosphere, oceans, microbial communities, and the production rates of biogenic gases. We must understand: (a) how a specific type of organism (e.g., methanogens and sulfate reducers) in a variety of microbial consortia responds to a change in a specific environmental parameter (e.g., atmospheric concentrations of CO_2, H_2, SO_2; SO_4^{2-} content and pH of oceans; types and flux of nutrients); (b) how the change in the nature of microbial communities will change the production/consumption rates of various biogenic gases; and (c) how the change in biogenic gases will change the chemistry of the atmosphere and oceans and climate.

The microbial consortia in these experiments will be primarily composed of anoxygenic photoautotrophs (green sulfur bacteria, green non-sulfur bacteria, purple sulfur bacteria, purple non-sulfur bacteria), fermentative microbes, methanogens, and sulfate reducing microbes. In some experiments, cyanobacteria, iron oxidizing bacteria, and methanotrophs will be added to the system.

For this work, we will investigate different principle atmospheric gases (N_2, CO_2 and H_2 of various mixing ratios) with small amounts (< 1%) of SO_2, H_2S, and/or O_2 added in some cases. The artificial seawater will be composed of mixtures of major salts (NaCl, $CaCl_2$, $MgCl_2$, and KCl), $NaHCO_3$ (together with the atmospheric pCO_2) to control the pH, Fe^{2+} (varied from 1 to 100 μ*M*), and SO_4^{2-} (0.05 – 30 m*M*). We will also add various mixtures of phosphate, nitrate, and ammonia with trace amounts of some bioessential metals (e.g., Ba, Mo, Ni, and Zn).

Samples of the artificial seawaters and sediments will be periodically withdrawn from the bioreactor, and analyzed for: (a) the abundance of various types of microbes; (b) carbon isotopic composition of the major individual microbial species; (c) chemical composition of solution and dissolved gases; and (d) carbon and sulfur isotopic compositions of solution and dissolved gases. Samples of the air will be periodically withdrawn for chemical and isotopic analyses.

4.3.1.2.Carbon isotopes (House and Freeman)

Evidence for past life is often based either on the carbon isotopic composition of bulk sedimentary organic mater or on the structures and carbon isotopic composition of preserved extractable lipids. The objective of this investigation is to continue past research identifying controls on microbial carbon isotopic fractionation. The proposed project will explore the carbon isotopic composition of bulk biomass or specific compounds produced by pure cultures and artificial microbial ecosystems produced in the laboratory. Previous research has identified the primary carbon fixation pathway as an influence on carbon isotopic fractionation from CO_2 with lower fractionation for the reductive TCA and the 3-hydrohxypropionate cycles (Sirevåg et al., 1977; Holo & Sirevåg, 1986; Preuß et al., 1989; House et al. 2003), and larger fractionations observed in biomass produced from the reverse pentose phosphate cycle and the acetyl-CoA pathway (Preuß et al., 1989; House et al., 2003).

However, it has been observed that the magnitude of isotopic fractionation observed in methanogen cultures is also related to the particular growth status that had been attained by the various cultures, with increasing fractionation as growth proceeded (House et al., 2003). Botz et al. (1996) obtained a similar result when they studied isotopic fractionation from CO_2 to methane. In order to understand carbon isotopic fractionation in methanogens, we will cultivate several different *Methanococcus* species in 500-mL bottles with a large headspace of gas, and in flow-though chemostats. We propose to investigate various different H_2 / CO_2 gas ratios, as well as the carbon isotopic composition of individual compounds as these experiments proceed to test whether changing degrees of isotopic fractionation are related to changes in the relative amount of different biomolecules in the bulk cell mass as the culture grows.

Recent advances in molecular biology and isotopic geochemistry have made the carbon isotopic analysis of nucleic acids, such as rRNA, possible (MacGregor et al., 2002, Pearson et al., 2001; Pearson et al., 2002). In principle, taxon-specific rRNA from a diverse microbial ecosystem can be extracted and analyzed to link microorganisms with their natural isotopic composition in a similar fashion as has been done by direct ion microprobe analysis of identified cells (Orphan et al., 2001; Orphan et al., 2002). However, important laboratory studies are necessary to understand what such results on the natural isotopic composition of RNA could reveal. We propose, therefore, to investigate the carbon isotopic relationships between whole cell biomass, lipids, and nucleic acids within a diverse set of methanogen pure cultures as a principle step toward understanding the isotopic relationship between these different biomolecules within methanogens. The work will provide a basis for using the carbon isotopic composition of natural nucleic acids and lipids of methanogens to study metabolism in a mixed environment.

In a separate set of experiments, we propose to investigate artificial mixtures of cultures created to explore the various different biospheres that could produce extremely ^{13}C-depleted carbon. The motivation for this work is that that extremely ^{13}C-depleted kerogen is an astrobiological signature observed in the Earth's record 2.7 Ga ago and potentially preserved in other planetary settings such as on Mars. A global ecosystem of methanogens and aerobic methanotrophs could be the cause of extremely ^{13}C-depleted kerogen at 2.7 Ga ago (Hayes, 1994). It is not, however, clear that extremely ^{13}C-depleted kerogen requires a global ecosystem of methanogens and aerobic methanotrophs. The conditions for producing such a signature may have occurred in restricted basins. It may also be possible to produce such a signature with acetogens that produce large carbon isotopic fractionations (Preuß et al., 1989) or with anaerobic methanotrophy under low sulfate conditions (Hinrichs, 2002). For these reasons, we propose to study artificially produced mixed microbial ecosystems containing methanogens and aerobic

methanotrophs, while also studying carbon produced by an ecosystem of acetogens and acetotrophs in the absence of methangens.

The results will be integrated with previous data on the carbon isotopic composition of marine anaerobic methane oxidizing microbes (Orphan et al., 2001; Orphan et al., 2002) to give reasonable constrains on how large a ^{13}C-depleteion might be expected from a world of methanogens, of methanogens and aerobic methanotrophs, of acetogens and acetotrophs, and of methanogens and anaerobic methanotrophs (considering different levels of sulfate).

4.3.1.3. Sulfur isotopes (Ohmoto)

Sulfur isotopic composition ($\delta^{34}S$ value) of pyrite in marine sedimentary rocks, or more precisely the difference in the $\delta^{34}S$ values between pyrite and sulfate minerals (anhydrite and barite) of the same geologic age, has been used by many investigators as an important guide for the activity of sulfate-reducing microbes and the sulfate content of coeval seawater (e.g., Hattori & Cameron, 1986; Ohmoto & Felder, 1987; Ohmoto et al., 1993; Canfield & Teske, 1996; Kakegawa et al., 1998; Shen et al., 2001). This is because the magnitude of sulfur isotope fractionation during biological reduction of SO_4^{2-} to form H_2S (and HS^-), which is expressed as $\Delta_{SO4-H2S} = \delta^{34}S_{SO4} - \delta^{34}S_{H2S}$, may depend on the SO_4^{2-} content of water and various other parameters, including the types of sulfate-reducing microbes (genera and families), types and concentrations of electron donors (e.g., H_2, lactate, acetate, alcohols, and CH_4) and temperature (Kaplan & Rittenberg, 1964; Kemp & Thode, 1968; Habicht & Canfield, 1997; Canfield, 2001; Brüchert et al., 2001). For example, Habicht et al. (2002) have concluded that the sulfate content of the Archean oceans must have been less than 1/100 of the modern oceans, because the small $\Delta_{SO4-H2S}$ values of <10 ‰, which may be required to explain the sulfur isotope characteristics of Archean sedimentary rocks, were produced only when the SO_4 contents of their experimental solutions were below ~200 μM. However, a serious problem in such an argument is that all previous laboratory experiments, including those of Habicht et al., were conducted using only one type of electron donor, most commonly lactate, in their experimental systems. In contrast, sulfate reducing microbes in natural environments always occur in consortia with various other microbes that produce and/or consume simultaneously a variety of electron donors. The principles governing the sulfur isotope fractionations in natural environments may be quite different from those in simplified laboratory systems.

The main purpose of our investigations is, therefore, to understand the relationships between the magnitude of sulfur isotopic fractionation and the nature of microbial consortiums, specifically the types of organisms and the rates of production and consumption of H_2, lactate, acetate, alcohols, CH_4 and other hydrocarbons. We will also focus our attention on the sulfur isotope fractionation between ^{33}S and ^{32}S isotopes (i.e., $\delta^{33}S$ value), as well as that between ^{34}S and ^{32}S ($\delta^{34}S$ value), in order to understand the magnitude of mass independent fractionation (MIF) of sulfur isotopes during microbial sulfate reduction (see Section 1 and 2 of this proposal).

4.3.1.4. Trace metals (Brantley and House)

All organisms need essential trace metals for active sites in enzymes, as well as for coenzymes and cofactors. While the relative proportion of metals needed by microorganisms roughly parallels their concentrations in seawater (Schlesinger, 1991; Frausto da Silva & Williams, 1991), there are notable exceptions. The concentration of Fe in oxic seawater is very low, making necessary the microbial production of high-affinity Fe-specific ligands (siderophores) by marine microorganisms. The problematic need to extract micronutrients from

earth materials and natural solutions may have created bottlenecks in evolution.

Of the ~10 or more bioessential trace metals found in rocks, however, only Fe minerals are known to be the target of metal-specific ligands secreted by environmental microbes (Hersman, 2000; Liermann et al., 2000). Although no metal-ophores (here defined as high affinity metal-specific ligands secreted by organisms for metal uptake) other than siderophores have so far been fully identified and documented, several lines of evidence, including findings based upon PSARC-funded research, suggest that these ligands may be important in natural systems. Several researchers have documented that strongly bound ligands found in seawater show great specificity for Fe, Cu, Zn, Cd, and Co (e.g. Coale & Bruland, 1990; Bruland, 1989, 1992; Saito & Moffett, 2002). Saito and Moffett (2002) have inferred the presence of organic cobalt ligands for the uptake of Co by a marine phytoplankton. Moffett has suggested that Cu-complexing compounds may be secreted by some organisms as a detoxification mechanism (Moffett & Brand, 1996; Croot et al., 2000). A variety of compounds secreted by microorganisms have also been identified that show strong and relatively metal-specific chelation capability with metals other than Fe (Stolworthy et al., 2001; Cortese, et al., 2002); however, the utility of these ligands for metal uptake by organisms has not been demonstrated. Thus, if metal-ophores other than siderophores are produced in natural systems, the nature and effect of these ligands is at present not known.

It has been hypothesized that the diazotroph *Azotobacter vinelandii* may produce a "molybdophore" to bind and transport Mo. *A. vinelandii,* a Gram-negative, obligately aerobic soil diazotroph (Page & von Tigerstrom, 1982; Duhme et al., 1996) produces the dicatecholate azotochelin, which acts as a tetradentate ligand ideally suited to bind the four vacant co-ordination sites of the MoO_2^{2+}-unit (Duhme et al., 1998). However, in tests with azotochelin incubated with a Mo- and Fe-containing glass in Co-PI Brantley's laboratory, no enhancement of Mo release to solution was observed. In contrast, incubation of cell-free supernatants from *Azotobacter* culture with the glass causes significant enhancement of the release rate of Mo, and we have identified the responsible ligand: the monocatecholate aminochelin.

A report of this research has been submitted for publication, and we seek further funding now to investigate whether such molybdophores are generally produced by diazotrophs. In particular, we plan to investigate Mo extraction from Mo-silicate and Mo-oxide by cyanobacteria grown under nitrogen-fixing conditions. We will investigate Mo uptake by *Anabaena*, a cyanobacteria which we are already growing in our laboratory, to determine whether this organism secretes a molybdophore.

Our first five years of work has also focused on whether methanogens also produce metal-ophores. Specifically, Ni is essential for many enzymes necessary for methanogenesis. By growing *Methanobacterium thermoautotrophicum* in Ni-depleted medium we have now shown that this methanogen can preferentially extract Ni from a Ni-containing glass. We are working to identify the nature of the ligand secreted. Now, we seek funding to use high performance liquid chromatography, nuclear magnetic resonance, and mass spectrometry to determine the mass and structure of the compound. Under favorable conditions, these techniques in combination can permit the full structural elucidation of novel natural products from crude extracts (Albert, 1999; Lommen et al., 2000; Bringmann et al., 2002). For example, we have previously used HPLC-MS to identify iron-chelating agents (Kalinowski et al., 1998).

When we have more information about the compound, we can continue to test our hypothesis by analyzing for it or seeking evidence of its importance in water or soil samples. To further the search for biosignatures, we also propose to continue to use x-ray photoelectron spectroscopy

(XPS) to search for depletion of Ni and Mo on silicate surfaces after incubation with Ni- and Mo-extracting bacteria. In our earlier work, we identified the development of Fe-depleted layers on hornblende crystal and glass after incubation with a siderophore-secreting soil micro-organism (Kalinowski et al., 1998). We seek to search for other similar chemical signatures on earth materials including exploring using trace metal isotopes as possible biosignatures (Brantley et al., 2001).

Despite being less sensitive to redox conditions, Se and W are scarce in basaltic environments. However, these elements are necessary for the growth of many microbes including hyperthermophiles (Adams, 1994; Kletzin & Adams, 1996). As part of the research funded by a supplemental NAI grant, we have observed W-extraction from basalt by *Pyrobaculum aerophilum* under hydrothermal conditions. This is our first evidence that microbial trace metal extraction may be necessary under certain hyperthermophilic conditions. We have sought NSF funding to continue this work on hyperthermophiles and extend it test whether certain hyperthermophiles extract Se as a trace metal from basalt. As part of PSARC's effort to understand the evolution of a habitable planet, we propose to now explore Se- and W-scavenging in non-hyperthermophilic marine microbes as W is necessary for the growth of methanogens (Kletzin & Adams, 1996) and Se is a growth requirement of a wide range of mesophiles as it is required by any organism that uses selenocysteine as an amino acid (Gladyshev & Kryukov, 2001; Kohrl et al., 2000; Rother et al., 2001).

The varying microbial needs for trace metals suggests that metabolisms that use one or more of the metal-specific enzymes may require elevated cellular concentrations of the cofactor metal. Previous work indicates that *E. coli* exerts tight genetic control over cellular metal concentrations (Outten & O'Halloran, 2001). These studies suggest that microbes contain a background metal content, termed the "metal quota" that is conserved regardless of the metal content of the media. This research seeks to investigate whether or not the trace metal quota of different cultured microorganisms varies with the metabolic capabilities of the species, and thus with the species particular trace metal needs for enzymes and cofactors. A variety of microorganisms will be grown in media of variable trace metal concentrations to correlate their cellular metal quota with their different metabolisms. Alternate culturing strategies will be used to minimize mineral precipitation (such as the use of Ti(III) citrate as a reducing agent in anaerobic media), and trace metal concentrations of microbial biomass will determined by ICP-MS, and tied to cell counts to produce per cell concentrations.

The validity of measured trace metal concentrations of cultured organisms will be examined by direct comparison of cultured bacteria with environmental microbes. This will be accomplished through the development of culture-independent techniques for determining microbial metal content in environmental samples. Fluorescent in situ hybridization has previously been coupled with secondary ion mass spectrometry (FISH-SIMS) to measure carbon isotopic ratios of single cells of environmental organisms (Orphan et al., 2001, 2002). In collaboration with other NAI member institutes, this technique will be modified and performed on contaminant-free polished silicon wafers to measure trace metal content of microbial cells from environmental samples (Fayetteville Green Lake, discussed in Section 5).

In sum, the results of this research will have important implications for astrobiology because (1) the results will reveal ligands for the leaching of metals other than Fe (e.g., Ni, W, Se, and Mo) for a variety of organisms, (2) the results will shed light on the extent to which common microorganisms are altering the chemical and isotopic compositions of mineral surfaces, (3) the results will provide measures of the lower limits of trace metals required by particular

microorganisms, and (3) the results will reveal potential biosignatures of microbial life. In addition to providing microbial biosignatures, this research will help us understand if, when, and how trace metals may have limited ocean productivity (Anbar & Knoll, 2002).

4.3.1.5. Archaea cell membrane lipids (Freeman)

The structures and abundance of microbial membrane lipids are known to vary with properties of the growth environment such as pH and temperature. The fatty acids of Eubacteria and the fatty acids and other waxy compounds (like alkenones) of Eukaryotes are most commonly studied in this context. Lipid structures of Archaea are also known to vary with environmental conditions, most notably temperature. For example, higher growth temperatures are associated with a greater number of cyclic rings in tetra-ether linked diphytanyl structures (DeRosa & Gambacorta, 1988; Gliozzi et al., 1983; Uda et al., 2001).

Recent analytical advances now permit relatively routine analyses of large lipid structures including tetraether compounds from the cell membranes of Archaea (Hopmans et al., 2000). Initial efforts reveal structural diversity among Archaea lipids, and provide an exciting opportunity to understand relationships between changes in membrane lipids and properties of the environment. The membrane lipids from both Crenarchaeota and the Euryarchaeota are distinctive and preserved in the sedimentary record. The two basic types of ether lipids, glycerol diphytanyl glycerol tetraethers (GDGT) and diphytanyl glycerol diethers (DGD), are produced found in a wide variety of environments as well as ancient sediments (Schouten et al., 2002). A new temperature proxy, TEX_{86} (TetraEthers indeX), uses the linear relationship (TEX_{86}= 0.015T + 0.28 (r^2=0.92)) between the relative concentration of cyclic GDGT structures and sea surface temperature (T°C) (Schouten et al., 2002). TEX_{86} has the potential to provide paleotemperature information that may be used in conjunction with other proxies to fine tune ocean reconstructions.

Cyclic GDGTs create a densely packed membrane structure to protect Crenarchaeota living at high temperatures (40-102°C). Mesophilic Crenarchaeota add a six-member ring to create a "kink" that ultimately increases membrane fluidity at lower temperatures (Damste et al., 2002). This type of GDGT is called Crenarchaeol because it is the dominant membrane lipid of pelagic Crenarchaeota. Two major types of DGD, archaeol and hydroxyarchaeol, are chemotaxonomic markers of Euryarchaeota. Two isomers of hydroxyarchaeol appear to be specific to methanogenic genera (Koga et al., 1993; Nishihara & Koga, 1991; Sprott et al., 1993). Carbon isotope analysis of these compounds often confirms that the methanogenic Archaea are active in anaerobic oxidation of methane (Hinrichs et al., 1999, 2000; Nauhaus et al., 2002; Orphan et al., 2002; Schouten et al., 2001). A new macrocyclic DGD containing cyclopentane rings has been attributed to methanogens in the Black Sea (Stadnitskaia et al., 2002). Finally, we note that halophilic Euryarchaeota produce only DGD (Kamekura, 1993; Kamekura & Kates, 1999; Kates, 1997). DGD have been found to document halophiles in ancient evaporite deposits (Barbe et al., 1989; Benali et al., 1995; Grice et al., 1998; Teixidor et al., 1993). Recent work by Schouten et al. (2002) has focused on calibrating the TEX_{86} proxy against annual sea-surface temperature. However, the relationships between lipid structures and salinity are essentially unexplored in both the field and in culture experiments.

We propose to construct a systematic study of lipid structures in a diversity of Archaea cultures, including both a range of growth temperatures and salinities. Experiments will include Euryarchaeota, and Crenarchaeota cultures, as available. In addition, we will evaluate lipid properties in suspended and sinking particles from a range of oceanic localities, including the

Black Sea, the Cariaco Trench, the Mediterranean Sea, the northwestern Atlantic (near Bermuda), and from coastal waters of Virginia and Maryland. These sampling localities will enable us to evaluate relationships between Archaea diversity (via selected analyses using FISH or sequencing methods), lipid chemistry and growth-environment properties, including specifically a range of salinities.

The cultures and field samples will be evaluated using methods that target both the diether and tetraether structures. We have optimized a LC-APCI-MS (liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry) method for evaluation of both types of structures, and we use this method for both structure identification and evaluation of the relative concentrations of both forms. We are also able to isolate diether structures by gas chromatography, coupled with mass spectrometry for structure determination. Our existing, state-of-the-art stable isotope facilities include numerous continuous flow inlets for C, D, O and N analyses on individual compounds and on microgram quantities of solid samples. Coupling molecular isotopic analyses (^{13}C, D) with structural analyses by LC-MS and GC-MS, allow us to link insights to controls on lipid properties with element flow (especially H and C) within cultures and natural ecosystems.

4.3.2. Microbial Response to Environment

By applying microbial culturing to investigate microbial response to differing environments, we propose to address the following important astrobiological questions: (1) How do anaerobes deal with O_2? (2) When did oxygen detoxification enzymes evolve? (3) Are there any biomarkers for microaerobic conditions? (4) How do aerobes (e.g., cyanobacteria and eukaryotes) deal with an anaerobic atmosphere or a sulfidic medium?

4.3.2.1. Oxygen metabolism in anaerobic microorganisms (Ferry and House)

Anaerobic prokaryotes, by definition, are sensitive to O_2; however, accumulating evidence suggests they evolved mechanisms for the utilization of O_2 in energy metabolism and response to oxidative stress. While free O_2 is not likely to have been abundant until the evolution of oxygenic photosynthesis, recent studies by PSARC have revealed that hydroxyl radical is produced at the surface defect sites of pyrite when pyrite is added to O_2-free water (Borda et al., 2003). This leads to the formation of H_2O_2 in a process that is expected for the early Earth (Borda et al., 2001, 2003). The goal of this research is to utilize geochemical, genetic, genomic, proteomic, and biochemical approaches to document the relative sensitivity of geologically significant anaerobes to O_2 and H_2O_2, and identify and characterize enzymes and proteins essential for adaptation to

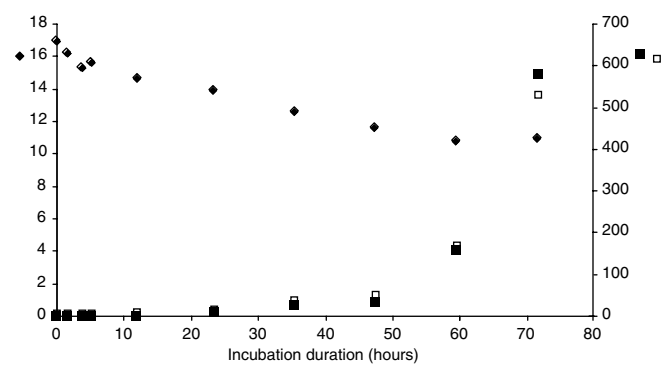

Figure 4.1. Headspace oxygen and methane partial pressures of a "microaerobic" culture of *M. acetivorans* grown in a 125-mL serum bottle. The 30 mL of media contained cystine (S source), but lacked sulfide. Decreasing oxygen is likely due to a reaction with cystine. The results show that exponential growth is obtained within hours of inoculation despite >1% O_2 in the headspace.

low levels of O_2 by anaerobic Archaea.

First, the geomicrobiological implications of O_2-metabolism in anaerobes will be explored. We will document the relative sensitivity of geologically significant anaerobes to O_2 and H_2O_2. Preliminary culturing results show that *Methanosarcina acetivorans* (a methane-producing microbe) will grow in bottles with >1% O_2 in the headspace (Figure 4.1). Using microbial cultivation, gas analysis, HPLC, and numerical modeling, we propose to determine the O_2 concentration in the media and reveal any products of growth that might lead to a biosignature for these conditions (such as a lipid modification or the production of a novel cofactor). For example, quinones are good oxygen scavengers and there may be novel archaeal quinones that are synthesized under oxidative stress that could serve as a biomarker for microaerobic conditions. Furthermore, our preliminary results suggest that the lipid ratio of hydroxyarchaeol to archaeol increases when *M. acetivorans* is grown in the presence of O_2. Additionally, growth in the presence of O_2 and H_2O_2 will be investigated for other organisms that were thought to be strict anaerobes (e.g., acetogens; Boga & Brune, 2003). The ability of oxygen-reducing Prokaryotes to metabolize H_2O_2 for energy will be explored as an explanation for the widespread phylogenetic distribution of this metabolism. Overall, this integrated project will reveal new modes of growth for prokaryotes previously considered strict anaerobes, provide data for considering the microbial implications of the oxidation of the oceans, and may reveal preservable biomarkers indicative of microaerobic conditions.

In contrast to enzymes from aerobic organisms, enzymes from strict anaerobes that reduce O_2, H_2O_2, or O_2^- do not produce O_2 as a product (Ward et al., 2001; Jenney et al., 1999; Gomes et al., 1997; Chen et al., 1993; Coulter et al., 1999; Lumppio et al., 2001) suggesting that these enzymes evolved as a response to free O_2. We propose to investigate this adaptive response to O_2 with genetic, genomic, and proteomic approaches using the model organisms *Archaeoglobus fulgidus* (a sulfate-reducing anaerobe) and *M. acetivorans.* We will exploit the genomic sequence of *M. acetivorans* (Galagan et al., 2002) to identify proteins specifically involved in the adaptive response to O_2. Cells will be cultured in the presence and absence of either O_2 or H_2O_2 to induce the synthesis of proteins expected for reduction and/or dismutation of oxidative species (O_2, H_2O_2, O_2^-), electron transport, and gene regulation. DNA microarray analyses and protein separation/identification methods (LC/MS-MS in collaboration with D. Jones, Penn State) will be employed to identify the proteins that are up-regulated in the O_2-challenged cells. Approximately 40 percent of the ORFs of *M. thermophila,* have no significant percent deduced identity to any known proteins; thus it is expected that novel proteins and enzymes will be discovered and that their characterization will uncover novel biochemical principals. The induced genes will be characterized by transcriptional mapping and the gene sequences will be analyzed to provide preliminary information regarding their potential function. To validate and further investigate the role in the oxidative stress response, the identified genes will be disrupted by established methods (Metcalf et al., 1997) and the mutant cells characterized. These experiments will also lay a foundation for understanding the molecular mechanisms of regulation that apply not only to *M. thermophila* and the methanoarchaea but also to the Archaea domain.

Additionally, we will characterize the newly discovered, and previously hypothesized, proteins involved in the O_2-adaptive response for *A. fulgidus* and *M. acetivorans*. We will ask if they either interact directly with oxidative species (i.e. catalyze reduction/dismutation to less toxic products) or function to supply electrons to the reductive enzymes. It is also planned to utilize biochemical and molecular approaches to determine if any of the up-regulated proteins are

redox sensors that could function to regulate the expression of genes involved in the adaptive response. The proteins will then be characterized kinetically and biochemically to determine the function and catalytic mechanism. The structures of proteins will be determined in collaboration with PSARC associate Dr. Cathy Drennan (MIT). The structural information together with sequence analyses and biochemical/physiological characterization will be used to predict the evolutionary history of ancient enzymes and metabolic pathways.

4.3.2.2. Response of a microcosm to changes in environment (Arthur and House)

As described later in the proposal (Section 5), Fayetteville Green Lake (FGL) is an ideal natural setting that in some ways approximates a Proterozoic ocean. In order to gain insight into the microbial biosphere of the Proterozoic and Archean, we propose to use samples from FGL to create microbial microcosm that can be perturbed in the laboratory. In parallel with field studies (described in Section 5), we will create microbial microcosms using the various microorganisms collected from FGL These relatively large-scale bioreactors will, in essence, be living box-models with-which to ground truth numerical modeling studies, as well as environments on which experiments can be conducted.

Our first objective will be to compare the geochemical attributes (dissolved oxygen, nitrate, ammonia, phosphorous, etc.) of such a microcosm as a function of time with those of FGL to assess how similar these artificial microcosms can be to a natural setting. After this initial work, we propose to study the effects of major perturbations to the microcosm including creating conditions that do not exist presently on Earth. One such experiment will be to alter the headspace to be consistent with postulated atmospheres of the early Earth (see Section 4.3.1.1 Biogenic gases) in order to investigate the response of cyanobacteria when exposed to high dissolved methane concentrations, an anoxic atmosphere, and/or a sulfidic medium. Experiments will also include observing the microbial response to changes in nutrients provided – such as nitrogen sources, phosphorous, and trace metals (Beck et al., 2002; Flemer et al., 1998). These experiments will provide important information with which to interpret the biogeochemical cycles of FGL, as well as with which to address the hypothesis that different nutrients have limited the productivity of the photic zone at various time in Earth history (Anbar & Knoll, 2002). Additionally, these FGL laboratory microcosms will be used to observe the effects of that microbial ecosystem when sulfate concentrations are greatly reduced. The importance of this research stems from the fact that the sulfate concentrations of Precambrian oceans are thought to have been significantly lower than those of FGL. The microcosm experiments coupled with the field experiments proposed later in this proposal will allow us to accurately extrapolate results (including sulfur isotopes) from FGL to allow geochemical biosignatures to be used for the examination of Precambrian ecosystems.

As with the work on biogenic gases described previously, samples of the artificial seawaters and sediments will be periodically withdrawn from the bioreactor, and analyzed for: (a) the abundance of various types of microbes; (b) carbon isotopic composition of the major individual microbial species; (c) chemical composition of solution and dissolved gases; and (d) carbon and sulfur isotopic compositions of solution and dissolved gases. Samples of the air will be periodically withdrawn for chemical and isotopic analyses. Additionally, genomic approaches (such as microarrays) will be employed as a means to monitor changes in species composition, as well as changes in gene expression during experiments.

V. MODERN ANALOGUES OF PRECAMBRIAN MICROBIAL ECOSYSTEMS

Kump (Leader), Arthur, Brenchley, Freeman, House, Capo, and Stewart

5.1. INTRODUCTION AND OVERVIEW

A strong motivation for conducting the microbial laboratory cultivation experiments of Section 4 is that the pervasiveness of eukaryotic life in natural environments makes modern ecosystems different than those of the Precambrian. With rare exceptions, only extreme modern environments (hydrothermal springs, highly evaporative lagoons, arid desert soils) provide modern analogues of Precambrian ecosystems that existed before the rise and spread of metazoans. Other NAI groups have been investigating these extreme environments, including the Ecogenomics Focus Group and the microbial mat studies being performed at NASA Ames. We propose to complement their investigations with numerical modeling of mat biogeochemistry (work in progress) and field studies of other modern settings where archaea and eubacteria dominate biological and biogeochemical processes. One such setting is a meromictic (permanently stratified) lake that supports a diverse microbial community of cyanobacterial, green and purple sulfur bacteria, sulfate-reducing bacteria, and methanogens and may provide a useful analogue for Precambrian pelagic ecosystems, of interest to the Mission to Early Earth Focus Group. Our studies of microorganisms in deep glacier ice cores will help meet the NASA objective of learning about the environmental limits of life, provide insight into how organisms could survive extended cold periods during the Snowball Earth, and serve as analogues to extraterrestrial cold habitats that might exist on Mars or Europa (Kerr, 2001; McKay et al., 1992). Finally, we will continue our investigation of modern soils forming in desert environments and developed on mafic and ultramafic parent materials to aid in the evaluation of early Earth land-atmosphere interaction and in the identification and interpretation of Precambrian terrestrial environments.

Through these investigations we will address the following major questions of Precambrian biospheric evolution:

1) What was the efficiency of the "biological pump" in Precambrian pelagic marine ecosystems? Was it sufficient to sustain a chemically stratified global ocean?
2) Were essential trace elements available to planktonic ecosystems living in an ocean with sulfidic deep waters?
3) What are the mechanisms of metabolism, long-term survival, and recovery of viable microbial cells in glacial ice?
4) Can we detect any organism or groups of organisms that could serve as biomarkers for specific geological or climatic events?
5) Are there diagnostic criteria for soils developed under anoxic atmospheres that are distinct from those of soils that are devoid of oxygen because they are water saturated?

5.2. A MODERN ANALOGUE OF PRECAMBRIAN PLANKTONIC ECOSYSTEM: FAYETTEVILLE GREEN LAKE, NEW YORK, USA

The primary focus of most research on Archean and Proterozoic life has been on microbial mats (including stromatolites), but it is likely that the pelagic oceans were productive, and some form

of "biological pump," the transfer of biological material from surface ocean to the deep through particle settling or advection, existed in the ancient ocean (e.g., Hotinski et al., submitted). The efficiency of the biological pump is uncertain: absence of fecal pellets and eukaryotes such as diatoms that enhance coagulation of small (non-settling) particles may have slowed the transfer of organic material to the deep sea, affecting the oxidant demand that presently creates the O_2 minimum in the ocean (Logan et al., 1995). However, microbes play a fundamental role in the formation of marine aggregates (Decho, 1990). Moreover, cyanobacteria are known to stimulate and perhaps regulate (Thompson & Ferris, 1990) the precipitation of $CaCO_3$, which serves as an effective ballast material in the modern ocean (Armstrong et al., 2002). The establishment of an anoxic and sulfidic deep ocean in the Proterozoic (Canfield, 1998), when atmospheric oxygen levels were significant, requires an efficient biological pump. We can only interpret the Archean and Proterozoic redox history of the atmosphere/ocean system from marine sedimentary rocks if we understand the effect that planktonic life had on the deep sea.

We will use a variety of approaches, including direct observation, manipulation using limnocorrals (e.g., Schindler, 1999), and numerical modeling to understand the controls on biological pumping in a modern, microbially dominated ecosystem. We have selected an ideal natural observatory: Fayetteville Green Lake (FGL) in upstate New York. In FGL surface waters, planktonic cyanobacteria dominate primary production and facilitate a seasonal precipitation of $CaCO_3$ in the water column (a "whiting"). Because it is meromictic and receives sulfate-rich groundwaters, FGL exhibits a strong chemocline (Figure 5.1) replete with the full suite of autotrophic and heterotropic sulfur bacteria so important in microbial mats and other anoxic, sulfidic environments (e.g., the Black Sea). The lake's small size (500m diameter) allows us to sample it comprehensively both in space and time, a situation not afforded by oceanographic studies of such basins as the Black Sea. However, in comparison to the microbial mat, the spatial scale of variation is large, so that we are able to detail its structure without having to resort to microelectrode techniques. In fact, although the focus of our study will be on

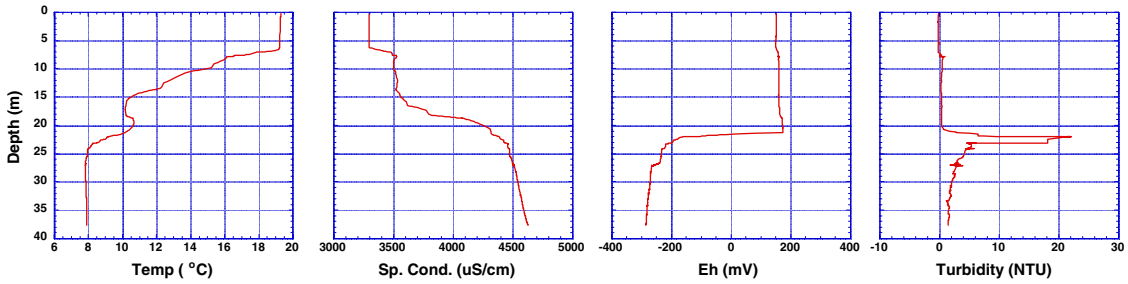

Figure 5.1. Characteristics of FGL on September 30, 2002. Temperature and conductivity profiles indicate that a pycnocline extends from 15-25m, while the Eh and Turbidity profiles reflect a strong chemocline at 21.5 m. Turbidity maximum results from high cell densities of purple and green phototrophic sulfur bacteria.

the pelagic ecosystem, FGL has its own well-developed carbonate bioherms and microbial mats (Thompson et al., 1990) which allow us to directly examine ecosystem function at a variety of spatial scales, from the millimeter scale of the mat to the meter scale of the water column. We will monitor intensive variables over the course of multiple seasonal cycles using a multiprobe sonde (YSI), a remote video camera for plankton observations, and a spectral irradiance monitor. Water and particle samples will be recovered by peristaltic pumping through the tubing system.

Samples will be processed and/or fixed immediately on board the pontoon vessel and transferred to the shore-based lab for analysis or frozen for later analysis.

5.2.1. **Nitrogen and Phosphate Cycling** (Kump and Arthur)

Phosphate is often the limiting nutrient in lacustrine systems, and it has been proposed as a limiting nutrient for the Precambrian marine biota as well (Bjerrum & Canfield, 2002). However, in meromictic lakes such as FGL, there is substantial recycling of phosphate that results from both sorption-desorption reactions on particles (especially Fe and Mn oxides) and organic phosphorus remineralization (Taillefert et al., 2000; Hongve, 1997). High concentrations of dissolved phosphate in the anoxic monimolimnion supplies phosphate by mixing and diffusion across the chemocline. Blooms of *Synechococcus* and their associated calcite precipitation may be effective in controlling phosphate concentration. Phosphate is known to have an affinity for calcite surfaces, and a high flux of micron-sized calcite particles from surface waters may limit phosphate availability and productivity during blooms (Otuski & Wetzel, 1972).

Little is known regarding these rates and processes of phosphate cycling in layered microbial systems. Our research will examine the role of particle fluxes, including organic particles, calcite grains and iron oxyhydroxides (which undergo reductive dissolution, perhaps mediated by bacteria) in phosphate transfer from shallow- to deep waters using sediment traps. We will monitor alkaline phosphatase activity and attempt to relate it to fluxes of organic phosphorus from grazing and transport from the chemocline.

Most sediments of ancient anoxic basins have average nitrogen isotopic values of +1‰. This relatively low N isotopic value has led some workers to propose an active role for cyanobacteria in fixing nitrogen within surface waters in these basins with substantial transfer of this carbon to sediments. However, there is little evidence for N fixation in oxic surface waters of modern anoxic basins, such as Framvaren Fjord (Velinsky & Fogel, 1999). It is more likely that particulate organic fluxes to sediments in such basins are dominated by production within the chemocline where N can be fixed by phototrophic anaerobic sulfide oxidizing bacteria and/or a reservoir of isotopically light ammonia is available. Depleted carbon and nitrogen isotopic values in sediments of anoxic basins probably indicate the presence of a chemocline within the photic zone, as shown for Framvaren Fjord by Velinsky and Fogel (1999). We need to understand the relative importance of carbon flux from oxygenic photosynthetic organisms vs. anaerobic phototrophic organisms in such environments. Thus, analyses of the nitrogen (and carbon) isotopic composition of DIN and particulates in the water column and sedimentary nitrogenous phases in Green Lake may provide a picture of Proterozoic planktonic ecosystems.

5.2.2. **Trace Metal and Sulfur Cycling** (Kump, Arthur, Capo, and Stewart)

Under euxinic conditions (including, perhaps, the deep oceans of much of the Proterozoic; Canfield, 1998) concentrations of redox-reactive metals, particularly Fe, Cu, Zn and Mo, can be limiting to productivity in the photic zone because of direct growth limitations and suppression of nitrogen fixation (Anbar & Knoll, 2002). Thus, it is important to examine the cycling of these trace metals through incorporation into organic matter, sorption onto particles, transport to the chemocline, particle dissolution and decomposition, metal desorption, and precipitation of metal sulfides (e.g. Suits & Wilkin, 1998; Wilkin et al., 1997). We will trace this cycling using large-volume pumping and filtering, sediment traps and by analysis of sediment metal accumulation

rates. We will also determine total dissolved reduced sulfur concentrations in the deep lake. The FGL water column will be sampled at discrete intervals and samples will be analyzed for dissolved and particulate Fe, Mo and S. To track particulate fluxes and Fe cycling processes, Nd and Fe isotope ratios will be measured on selected samples and on lake input sources. FGL sediments and particulate matter will be sequentially extracted to verify the dominant form in which the metals and S are sequestered.

The sulfur isotopic composition of dissolved sulfate and sulfide (and individual reduced sulfur species when possible), organic sulfur, and sulfide minerals such as pyrite and/or monosulfides will allow characterization of sulfur species in the water column and sediments (Fry, 1986). We also plan to track the isotopic composition of sulfate in surface waters via analyses of sulfate found in calcite precipitated in the mixolimnion. The latter analyses will help validate the technique for tracking ancient sulfur cycling.

5.2.3. **Genetic Approaches** (House in collaboration with D. Bryant, PSU)

Complete sequencing of microbial genomes has dramatically altered the approaches that are available for analyzing the total responses of organisms to their environment. An important part of our research is to identify gene products that are associated with these biogeochemical processes. To this end, we will develop and employ several different environmental genetic approaches.

Polymerase-chain-reaction (PCR)-based amplification of 16S rRNA sequences will be used to identify closest relatives of the phototrophic and heterotrophic organisms of the lake community that have been well-studied in the laboratory. Complete genomic sequences are already available or in progress for cyanobacteria (*Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120, *Nostoc punctiforme*, *Prochlorococcus marinus*, *Synechococcus* sp. PCC 7002, *Synechococcus vulcanus*, and *Synechococcus* sp. PCC 7942), a green sulfur bacterium (*Chlorobium tepidum*) and purple non-sulfur bacteria (*Rhodobacter capsulatus*, *Rhodobacter sphaeroides*). This sequence information, as well as that from other bacteria, can be used to identify specific genes that can serve as reporters of nutrient status or activity status for various biochemical pathways. The activation of these targeted reporter genes will be detected using DNA microarray methods (Taroncher-Oldenburg et al., 2003; Dennis et al., 2003), and thus the nutritional status and biochemical activities of the major photoautotrophs in FGL will be monitored throughout the year. These season-long microarray experiments will (1) provide complete seasonal profile for the genetic regulation of the genes identified as being important to the biological response to the seasonal cycle, and (2) provide adequate time dependent data for analysis of the statistical significance of the genetic results identified during this project. The available sequence information for model organisms will also be used in leveraged sequencing approaches to identify genes conferring unique capabilities to the organisms of FGL.

5.2.4. **Numerical Modeling** (Kump and Arthur)

Our goal here is to adapt a simple, one-dimensional model of the physical mixing, temperature and density structure of the lake, and to then add biogeochemical and stable isotopic components to the model to investigate the interplay between the biota and their physical environment and its isotopic signature. The model will incorporate our understanding of the couplings among system components. Simulations will be performed for each of the limnocorral

experimental conditions and for the annual cycle of light and temperature. Toward this end we will begin with the lake model developed by Jewell (1992). Adequate treatment of trace-metal cycling, however, requires that the water-column model be modified. It must account not only for active trace-metal uptake by the biota, but for the nutrient or toxic effects of some metals and their tendency to be scavenged onto small particles, both of which are dependent on their speciation (e.g., Clegg & Whitfield, 1990, 1991).

5.3.MICROBIAL DIVERSITY IN GREENLAND GLACIAL ICE (Brenchley)

5.3.1. Introduction

Compared to other cold environments, glacial ice sheets are special habitats that have preserved microbial life (including *Proteobacteria, Cytophaga/ Flavobacterium/ Bacteroides* and Gram-positive bacteria) chronologically for geologically significant times (Abyzov, 1993; Christner. et al., 2000; Christner, 2002). These results suggest that microorganisms survive for long periods in ice and may be metabolically active at subzero temperatures, possibly in proposed liquid veins along three-grain boundaries in ice (Price, 2000; Junge et al., 2001). In order to investigate microbial survival during these extreme conditions of low temperature, nutrients, oxygen, and desiccation, we will expand our investigation of the abundance, viability, diversity and recovery of microorganisms in Greenland glacier ice cores. The proposed research addresses the following questions: (1) Do different physiological groups predominate at different depths in the ice sheet? (2) What are the mechanisms of long-term survival and recovery of viable microbial cells?

Our previous investigation demonstrated the presence of an abundant viable (10^7 cells/ml) microbial community trapped for over 100,000 years in the basal portion of a Greenland glacier (GISP2) ice core (Sheridan et al., in press). Clone libraries of 16S rDNA were obtained from anaerobic psychrophilic enrichment cultures started from the ice core. Approximately 60 Bacterial inserts were screened and 24 representative sequences compared. Diverse Bacterial sequences represented major phylogenetic groups including *Alpha-, Beta-* and *Gamma-Proteobacteria* as well as relatives of the *Thermus*, *Bacteroides*, *Eubacterium*, and *Clostridium*. Seven sequences may reflect new genera and one was over 12% distant from its closest relative and may represent a novel Order (Sheridan et al., in press). In addition, nearly 800 isolates were recovered from the melted ice and aerobic and anaerobic enrichments. The phylogenetic and physiological analyses of the isolates showed a diverse range of physiological groups, dominance of psychrophilic and pigmented organisms, and a higher representation of low G+C Gram positive bacteria as

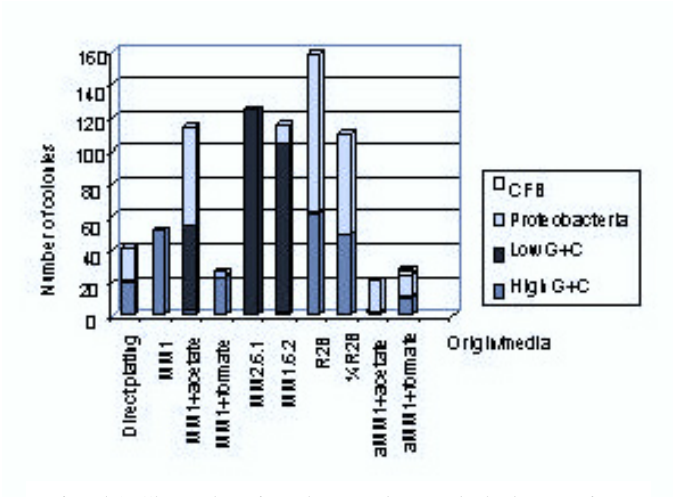

Fig. 5.2 Chart showing the number and phylogenetic distribution of colonies obtained after directly plating a sample from the ice core and after enrichments in a variety of anaerobic media.

compared to the clone libraries (Figure 5.2). Only three isolates had 16S rRNA gene sequences corresponding to those from the clone library. These results demonstrated the value of using both culture-dependent and culture-independent approaches to obtain a more comprehensive view of the ice core community. Of particular importance was the discovery of many dwarf cells (< 1 micron) so small they would have been excluded by the standard methods for concentrating cells. Because this finding is significant to detecting microorganisms in all environments, including extraterrestrial habitats, one of our future goals includes developing methods for examining and identifying these ultra-small cells.

5.3.2. Objectives, Goals, and Approaches

In order to address questions relevant to our NASA mission, we will investigate both the Bacterial and Archaeal diversity of Greenland ice cores and other glacial environments. Our objective will be to reveal their community structure, identify the ultra-small microbial population, and test proposed mechanisms of survival and recovery of damaged and stressed cells. Future success in the recovery and cultivation of environmental isolates will largely depend on our knowledge of the mechanisms these organisms use to deal with the multiple stress factors associated with life in one of the most extreme environments on Earth.

Goal one: Examining the community diversity. Ice core sections will be treated to remove contaminated layers and aseptically sampled using procedures developed in our laboratory. The morphological diversity will be examined microscopically and the ratio of live/dead individual cells determined by epifluorescence microscopy. We will also use flow cytometry to estimate cell abundance and cell size and FISH (fluorescent in situ hybridization) to determine the *in situ* presence/absence of *Bacteria* and *Archaea* and of key physiological groups, especially ones of geochemical interest in other sections of this proposal. The prokaryotic diversity in these new ice core samples will then be analyzed using both culture independent and culture dependent analyses to obtain a comprehensive view of the populations. The first approach will include extracting DNA directly from different sections of the ice core followed by PCR amplification, cloning, and sequencing the 16S rRNA genes for phylogenetic comparisons. The culture dependent examination will recover and isolate organisms from different ice samples and enrich for physiological groups targeted in the microscopic and culture independent studies. Novel isolates will be characterized physiologically and phylogenetically.

Goal two: Studying ultra-small microorganisms. Extremely small cells dominate oligotrophic terrestrial habitats (Torrella & Morita, 1981), it has been proposed that they represent a novel group of organisms (Schut et al., 1997). The presence of small but viable cells in the melted ice and other oligotrophic environments is also consistent with the concept of adaptation to stress, including cell size reduction, cytoplasm condensation, permeability loss, RNA content decrease, inability to multiply, and a metabolic reduction leading to viable but not culturable (VBNC) state (McDougalda et al., 1998). Our work will separate and characterize these dwarf cells and determine whether they represent one dominant type or a heterogeneous population.

Goal three: Determining factors that affect survival, recovery and culturability of microorganisms. Our observations suggest that microorganisms trapped in the ice are dormant or injured and require special conditions for resuscitation. Others suggested that stressed cells undergo oxidative suicide after transfer to a rich media (Bloomfield et al., 1998). We will design media and incubation conditions to examine the critical factors needed to recover stressed cells. We also observed that over 75% of the isolates were pigmented, as seen in other cold

environments (Bowman et al., 1997; Christner et al., 2000; Christner, 2002; Dancer et al., 1997; Smith et al., 2000). We have obtained a non-pigmented variant of one isolate and will test the hypothesis of Fong et al. (2001) that pigments enhance survival. The pigmented isolate and its variant will be subjected to freeze-thaw treatments, UV irradiation, and desiccation and the survival of the cells compared. Our work will answer questions about life that survives *under extreme conditions and explore the biochemical strategies that push the physical-chemical limits of life.*

5.4. MODERN SOIL ANALOGUES FOR PRECAMBRIAN TERRESTRIAL ENVIRONMENTS (Capo and Stewart)

Soils harbor primitive life forms on the early Earth and other planetary bodies. Preserved ancient soils (paleosols) also offer one of the few records of direct lithosphere-atmosphere interaction, and have often been used to evaluate the composition of Earth's early atmosphere (e.g., Holland, 1984; Ohmoto, 1996). We propose to investigate modern soil profiles, with a particular emphasis on those aspects that are relevant for interpretation of Precambrian paleosols and extraterrestrial weathering processes. In particular, the problems to be addressed include: (1) the behavior of redox-sensitive elements in soils developed under differing Eh-pH conditions; (2) the relationship between bulk soil chemistry and the evolutionary history of the soil; (3) the impact of multiple sources of material (e.g., fluvial, eolian) on the geochemistry of a soil profile; and (4) radiogenic and Fe isotope systematics of modern soils and implications for soil chronology and evolution.

In collaboration with soil scientists and R. Graham and O. Chadwick, (PSARC Associate Members), we will focus on soil profiles that allow the best comparison with the pedosphere of early Earth and that of other terrestrial planetary bodies (e.g., Mars). Soils developed on mafic and ultramafic substrate would be appropriate analogues for many early Precambrian terrestrial paleosols, and common on extraterrestrial surfaces; soils developed on ultramafic rocks such as serpentinite also tend to be sparsely vegetated and thus better analogs for Precambrian soils. Pedogenic carbonate has been documented in Precambrian paleosols (e.g., the Schagen paleosol, South Africa; Martini, 1994; Watanabe et al., 2000) and could be a significant component of near surface deposits on Mars (Knauth, 2001). Profiles developed under locally anoxic conditions provide insight into the geochemistry and texture of soils that formed in an anoxic atmosphere vs. those that formed under water-saturated conditions. Candidate sites for detailed field, micromorphologic, elemental and radiogenic isotope tracer studies study include well characterized weathering profiles developed on serpentinized ophiolite units in Northern California (e.g., Alexander et al., 1990; Graham et al., 1990), hydromorphic, anoxic soils developed on basalt in Hawaii (Stewart et al., 2001), and desert soils containing calcite and dolomite from Hawaii (Capo et al., 2000; Whipkey et al., 2002) and New Mexico (Capo and Chadwick, 1999).

VI. PLANETARY HABITABILITY AND LIFE DETECTION

Sigurdsson (Leader), Wolszszan, Kasting, Freeman, Capo, and Stewart

INTRODUCTION

The earlier sections of this proposal focus on efforts to understand the earliest stages of the evolution of the atmosphere, the role of biochemical processes in modifying the Archean surface, and the characterization of the early evolutionary processes that lead to the development of life, and the interaction of life and planetary geochemical processes. In this section we propose to initiate a new effort to make some additional progress in understanding how astrophysical processes give rise to planets elsewhere, the dynamical evolution of planetary systems, and investigations both to improve our understanding of prospects for the detection of habitable planets, and the actual detection of biosignatures. This involves integrating ongoing research in the Penn State astronomy department with the existing Astrobiology effort, and expanding astronomical research into new areas, resources permitting. We note that the Astronomy department has made an offer of a tenure-track position to a planetary astronomer who is a member of the Terrestrial Planet Finder (TPF) science working group.

The research proposed in this section complements work at other NAI institutions and ongoing research which is beyond the current scope of the NAI. With the proposed formation of a new NAI Astronomy Focus Group, it is important to have a local astronomical focus at PSARC and to integrate astronomical research into complementary ongoing research in planetary sciences and the evolution of the early Earth. PSARC provides a valuable nexus for cross-disciplinary communication, and through its student support it provides a basis for the long term presence of a group of astronomers with formal education and network connections in the planetary sciences, geosciences, chemistry and biology. It is the establishment of such informal networks that will lead to long term synergy between the currently somewhat disconnected fields.

A major focus of our research efforts over the next five years will be to investigate the chances of finding habitable planets around different types of stars and to aid in the search for life on such planets and on planets in our own Solar System. This work will involve a combination of theoretical, observational, and laboratory-based research. The following section describes those efforts.

6.1. TESTING THE "RARE EARTH" AND PERSISTENCE IN THE HZS (Sigurdsson)

Is the Earth Rare? Relatively little theoretical consideration has been given to the question of planet formation around non-solar like stars, although a few authors have discussed this problem (Sigurdsson, 1992; Lineweaver, 2001; Zinnecker, 2002). This lack of attention is in part because our understanding of the formation of the Solar System itself has been limited and has absorbed much effort. The older stars in the Galaxy are systematically more metal-poor than the Sun. Considering the length of time it took for multi-cellular life to develop on Earth, and that for 98% of the age of the Earth evolution was not at a stage where intelligent life could plausibly have developed, long term prospects for detection of complex life outside the solar system must

be sensitive to how late in the history of the Galaxy the opportunities for terrestrial planet formation in the habitable zone around main-sequence stars became possible. This tests some of the key assumptions of the "Rare Earth" hypothesis put forward by Ward and Brownlee (2000). This hypothesis suggests that complex life (defined as multicellular animal life) is rare because the conditions required for its evolution are rare. One aspect of this hypothesis concerns stellar metallicities. We would like to understand whether Earth-like planets could have formed around the earlier generations of stars.

6.1.1. Role of Stellar Metallicity in the Formation of Habitable Planets

Planet formation does not necessarily take place exclusively in solar, or near-solar, composition protoplanetary disks. How does stellar metallicity, particularly carbon and oxygen abundances, affect terrestrial planet formation and primordial atmospheric composition? It has been argued that anomalously high metallicity is critical to planet formation in the habitable zone (Gonzalez, 1999; Gonzalez et al., 2001; Lineweaver, 2001), but other considerations suggest that planet formation may generically take place over a wide range of initial composition of the proto-planetary disk (Gaidos, 2000; Sigurdsson, 1992; Miller & Hamilton, 2001). There is little observational evidence for planets around non-solar like stars (cf. Zinnecker, 2002; Gilliland et al., 2000; an exception is the planet in M4 [Thorsett et al., 1999]). If top-down formation of Jovian planets, through a disk gas instability, is a common formation mechanism, then Jovian planet formation may be insensitive to metallicity, but terrestrial planet formation may be sensitive to it (cf. Boss, 2002). Gaidos (2000) noted the importance of C/O abundance ratios, which correlate with mean metallicity, but with large stochastic scatter for individual stars. This determines the oxidation state of metals in the protoplanetary disk and the water abundance. We plan further investigation of the impact of different ice abundances and oxidation states as initial conditions for the formation of terrestrial planet mantles, and the late bombardment enrichment of the surface. The prospects for life and early evolution of the atmosphere will depend on the initial composition, and some exploration of the range of possibilities will make an interesting student project.

We have two fundamentally conflicting lines of evidence: On the one hand, some authors argue that anomalously high metallicity may be critical to planet formation and persistence within the habitable zone (but see Heiter & Luck, 2002). On the other hand, the Copernican Principle would suggest that planets form easily and persist robustly in the most extreme range of metallicity environments probed. Resolution of this issue is critical to our understanding how common terrestrial planets with liquid water are, how stably they remain in habitable zones, and hence the number of planets on which life may evolve and persist for significantly long times. To investigate this issue we propose to first extend modeling of the "branching ratio" for solar impact of planetesimals and planetary embryos during the dynamical evolution of young model planetary systems. Even if the degree of pollution is inadequate to explain the observed planet-metallicity correlation, we still expect potentially quantifiable accretion of metals onto the star; this may also provide a signpost for searching out planetary systems. The mechanism described in Debes and Sigurdsson (2002) can selectively enhance stellar metal accretion, providing a signpost for multi-planet systems that may be especially interesting as sites for life bearing terrestrial planets (cf. Chambers, 2002). Secondly, we will model the formation and evolution of, and look for planets around, non-solar like stars. Most of the observing effort involved in this will be supported by independently of NAI (HST proposal, submitted), the students supported by

this grant are expected to take part in the observing effort and in modeling and analysis of the data.

6.1.2. Geochemical Initial Conditions and Atmospheres

What is the probability distribution of observing the atmosphere at different stages, and hence the probability of detecting given presumed biomarkers? If terrestrial planets formed early in galactic history, then this both affects future strategies for which stars to search for habitable planets, and the duration that has been available for a "typical" habitable planet to evolve life and imprint biosignatures on its atmosphere. We will model probability of seeing an atmosphere at different stages (oxidizing, reducing, inert, etc.) depending on formation scenarios and the composition of the planetary nebula. This investigation is planned to form a major part of A. Mandell's PhD thesis.

6.1.3. Spectroscopic Studies of Parent Stars

Are the spectroscopic "smoking gun" signatures of planet formation in the photospheres of host stars? We have been carrying out simulations of asteroid scattering due to jovian planet migration and evaluating rates of photospheric contamination by planetesimal and planetary collisions. The resultant anomalous metallicity can provide a spectroscopic signature of a planetary system and a diagnostic of past planetary dynamical evolution (Figure 6.1).

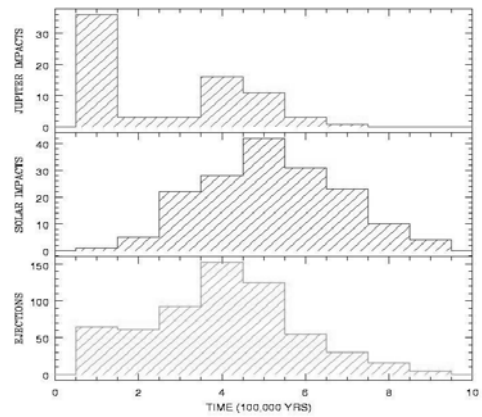

Figure 6.1. Relative frequency of ejection, solar impact and Jupiter impact for a proto-asteroid belt perturbed by a migrating Jovian planet for a solar-like system. The resultant metal accretion onto the parent star can lead to a detectable spectroscopic signature of due to metal contamination of the stellar photosphere, such a signature must be present even if it is not the cause of the observed correlation between metallicity and close Jovian planets (Mandell et al., in preparation).

6.1.4. Habitable Moons and Post-migration Planets

Currently known planetary systems are unlike the solar system, and have probably undergone substantial dynamical modification due to jovian migration. What are the prospects for habitable moons, and habitable planets in post-migration planetary systems? We have been creating models of the dynamical evolution of systems of inner terrestrial planets in response to the onset of rapid migration of outer Jovian planets. Intriguing preliminary results (Figures. 6.2 and 6.3) suggest that there is a significant probability for a terrestrial planet to survive Jovian migration, enabling post-migration circularization of terrestrial planets in

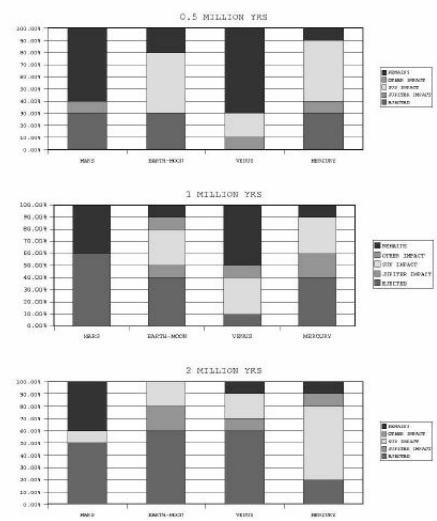

Figure 6.2. Survival probability for terrestrial planets in a Jupiter migration scenario. Note there is a significant probability for survival in a stable orbit. There is also a significant solar impact probability, with possible ensuant detectable photospheric metal pollution (Mandell & Sigurdsson, in preparation).

dynamically stable orbits in the CHZ, outside the now close orbiting "hot Jovian." This suggests that some of the already detected planetary systems may be good prospects for terrestrial planet detection, despite the probable low formation efficiency of terrestrial planets post-migration (Armitage, 2002).

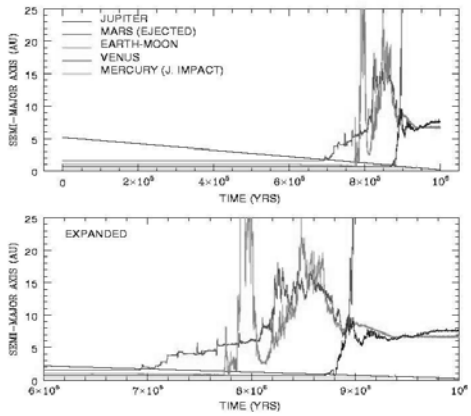

Figure 6.3. Final semi-major axis and eccentricity of a Solar-like planetary system after post-formation Jovian migration. Note that some of the terrestrial planets survive the migration in eccentric, moderately widened orbits (Mandell & Sigurdsson, in preparation).

6.2. SEARCH FOR PLANETS AROUND WHITE DWARF STARS (Wolszczan)

The possible existence of planets around white dwarfs (e.g., Livio et al., 1992) has been seriously considered since the time of the discovery of the first extrasolar planets orbiting the neutron star PSR B1257+12 (Wolszczan & Frail, 1992). Although discoveries of planets around solar-type stars are of more immediate interest (e.g. Marcy et al., 2000), a thorough search for and study of planetary survivors of the post-main sequence evolution of stars of various types is necessary to gain a global understanding of planetary systems and their fate over timescales comparable to the stellar lifetimes.

Possible detections of white dwarf planets would have a number of important consequences. They would provide unique information on planets around stars of spectral types A-F which are difficult, if not impossible to study by means of Doppler spectroscopy. Such planets would also be of great interest from the point of view of planet formation around previous generations of stars. Since it is clear that planets surviving around post-main sequence stars would undergo a significant dynamical evolution (e.g., Debes & Sigurdsson, 2002, and references therein), this process could be studied and understood on the basis of direct or indirect observations. Finally, as there is a possibility that planets can form as a byproduct of white dwarf mergers (Livio et al., 1992), such second-generation planets could be detected by properly designed surveys and, as in the case of neutron star planets, they would provide clues concerning planet formation in circumstellar disks consisting of an evolved stellar material.

Alongside with the studies of various detection methods of planets around solar-type stars, similar approaches have been proposed for the corresponding white dwarf surveys. These include means such as direct detection (e.g., Burleigh et al., 2002), Doppler spectroscopy and astrometry (e.g., Chu et al., 2001; Ignace, 2001), and the timing of stellar oscillations (J. Goodman, 1995, private communication; Provencal, 1997). We are planning to employ our extensive experience in using the pulsar timing as a tool to study various processes in physics and astrophysics in a program of a precision photometry and timing of oscillations in a selected group of white dwarfs that exhibit a sufficient stability of this phenomenon. To our knowledge, the only existing program that seriously exploits this intriguing possibility has been recently implemented at the McDonald Observatory (Ann. Rep. Astro. Obs. Dept., 2002).

We intend to begin this project with a study that will lead to a selection of an appropriate group of target stars using their oscillation stability as a criterion (e.g., Winget, 1998). We will also conduct a search for appropriate telescopes that will be large enough to ensure a << 1 s timing precision of the photometric oscillation curves of >17 mag stars, as white dwarfs are very

faint objects. A ~1 s timing accuracy would give us access to Jupiter-mass planets, but our goal is to reach all the way down to terrestrial mass bodies, which will require a millisecond timing precision. Simple calculations show that we should secure access to one or more 4-6 meter-class telescopes, in order to have a chance to achieve this precision goal. We are planning to complete these two initial stages of the project within the first two years and spend the remaining three years on initial observations that should lead to the establishment of a long-term survey that will cover a wide range of orbital periods and masses of the hypothetical white dwarf planets.

6.3. FINDING PLANETS (Sigurdsson)

In collaboration with J. Debes and other PSU students, we are considering alternative detection strategies for planet detection (cf. Gilliland et al., 2000; Debes & Sigurdsson, 2002). Strategies for direct imaging of jovian planets around non-solar like stars using space based infrared imaging, ground based adaptive optics and apodized masks are in progress or proposed. Sigurdsson was part of a previous effort to search for planetary transits around population II stars (HST GO-8267), and further work to consider improved strategies for detection of transits of planets in clusters is planned. New HST data has provided strong evidence for a Jovian planet orbiting a population II star, possibly providing a strong test of Boss's (2002) scenario for Jovian planet formation through top down collapse (Sigurdsson et al., in preparation).The actual observational efforts are beyond the scope of this proposal (funded by a GSRP to J. Debes [Sigurdsson supervising PI] a HST GO proposal [submitted], and other collaborative proposals beyond the scope of the PSARC).

6.4. INDEPENDENTLY FUNDED DEVELOPMENT OF PLANETARY EXPLORATION INSTRUMENTS

6.4.1. Instrumentation for Detection of Biosignatures on Mars (Freeman)

The search for life outside of Earth is at the core of the goals of the field of Astrobiology. Based on research activities employing elemental, molecular and isotopic signatures of organic matter on the early Earth, Penn State researchers have developed expertise in the preservation of life materials on this planet, and these insights are proving useful in the development of strategies for extraterrestrial exploration. In particular, K. Freeman is a member of a research team that is designing a coupled pyrolysis/extraction-gas chromatograph-mass spectrometer system for the study of life signatures on future lander missions to Mars, Titan, Comets and Europa. Continued development of the pyrolysis/extraction and gas chromatography sub-systems were the subject of a recent proposal to the ASTID program. The mass analyzer is a Time-of Flight (TOF) mass spectrometer, along with the sample acquisition delivery sub-systems, are being studied separately in collaboration with Southwest Research Institute and NASA/JPL, respectively. The team is centered at the University of Michigan, and is led by J. H. Waite. The compounds selected for performance characterization come from a parallel laboratory program of organic synthesis and analysis of target organics, led by S. Benner of the University of Florida working with K. Freeman of Penn Sate University and Dr. George Cooper of NASA/AMES.

This parallel laboratory effort provides the intellectual direction for verifying the application of the proposed techniques to future astrobiology missions.

The environment is an important constraint on both the types of organisms that contribute organic matter, and the nature of the reactions that determine their fates. On Earth, more oxidizing environments provide relatively more aggressive means, both chemically and biologically, for the degradation of organic materials. For example, anoxic settings are characterized by higher preservation of organic matter relative to input fluxes. Polymeric compounds such as proteins, nucleic acids and carbohydrates dominate inputs of organic materials from living cells. The oxidation state of carbon in living cells depends on the constituent compound, with carbon in carbohydrates and proteins having average values near zero, while it is above zero for nucleic acids, and about –2 for lipid carbon. Carbon in a living

Table 6.1. Prospective pyrolysis methods for elemental and molecular analyses of organic materials on lander missions.

Pyrolysis Methods		Products			
Technique	Applications	50-300 °C	300-500 °C	> 500 °C	>1000 °C
Inert (N_2): Temperature programmed or Flash-heated to > 500 °C	Widely used, and commonly coupled with direct GC-MS analyses; high-temperature conditions for elemental analyses	Free compounds (held by hydrogen bonds or other weak forces)	Compounds held by lower energy covalent bonds (i.e., C-S, C-O); or single C-C linkages; products can cross-react	Compounds held by multiple covalent bonds	CO, H_2 These are used to quantify total O and H in the organic matrix
Hydropyrolysis (H_2)	Increases yield relative to inert method for lipid biomarkers; product are hydrogenated which protects stereochemistry	Free compounds (held by hydrogen bonds or other weak forces)	Compounds held by lower energy covalent bonds (i.e., C-S, C-O); or single C-C linkages; hydrogenation limits cross-reactions	Compounds held by multiple covalent bonds	
Hydrous Pyrolysis (H_2O)	Increases yield of 'petroleum' like materials; widely used in oil exploration research; water serves as H source	Free compounds (held by hydrogen bonds or other weak forces)	Compounds held by lower energy covalent bonds (i.e., C-S, C-O); or single C-C linkages	Compounds held by multiple covalent bonds	
Oxidative pyrolysis or Combustion (O_2)	High- temp conditions used for elemental and isotopic analyses				CO_2 , NO_x, SO_2 These are used to quantify total C, N and S in organic matrix

cell has an average oxidation state near zero. As fresh biological inputs are degraded in surface environments, the materials delivered to natural sediments becomes increasingly more difficult to analyze with conventional methods due to the removal of dominant compound classes, the formation of oxidized degradation products, and the cross reactions of biochemicals with other compounds and with mineral surfaces. As a consequence, materials that resist degradation, such as lipids and refractory polymers like lignin, dominate organic matter delivered to and preserved in sediments.

6.4.2. Chronology of Planetary Surfaces (Stewart and Capo)

Absolute time scales for planetary processes are difficult to obtain without dated samples from clearly identified features. Such samples are currently available only for the Earth and Moon, while events on other planetary bodies can only be "dated" using the calibrated lunar impact cratering record. Nonetheless, understanding the timing of formation of surface features is critical for our interpretation of planetary history and evolution. Efforts are underway at NASA to develop plans both for sample return and for *in situ* analysis of planetary surfaces. With funding from the NASA PIDDP program and Jet Propulsion laboratory (JPL_ subcontracts, Stewart and Capo are collaborating with G. Cardell (JPL) to design and build an instrument that could ultimately be used to make *in situ* measurements of the crystallization age of rocks on planetary surfaces (Stewart et al., 2001). In addition, Stewart is collaborator on another JPL project (ASTID funding pending) to build a lander-based instrument capable of measuring metal isotope biosignatures. Both instruments are geared toward the astrobiological exploration of the surface of Mars.

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Decay, selective preservation, and cross-linking reactions render molecular characterization difficult, despite the rewarding richness of information potentially inferred from structures, isotopic signatures and stereochemistry. A rich array of information can be drawn from analyses of bulk fractions of organic materials, and thus the team has proposed a tiered approach to analyses of planetary surface materials. In particular, bulk characterization of the quantity and elemental abundances (and therefore elemental oxidation state) of organic material (i.e., ratios of C to H, N, O, S, P and other elements) will provide important base information, from which more detailed analyses targeting molecular constituents can be directed. Molecular studies will focus on compounds released by an array of methods, including a solvent extraction and a variety of pyrolysis methods (see Table 6.1), with analysis of the quantity and structures of the products conducted by a TOF mass spectrometer. Isotopic analyses of C and other life-related elements will also be conducted on the bulk and molecular materials.

6.5. THEORETICAL MODELING OF ATMOSPHERIC BIOSIGNATURES ON PLANETS AROUND OTHER STARS (Kasting)

We conclude with a brief discussion of how our efforts here at Penn State relate to the larger NASA mission of searching for life on planets around other stars. One of us (Kasting) has been involved with NASA's Terrestrial Planet Finder (TPF) mission for several years. Although he is not currently a member of the TPF Science Working Group, Dr. Kasting helped to draft the section on atmospheric biosignatures in the TPF book (http://planetquest.jpl.nasa.gov/TPF/tpf_book/index.html) and was a coauthor on the report of the TPF Biomarkers Subcommittee (Des Marais et al., 2001). Kasting is also an (unpaid) member of the JPL NAI group led by Victoria Meadows, whose goal it is to develop a library of synthetic spectra of possible Earth-like planets for future reference by the TPF mission. Kasting and his students have been holding weekly videoconferences with the JPL group and are in the process of writing up the results of one initial investigation (Kasting et al., in preparation). In this study, the Penn State group created coupled photochemical/radiative-convective models of Earth-like planets with various amounts of atmospheric O_2 circling F2V, K2V, and G2V stars. JPL collaborator Martin Cohen from U. C. Berkeley provided help with accessing the stellar UV fluxes from the IUE (International Ultraviolet Explorer) satellite, and Dr. Meadows and her JPL colleague David Crisp calculated synthetic spectra of the model atmospheres that were generated.

We plan to continue the collaboration with JPL over the next five years. As the JPL group gets their own coupled photochemical/climate model on line, our focus will switch—indeed, already has switched—to the difficult "Solid Planet" and "Life" modules that the JPL group hopes to develop. Currently, one of Kasting's graduate students, Pushker Kharecha, is trying to develop better estimates of biotic and abiotic methane fluxes on the early Earth. Methane has already been identified as a key potential biomarker on early-Earth type planets (Schindler & Kasting, 2000; Des Marais et al., 2001), but questions remain as to exactly how much CH_4 must be present in order to constitute a biotic signal. Once we have done what is possible with methane, our focus will likely shift to biogenic sulfur gases (CH_3S, OCS, and DMS) to see if any of these might be possible biomarker candidates.

We are also collaborating with W. Traub at the Harvard Smithsonian Observatory on a similar project. Kasting is an (unpaid) consultant on a recently funded NASA Origins of Solar Systems proposal to perform similar types of spectroscopic studies on hypothetical Earth-like extrasolar atmospheres. Traub's largely independent radiative transfer model will provide a key check on the results generated by Meadows and Crisp at JPL. By involving as many members of the TPF community as possible, we hope to create a robust theoretical groundwork so that we will be ready to analyze the results once the difficult technological hurdles of designing the mission are overcome.

SUMMARY

The goals of PSARC's research activities, described in the preceding six Sections, were developed in coordination with the following goals of the NASA Astrobiology New Roadmap:

Goal 1: Understand the nature and distribution of habitable environments in the Universe.

Goal 2: Explore for past or present habitable environments, prebiotic chemistry and signs of life elsewhere in our Solar System.

Goal 4:Understand how past life on Earth interacted with its changing planetary and Solar System environment.

Goal 5: Understand the evolutionary mechanisms and environmental limits of life.

Goal 7: Determine how to recognize signatures of life on other worlds and on early Earth.

Our group represents a unique blend of scientists whose *coordinated, multi-dimensional, and multi-disciplinary* efforts (Sections I through VI; Fig. II) are directed toward achieving these goals through (1) improving our understanding of the early co-evolution of life and surface environment on Earth, and (2) providing a comprehensive framework for the future recognition of habitable and inhabited planets. We use a multitude of approaches (experimental, observational, and modeling) and state-of-the-art instrumentation drawn from the represented disciplines to tease out the nature of the intimate linkages between the biota and their environment as they interact on a variety of timescales. Although a strong focus of our proposed research, as in the previous funding interval, is on early Earth, we here exploit modern environmental analogues and controlled laboratory experiments to develop a deeper understanding of these interactions. We also avail ourselves of developing collaborations in chemistry and astronomy and astrophysics to extend our research into new areas, including the search for habitable planets outside of our solar system and the detailed mechanisms for the origin of isotopic signatures of environmental evolution.

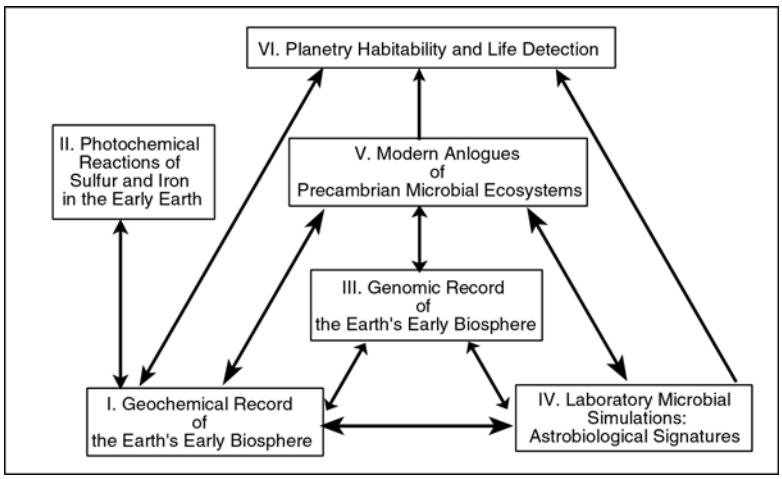

Fig. III Connections of research focuses described in six sections

Executive Committee: Following the administrative structure of the NAI, PSARC has established an Executive Committee, which is composed of the six Section Leaders (Ohmoto, Kasting, Hedges, House, Kump, and Sigurdsson). The Executive Committee will advise the Director on major issues related to the operation of PSARC, including, but not restricted to: (i) the selections of the postdoctoral fellow, graduate fellows, and graduate research assistants who are supported by funds from PSU; (ii) the allocation of undergraduate wages to individual (Co-)PIs; (iii) the allocation of travel funds for Associate Members; and (iv) new initiatives in research, education, and public outreach programs.

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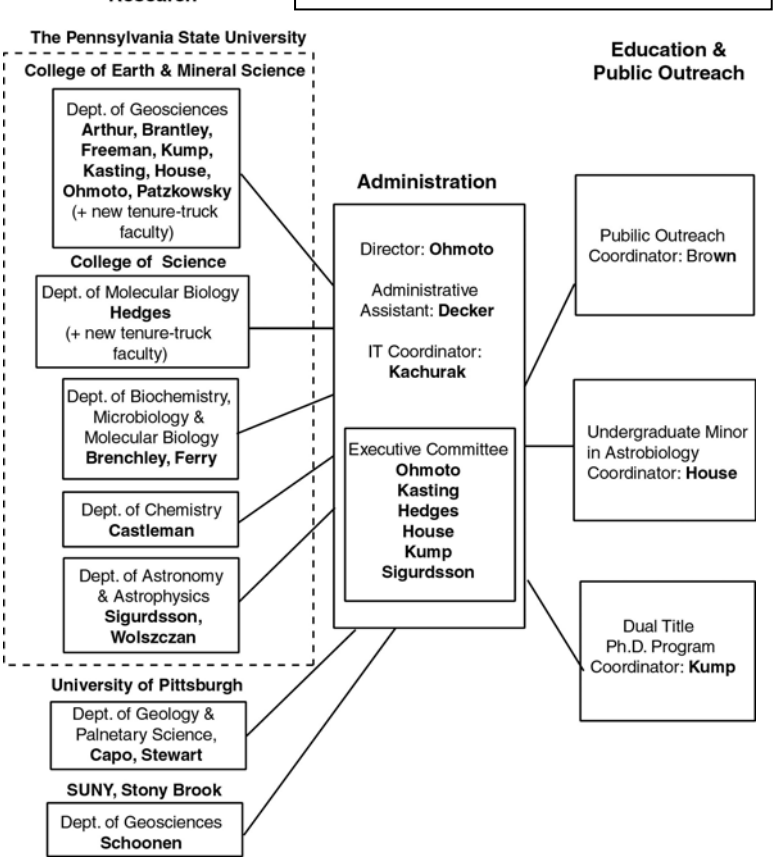
PLAN FOR STRENGTHENING THE ASTROBIOLOGY COMMUNITY

INTRODUCTION AND OVERVIEW

The Penn State Astrobiology Research Center (PSARC) was created in July 1998 by 15 investigators (13 from The Pennsylvania State University (PSU), two from The University of Pittsburgh, and one from SUNY Stony Brook) as one of eleven initial Lead Institutions (Teams) of the NASA Astrobiology Institute (NAI). To support the NAI/PSARC activities to promote astrobiology, ***PSU created a new tenure-track faculty position in the Department of Geosciences to hire Dr. Chris House (astrobiology/geomicrobiology) in 1999.*** The proximity of all members has enabled close interaction and a variety of collaborative research, teaching, and public outreach programs. During the past five years, PSARC has supported all or part of the research/education/PO activities carried out by 142 persons (16 (Co-)PIs, 22 research associates and postdoctoral fellows, five research assistants, two technicians, 49 graduate students, 37 undergraduate students, and five staff in administration/IT/EPO).

With the retirement of two Co-PIs (one in Evolutionary Genomics and the other in Atmospheric Chemistry) and the addition of three new Co-PIs (one in Experimental Chemistry/Physics and two in Astronomy and Astrophysics), PSARC will be operated by 17 (Co)-PIs from two departments of the College of Earth and Mineral Sciences of PSU (Geosciences and Meteorology) and four departments in the Eberly College of Science (Biology, Biochemistry and Molecular Biology, Chemistry, and Astronomy and Astrophysics) at PSU, the Dept. of Earth and Planetary Sciences at the Univ. of Pittsburgh, and the Dept. of Geosciences at SUNY Stony Brook (Fig. III). As part of new commitments to promote Astrobiology, ***PSU will create two additional tenure-track faculty positions***, one in the Department of Geosciences and the other in either the Department of Biology or the Department of Biochemistry and Molecular Biology, pending the funding of this grant proposal (see Section E-5.

Fig. III. Organization of new PSARC.



PSARC remains committed to its efforts to expand and strengthen the astrobiology community and to share the benefits of its research endeavors with students of all grade levels as

well as the general public. PSARC activities in education and public outreach programs are described under the following sections: education and public outreach to the K-14 community and the general public (Section E-1); programs directed toward training young scientists and educators in astrobiology (Section E-2); the utilization of information technology (Section E-3); and efforts to strengthen and promote the professional astrobiology community (Section E-4). The University's strong commitments to support the activities of PSARC are presented in Section (E-5).

SECTION E-1. THE K-14 EDUCATION AND PUBLIC OUTREACH PROGRAMS

(Coordinator: Dr. Lisa Brown)

Being the state's Land Grant Institution, PSU has a long tradition of outreach and extension into communities across the state. As such, we have a wealth of education and outreach resources that we can leverage for maximum impact. Key resources include the Pennsylvania Space Grant Consortium (PSGC), Penn State Public Broadcasting, and the Women In Science and Engineering (WISE) Institute.

The PSARC Lead for Education and Public Outreach (E/PO) will be Dr. Lisa L. Brown, Director of PSGC. PSGC is part of the National Space Grant College and Fellowship Program, a national network of colleges and universities working to expand opportunities for Americans to understand and participate in NASA's aeronautics and space programs by supporting and enhancing science and engineering education, research, and outreach programs. Dr. Brown has fulfilled the E/PO Lead role for the past three and a half years. During this time she has been assisted in all programs by Angela Phelps, Assistant Director for the PSGC. Dr. Brown earned her Ph.D. in Geosciences from PSU, working with Dr. Jim Kasting (Co-PI) to study the ancient atmospheres of Earth and Mars. Angela Phelps earned her BS degree in Elementary Education from Southern Illinois University. Over the past three and a half years Dr. Brown and Angela have had extensive interactions and collaborations with the other E/PO teams of the NAI and will continue to do so.

E-1.1. Recruitment and Retention of Women in Science

The future strength of the astrobiology community requires a diverse and talented pool of scientists and engineers. PSARC will make a concerted effort to recruit more women into the field of astrobiology by leveraging an existing program that is sponsored by the PSGC. ***The Women In Science and Engineering Research (WISER) program*** provides research internships to first-year undergraduate women students at PSU. Students will work alongside PSARC faculty, research associates, postdoctoral fellows, and graduate students, receiving a hands-on introduction to academic research. The WISER program is designed to increase the retention rate of women students in science and engineering by intervening during the critical first year of their undergraduate careers, a time when many women students leave the sciences, to provide one-on-one mentoring in a stimulating and supportive research environment. PSARC faculty hosted fifteen WISER students during its first five years; we will work to expand this participation in the next five years.

Research has shown that in order to effectively increase the number of women students in the science and engineering pipeline we need to reach down into the K-12 grades with programs designed to stimulate and nurture girls' interest in science. PSARC will work to engage young women students in astrobiology topics through two existing programs that aim to introduce young women to science and engineering as college majors and careers. The first, ***WISE Camp***, is a one-week residential career awareness program for women in their junior year in high school. The second program, ***Nittany Science Camp for Girls*** is a one-week day camp for students in grades six through ten. During both camps, students participate in a variety of educational and technical activities, including hands-on laboratories in several different science and engineering fields; interaction with role-models from academia and industry; and discussions about educational opportunities at PSU. PSARC faculty and graduate students will introduce students to the field of astrobiology through hands-on investigations and demonstrations. The camps are sponsored and managed by the WISE Institute and by the College of Earth & Mineral Sciences Office of Diversity Enhancement, respectively.

E-1.2. Professional Development for Science Teachers

The PSARC E/PO team has partnered with the teams at NASA Ames, NASA Johnson, and The Marine Biological Laboratory to deliver high quality professional development for science teachers using a variety of existing astrobiology educational products. As an integrated team we are known for consistently modeling science as inquiry and connecting educators to standards-based resources and to the world of professional science. As lead team members that have interactions with NAI researchers we are able to engage astrobiologists directly in our teacher enrichment programs nationwide as speakers, panelists, and discussion facilitators. We each offer ***unique week long programs for educators*** at our sites or in the field, but we have also presented jointly at NSTA, NABT, TOPS, the NAI Executive Council, and the NAI General Meetings. Our E/PO teams include Ph.D. scientists currently involved in the research, university instructors, former school principals, textbook and curriculum publishers, and classroom educators with experience from the elementary to high school levels. This blend of skills gives our integrated team a broad perspective when developing and providing workshops and presentations for educators. All of our professional development programs provide a distribution mechanism for astrobiology-related educational materials, such as the NAI Educator Resource Guide, AstroVenture, Voyages Through Time, the micro-scope online database, as well as other classroom lessons developed by educators and scientists at NASA JSC, NASA Ames, and Yellowstone National Park. We propose ***to expand this multi-team partnership*** to include development of workshops at informal science centers and museums, particularly those that are ***developing new astrobiology exhibits*** (e.g. The New York Hall of Science) or centers that will host traveling astrobiology exhibits.

For the last four years, PSARC has offered a one-week summer workshop for in-service teachers (~24 teachers each year) entitled "Astrobiology: The Origins and Early Evolution of Life". This five-day course is designed to expose middle and high school science teachers to current thinking about the origin and early evolution of life in a way that they can pass on to their students. The course combines lectures by several PSARC faculties, hands-on classroom activities, lab tours, and a field trip to a local bog. PSARC Co-PI, Jim Kasting, is the Lead Instructor and is present throughout the week to facilitate discussion and provide content

expertise. In addition to activities created by PSARC members, we have incorporated classroom activities from the NAI Educator Resource Guide "Life On Earth…and elsewhere?" and from TERC's draft High School curriculum. In 2002, a PSARC graduate student in microbiology worked along with a student in Science Education to identify and lead additional activities such as extracting DNA segments from strawberries and collecting and observing magnetotactic bacteria. Evaluations were conducted using daily participant surveys. Following each workshop, teachers are added to our existing listserv discussion group to facilitate and track the application of knowledge from the workshop back to the classrooms. The listserv is also a means for us to continuously disseminate astrobiology-related information and opportunities to the workshop participants. During summer 2003, we will work with a graduate student in Science Education to develop and implement additional evaluation tools. PSARC and PSGC provide funding for the workshop. Housing, meals and some travel support are provided. Teachers are charged tuition for two graduate credits from Penn State; the workshop is approved by the Pennsylvania Department of Education for Act 48 Professional Development credit.

Every year, PSGC exhibits at the Pennsylvania Science Teachers Association convention. Part of the exhibit includes extensive information and resources about NAI and PSARC. Curriculum resources and brochures are distributed. PSGC recruits heavily for all of their Penn State Educator Workshops, including the Astrobiology Educator Workshop. In 2002, Judith Elliott, a middle school educator who taught an Astrobiology unit in her class after attending the Penn State Astrobiology Workshop during June 2001, shared some of her students' projects as part of our exhibit.

E-1.3. Outreach to K-12 Classrooms

In collaboration with Penn State Public Broadcasting, we have produced two 15-minute television segments on Astrobiology for the Emmy Award winning multimedia current events program, What's in the News (WITN). The weekly WITN programs reach approximately 5.7 million children nationwide, primarily in grades 4-7, and are distributed nationally via satellite and videotape by International Telecommunications Services, Inc. (I.T.S.). In addition to the video segment, the program employs interactive components to stimulate classroom discussion. The first segment, "Alien Hunting", featured PSARC faculty members (Hiroshi Ohmoto and Jim Kasting) and gave an introduction to the field of astrobiology and the search for life beyond Earth. The second broadcast, "The Stories Rocks Tell: Learning About Early Earth," focused on the oxygenation of Earth and how geologists study oxygen clues in rocks to learn how the planet developed an atmosphere that supports life. We will produce two additional segments in the next five years.

Dr. Martin Schoonen, Co-PI at SUNY Stony Brook, has developed a PowerPoint slide show that he has used in presentations to pre-K, K, and first grade students. The topic is planetary comparison. He will be engaged in similar presentations in the future, and continue to improve the PowerPoint slide set, which will be made available to the astrobiological community.

E-1.4. Increasing Public Awareness

PSARC has offered or sponsored numerous lectures and presentations on Astrobiology topics for the general public at PSU, the Carnegie Museum of Pittsburgh, and SUNY Stony Brook. The Earth Explorers Lecture Series at the Carnegie Museum of Natural History offered free public lectures with an earth science theme. The series contained a significant astrobiology component, including presentations by NAI members James Kasting (PSARC) and J. William Schopf (UCLA). The lectures were sponsored by the Museum and the Department of Geology & Planetary Science, University of Pittsburgh, through NSF funding to Co-PI Rosemary C. Capo; plans are underway to continue these activities. PSARC Co-PI Martin Schoonen has been a regular featured speaker in the "Geology Open Night" lecture series at SUNY Stony Brook. These lectures, open to the general public, are held three to four times per semester and draw 100 to 200 people per session.

PSARC will continue to participate in Space Day at PSU, an annual one-day event, sponsored by the PSGC, which is designed to showcase the exciting space-related research being carried out at PSU. PSARC faculty, postdoctoral fellows, and graduate students exhibit posters describing their Astrobiology research and work with students in Science Education to develop engaging hands-on activities that are designed to help translate their research to a general audience, as well as create lesson plans that are aligned with national science education standards and the Pennsylvania Academic Standards for Science and Technology and. We distribute educator kits that include the NAI Educator Resource Guide “Astrobiology in Your Classroom: Life on Earth…and Elsewhere?” as well as the following brochures: NASA Online Educational Resources; How to Access Information on NASA’s Education Program, Materials, and Services; NASA – Central Operation of Resources for Education; Space Science Education Resource Directory; PSGC; Penn State Workshops for Science Teachers (one of which is Astrobiology).

E-1.5. Informal Education

PSARC will collaborate with Penn State’s College of Earth & Mineral Sciences and the Whitaker Center for Science and the Arts (Harrisburg, PA) to develop a small, interactive traveling museum exhibit on astrobiology. The traveling exhibit will be designed for small museums and science centers, venues that cannot accommodate the large astrobiology exhibits that are currently under development (for example, by the New York Hall of Science). We have an opportunity to take advantage of investments being made by PSU’s College of Earth & Mineral Sciences in their newly renovated museum space. ***The College has committed to provide support for a full-time museum Director, to be hired later this year.*** The Director will work with PSARC faculty, who will provide content expertise, and with the staff of the Whitaker Center to develop an exhibit that highlights the astrobiology research focus of the PSU team. Staff from the Whitaker Center will provide expertise to design and fabricate an engaging and educational display. The exhibit will initially be housed in the museum within the College of Earth & Mineral Sciences, but will travel to the Whitaker Center in Harrisburg, PA and to other small museums across Pennsylvania to bring the science of astrobiology to underserved communities outside the major population centers of Pittsburgh and Philadelphia. Likely venues for display include The Discovery Center of Science & Technology, Bethlehem, PA; The North Museum of Natural History and Science, Franklin & Marshall College, Lancaster, PA; The

Reading Public Museum, Reading, PA; The Venango Museum of Art, Science, and Industry, Oil City, PA; and The Erie Historical Museum and Planetarium, Erie, PA.

The Whitaker Center for Science and the Arts and its exhibits and education branch, HARSCO Science Center, have extensive experience in exhibit design and development. They currently maintain over 240 interactive exhibits related to nine discreet scientific fields. They have created in-house exhibits on a range of scientific topics including space exploration and exobiology. Based on their experience and their in-house fabrication capabilities we have budgeted sufficient funds to create an exhibit approximately 1300 square feet in size. However, we plan to partner with the Space Science Institute (Boulder, CO) to pursue additional funding from the National Science Foundation to allow for the development of complimentary educational materials and professional development opportunities for museum staff. We will seek additional partnerships and supplemental funding within the NAI.

One possible element of the traveling exhibit is a replica of the large-scale bioreactors, ***"Simulated Precambrian Biosphere"***, that are described in Research Section IV. The bioreactors could be used to illustrate the structures of microbial ecosystems on the early Earth and of modern ecosystems (e.g., Fayetteville Green Lake, N.Y.: see Research Section V) that PSARC researchers are studying. The bioreactors could also be utilized during the one week teacher workshops, representing sets of 5-foot vertical "Winogradsky-type columns" which many of our workshop participants already utilize in their classrooms. The experimental bioreactors will allow for sampling of various geochemical parameters through the columns, allowing students to understand the connections between microbial consortia and their environments.

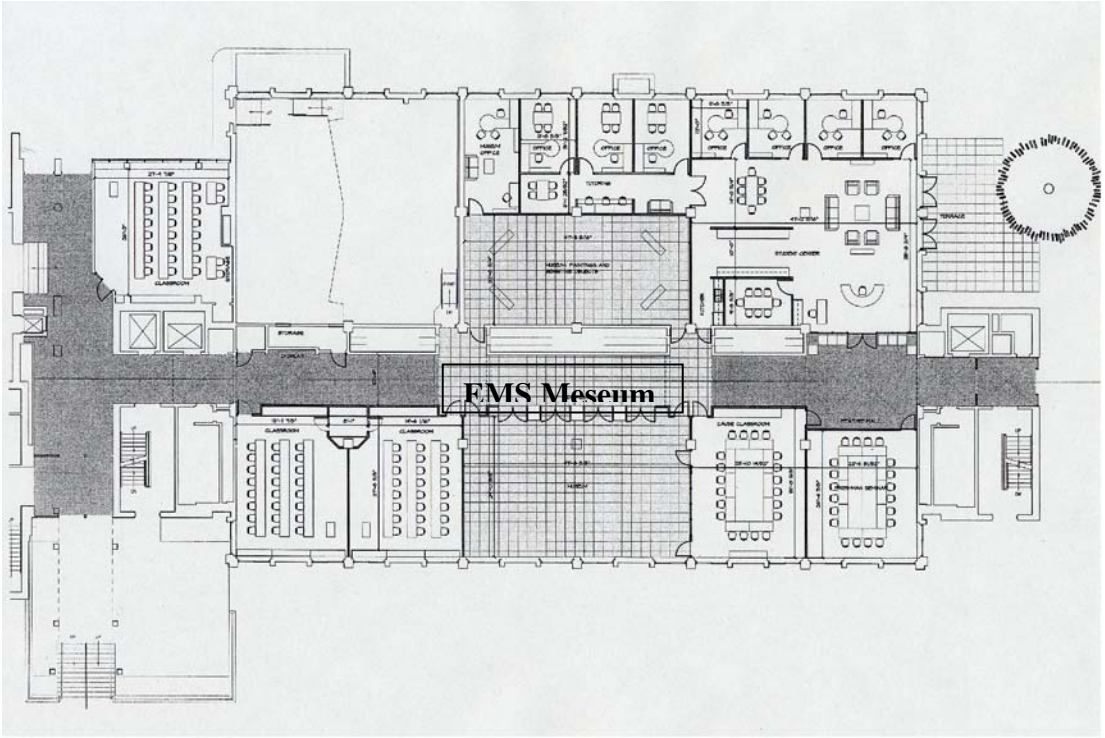


Figure 1: This figure shows a draft blueprint of the groundfloor of Deike Building which will include the new Earth & Mineral Sciences museum at PSU. Our proposed traveling astrobiology exhibit will be displayed in the room at the bottom center of the figure. This exhibit space measures approximately 29 feet by 48 feet.

SECTION E-2. TRAINING YOUNG SCIENTISTS AND EDUCATORS IN ASTROBIOLOGY

The training of new scientists and educators through undergraduate and graduate education and research in astrobiology and related fields has been (and will continue to be) an important objective of PSARC. During the past five years, we have fully or partially supported 49 graduate students and 39 undergraduate students using the funds provided by NASA (graduate assistantships and wages) and PSU (about three graduate fellowships/assistantships and support for undergraduate students). We have graduated ten Ph.D. students and nine MS students who have conducted astrobiology-related research as main parts of their theses. The ten completed Ph. D. students are: R. Hotinski, ’00; A. Pavlov, ’01; B. Borup, ’01; S. Ono, ’01; M. Van Tuinen, ’02; K. Yamaguchi, ’02; Y. Watanabe, ’02; H. Pointkiviska, ’03; M. Borda, ’03; and M. Hurtgen, ’03). The current graduate students who are conducting astrobiology-related research totals 22. PSU has established **an intercollege undergraduate Minor in Astrobiology** (E-2.1); the proposal to create the **Dual-Title Ph. D. Degree Program in Astrobiology** (E-2.2) is currently under review by the Faculty Senate. Other educational programs include the Astrobiology Summer Program at Penn State (E-2.3) and Satelite Courses (E-2.4), and they are also closely connected to The Penn State Biogeochemistry Reserch Initiative in Education (BRIE), which has been largely funded by IGERT (NSF) (Section E-2.5). Collaboration in research and education with Minority Institutions are described in Section (E-2.6).

E-2.1. Undergraduate Minor in Astrobiology (Coordinator: Prof. Chris House)

An inter-college undergraduate program in Astrobiology, headed by Dr. Chris House (PSARC Co-PI), was established in the Fall 2000 semester. The Astrobiology Minor is designed to educate students in this interdisciplinary field covering the varied scientific disciplines that contribute to our general understanding of life, the origin of life, the past history of life on Earth, possible futures for life on Earth, and the possible existence of life on other planetary environments. The principle goal of the minor is to develop student's literacy in astrobiology so that they can critically evaluate claims related to this field that they encounter well after their college education has ended. So far six students from the departments of Astronomy, Microbiology, Biology and Mathematics have completed the requirements and received this BS minor and four students are expected to graduate in May 2003. Student recruitment efforts are in progress to expand this program.

Requirements

To qualify for the Minor students are required to take 18 course credits from the list below, with at least six credits at the 400-level. (A grade of C or better is required for all courses in the minor.)

One of the following REQUIRED Introductory courses (3 credits, semesters 1-6):

<u>EARTH 002</u>: The Earth System (3)

<u>GEOSC 021</u>: Biodiversity and Earth (3)

One course from each group of the following REQUIRED courses (9-10 credits, semesters 5-8):

- ASTRO 140: Life in the Universe (3)
- ASTRO 291: Astronomical Methods and the Solar System (3)
- GEOSC 204: Geobiology (4)
- BIOL 427: Evolution (3)
- BIOL/GEOSC 474: Astrobiology (cross-listed course)

Additional courses (5-6 credits, semesters 5-8):

- ASTRO 475W: Stars and Galaxies (3)
- BIOL 405: Molecular Evolution (3)
- BMB 401: General Biochemistry (2)
- BMB 402: General Biochemistry (3)
- GEOSC 416: Stable and Radioactive Isotopes in Geoscience (3)
- GEOSC 419: Organic Geochemistry (3)
- GEOSC 481: Solid Earth and Planetary Geophysics (3)
- METEO 466: Planetary Atmospheres (3)
- MICRB 201: Introductory Microbiology (3)

E-2.2. Dual-Title Ph.D. Degree Program in Astrobiology (Coordinator: Prof. Lee Kump)

A proposal to create a dual-titled Ph.D. degree program in Astrobiology at PSU is now under review by the Faculty Senate, and we expect the approval shortly. Currently, about 10 graduate students in Geosciences, Biology, Chemistry, and Astronomy & Astrophysics departments are expected to enroll beginning with the Fall 2003 semester in the dual-title program. The Graduate Bulletin at Penn State will have the following section describing this program:

Degree Conferred: Students electing this degree program through participating programs earn a degree with a dual title in the Ph.D., e.g., Ph.D. in (graduate program name) and Astrobiology.

The Astrobiology dual-title degree program is administered by Dr. Lee Kump (PSARC Co-PI) under PSARC. PSARC maintains program definition, identifies courses appropriate to the program, and recommends policy and procedures for the program's operation to the dean of the Graduate School and to the deans of the participating colleges. The dual-title degree program is offered through participating programs in the College of Earth and Mineral Sciences and the Eberly College of Science and, where appropriate, other graduate programs in the University. The program enables students from several graduate programs to gain the perspectives, techniques, and methodologies of Astrobiology, while maintaining a close association with primary program areas of application.

Astrobiology is a field devoted to the exploration of life outside of Earth and to the investigation of the origin and early evolution of life on Earth. For admission to pursue a dual-title degree under this program, a student must apply to (1) the Graduate School; (2) one of the participating primary graduate programs; and (3) the Astrobiology program committee.

Admission Requirements

In addition to the admission requirements set forth by the Graduate School and the primary program, program candidates will be required to take the Graduate Record Examination and, where appropriate, provide a satisfactory TOEFL score. Candidates must also submit a written personal statement indicating the career goals they hope to serve by attaining an Astrobiology dual-title. A strong undergraduate preparation in the basic sciences is expected, with evidence of an interest in multiple disciplines. Participation in research at the undergraduate level is encouraged.

Degree Requirements

To qualify for a dual-title degree, students must satisfy the requirements of the primary graduate program in which they are enrolled, in addition to the minimum requirements of the Astrobiology program. The minimum course requirements for the dual-title in Astrobiology are ABIOL 574 Planetary Habitability (3 credits), ABIOL 590 Astrobiology Seminar (2 credits), ABIOL 570 Astrobiology Field Experience (2 credits), and at least two credits of 400- or 500-level coursework outside of the student's primary program in an area relevant to Astrobiology (through consultation with the advisor). The primary program administers the candidacy examination, but at least one member from the committee must be on the Astrobiology program faculty[1]. Similarly, the doctoral committee should include faculty from the Astrobiology program. In both cases the Astrobiology representative may be the advisor and have an appointment in the primary program of study. The field of Astrobiology should be integrated into the comprehensive examination. A Ph.D. dissertation that contributes fundamentally to the field of Astrobiology is required, as is a public oral presentation of the dissertation.

Financial Aid

Financial aid is generally available through the primary program and through highly competitive University Graduate Fellowships. However, PSARC provides support for students through research assistantships and graduate fellowships. Typically students in Astrobiology are supported 12-months per year on some form of assistantship, fellowship, or summer wages.

Other Relevant Information

Students intrigued by the possibility of pursuing research in Astrobiology should visit the PSARC website (http:// psarc.geosc.psu.edu/) and the NASA Astrobiology Institute website (http://nai.arc.nasa.gov).

Synopsis of the Required Courses

ASTROBIOLOGY (ABIOL)

570. ASTROBIOLOGY FIELD EXPERIENCE (2) Field excursions to sites where the early evolution of life and the environment is revealed in the fossil, geochemical, and sedimentary record or in modern analogue. Possible sites include the Huronian Supergroup of Ontario, Canada, Precambrian rocks of Virginia, West Virginia, or Maryland, Precambrian paleosols of Michigan, modern microbial ecosystems in the Bahamas or at Green Lake, NY State.

[1] This requirement is waived for departments that do not have individual candidacy committees or where departmental policies preclude this requirement.

574. PLANETARY HABITABILITY (3) This course provides the scientific foundations upon which the search for life on other planets is based, including aspects of Earth history, evolutionary biology, atmospheric chemistry, life in extreme environments on Earth, and planet and life detection.

590 ASTROBIOLOGY SEMINAR (2) Student-led presentations and discussions of current and classic literature relevant to the themes of Astrobiology.

E-2.3. Astrobiology Summer Program (ASP) at Penn State (Prof. Blair Hedges – Coordinator)

An undergraduate summer training program in astrobiology was established at Penn State, with the help of a 3-year NSF REU-Site award (S. B. Hedges, PI). Each summer, it supports 10 students (sophomores, juniors) from colleges and universities other than Penn State to participate in research training under the guidance of Penn State NAI faculty mentors. All expenses associated with the travel, room, and board for each student are covered by the program ($2300 per student), and students are additionally given a stipend of $3500 for the summer. Women and minorities are encouraged to apply. The program has a director (Hedges) and a coordinator (Dr. Carla Hass). The ASP web site provides a full description of the program (http://evo.bio.psu.edu/asp/).

Besides laboratory research, ASP students also have the opportunity to participate in a research excursion to a modern analog of a Precambrian ocean (Green Lake, New York), attend weekly seminars and laboratory tours, stargaze, and participate in a discussion group and a research symposium.

Thirty applications have been received and are under review for the first summer (2003). The applicants represent a diversity of majors (biology, geology, chemistry, physics, astronomy, geology) and are uniformly high in quality. We anticipate renewing this program support from NSF in 2005. Also, we propose here to strengthen this program by using PSARC funds to support two additional students (women or minority) each summer.

E-2.4. Satellite Courses (Coordinators: Prof. Brian Stewart and Rosemary Capo, Univ. of Pittsburgh, PSARC Co-PIs)

Through NAI-provided videoconference facilities, PSARC member institutions the University of Pittsburgh and SUNY Stony Brook have been able to offer successful, full-credit graduate courses in Astrobiology, with real-time guest lectures and discussions from all of the PSARC investigators. Co-PIs B. Stewart and R. Capo developed a graduate course in Astrobiology at the University of Pittsburgh in Spring of 2002. The 3-credit course (GEOL 3953, Topics in Geochemistry: Astrobiology) was taken by eight graduate students, and it featured lectures by Stewart and Capo followed by weekly videoconferences with PSARC researchers at Penn State and Stony Brook. Using the equipment, students from Univ. of Pittsburgh, Penn State and SUNY Stony Brook were able to ask questions and freely interact with the presenters. In Fall of 2002, students from the University of Pittsburgh and SUNY Stony Brook participated by videoconference in an Astrobiology Seminar run by Penn State. We expect to continue offering these courses over the next five years.

E-2.5. The Pennsylvania State University Biogeochemistry Research Initiative in Education (BRIE) (Director: Prof. Susan Brantley, PSARC Co-PI)

An award from IGERT (NSF) was provided in 1999 in order to support cross-disciplinary graduate training in biogeochemistry (S. Brantley, PI; J. Brenchley, K. Freeman, co-PIs). This program funds Ph.D. students who receive training in at least two of the following areas: geochemistry, microbiology, soil science, environmental engineering, meteorology and chemistry. Students are co-advised by faculty in different disciplines, and are provided with research, travel and teaching opportunities that enrich their training experience. Students are engaged in a rich array of research topics, many of which are directly aligned and complementary to the interests of Astrobiology. Many of the BRIE graduate students are working on aspects of the interactions of microbial life with its geochemical environment. This work includes the strategies employed by microbes to contend with salt, oxygen or other stresses in their environment, and the means by which microbes extract of necessary nutrients from minerals. In practical terms there are close links between BRIE and PSARC, as many of the BRIE faculty are also investigators in PSARC.

E-2.6. Teaming with Minority Institutions (Collaborators: Profs. David Shwartzman, Howard University, PSARC Associate; Prof. Warren Gooden, Cheyney University, PSARC Associate)

PSARC faculty have research connections with universities that are predominantly African American (Howard University, Washington, D.C.) and predominantly Hispanic (University of Puerto Rico). Dr. David Schwartzman (Howard U., Biology Department) is an associate member of PSARC and studies the early history of the biosphere (e.g., Life, Temperature, and the Earth; Columbia Univ. Press, 1999). Besides his research collaborations with Penn State, he will bring undergraduate and graduate students from Howard University to annual PSARC meetings for poster presentations and interactions with our faculty and students. Several colleagues of PSARC Co-I Hedges are on the biology faculty at the University of Puerto Rico (J. Ackerman, J. Mutt, R. Thomas) and provide a link for directing interested undergraduates to our Astrobiology Summer Program or PhD program. One such student, Ms. Maraliz Vazquez, participated in our first (trial) 10-week summer program in 2002 and this experience persuaded her to apply to PhD programs in astrobiology this year.

PSARC faculty and staff have also established connections with Cheyney University in Philadelphia, PA; the oldest of the Historically Black Colleges and Universities in America. Cheyney University offers baccalaureate degrees in more than thirty disciplines and the masters degree in Education. Dr. Warren Gooden, Professor of Chemistry, is an associate member of PSARC and has developed an introductory astrobiology course for undergraduate students at Cheyney. The course is offered to science majors and to education students working toward their science teacher certification. Dr. Gooden will collaborate with PSARC faculty to expand this introductory course and to develop a workshop for in-service science teachers modeled after the existing one-week PSARC summer workshop. Virtual connections between Cheyney University and the PSARC campuses can be easily established since Cheyney is a member of the Pennsylvania State System of Higher Education schools, all of which have extensive distance learning technologies. This would give faculty and students at Cheyney an entrance way into the virtual NAI research community.

SECTION E-3. UTILIZATION OF INFORMATION TECHNOLOGY

(IT Coordinator: Frank Kachurak)

Effectively utilizing collaborative technology is one of the key priorities of the Penn State Astrobiology Research Center Information (PSARC) Technology Team. Integrating the technology into educational, research, and administrative functions is the constant goal of the team.

One of the major projects that successfully integrated collaborative technology into the educational aspect of the Research Center is the Astrobiology Seminar Series. A test-pilot supported by the NASA Astrobiology Institute and PSARC, this program deployed a video conference system to Penn State, the University of Pittsburgh, and The State University of New York at Stony Brook. Each Friday during the semester, a professor from one of the universities would give a simultaneously-broadcast one-hour lecture that was followed by a question and answer session. Other than the initial cost of the systems, there was little residual cost as the conference was held via IP. After a full semester of classes, this program is considered to be a success, and both students and professors are looking forward to the seminar class next fall.

Along with larger projects comes the day-to-day support of the members of PSARC. System administration, web design assistance, and IT equipment management are some of the services provided by the IT Team members on a daily basis. A close relationship has also been developed with the NAI Digital Video Expert Center (DVEC) team, and PSARC IT Team members often work with DVEC team members when setting up conferences and scheduling collaboration technology resources.

Future IT plans for the PSARC will be based on a number of factors, including the goal of the NAI to promote collaboration through the use of electronic tools.

Current collaboration takes place mainly via e-mail, video conferences, and telephone calls. Future plans include the implementation of a document sharing system, set up similar to an electronic message board, which will allow asynchronous communication among PSARC members.

Further development of the Astrobiology Seminar Series will include the ability to attend the class from any IP-connected computer. This will enable researchers in the field as well as students who don't have access to the PolyCom systems to attend and participate in the class activities.

Migration from the current MacOS 9 and Windows 2000 environment to MacOS X and Windows XP is already beginning, but will require additional user training as the changeover takes place. One-on-one user training sessions will provide users the necessary training for this migration.

Finally, the current set-up of the PSARC Communications Center is in the form of a conference room complete with a large table in the center. To enhance the usability of the room for larger video conferences, and to provide a comfortable area for attendees of the NAI Directors Seminar Series, NAI Executive Council meetings, and other popular seminars, the feasibility of redesigning the Communications Center will be investigated. Plans will include the transformation from the standard conference type setup into a miniature auditorium setting, complete with large screen projection of both the video conference and the desktop sharing screens.

See also Section E-2.4 for Satellite Courses.

SECTION E-4. PROMOTING THE PROFESSIONAL ASTROBIOLOGY COMMUNITY

PSARC investigators have played major roles in promoting the new science of Astrobiology through: publications of new ideas and important data in major scientific journals and at major national and international conferences (E-4.1); organizing special sessions related to Astrobiology at major international conferences (E-4.2); serving on editorial boards of major journals related to Astrobiology (E-4.3); serving on national and international committees related to Astrobiology (E-4.4); participation in NAI Focus Groups (E-4.5); and the collaboration with scientists both within and outside the NAI (E-4.6). Our efforts in all these areas will continue to grow in the future.

E-4.1. Research Publications

The scientific impacts of research carried out by the 16 (Co-)PIs of PSARC and their research teams during the last five year period may be best measure by the numbers of papers published in major, refereed journals and books, which are summarized in the following table.

Summary of NAI Publications by PSARC Investigators
(1998-2003)

Journal	Published	In Press	Submitted (in review)	Total
Science	9		1	10
Nature	5		2	7
Proc. Natl. Acad. Sci. USA	9			9
J. Bacteriol.	8			8
Biochemistry	6			6
Mol. Biol. Evol.	5	1	1	7
J. Biol. Chem.	5			5
Other biological journals	26	6	3	35
Astrobiology	4			4
Geology	12		1	13
Geochim. Cosmochim. Acta	8	1	3	12
Chem. Geol.	6			6
Earth Planet. Sci. Lett	2	1	1	4
Other geological journals	23	4	4	31
Ann. Rev. Astron. Astrophys			1	1
Book Chapters	8	3		11
Total	**136**	**16**	**17**	**169**

The full titles of these papers are presented in “List of Publications” in Volume II. Abstracts of papers presented at professional meetings are excluded from the list because there are more than 1,000 entries.

E-4.2. Organizing Special Sessions on Astrobiology

Several PSARC investigators have organized special sessions and symposia at major international scientific conferences to promote Astrobiology, including:

Ohmoto and Schoonen co-organized a special session on "Astrobiology" and House organized a special session on "Genomic meets Geochemistry" at the 11th Goldschmidt Conference of the Geochemical Society at Hot Springs, VA, in May, 2001.

Ohmoto organized a Pardee Symposium and a Pardee Technical Session, "Evolution of the Early Atmosphere, Hydrosphere, and Biosphere 1: Constraints from Ore Deposits", at the Annual Meetings of the Geological Society of America in Denver, CO, October, 2002. This symposium and technical session were sponsored by the NAI, NASA Exobiology Program, The Geological Society of America, Geochemical Society, and the Society of Economic Geologists. Ohmoto is also a co-convener of a technical session, "Co-evolution of the atmosphere, oceans, and biosphere on early Earth", at the 13th Goldschmidt Conference of the Geochemical Society in Kurashiki, Japan, September, 2003.

Brantley is President and Schoonen is Scientific Program Chair of the Water-Rock Interaction Conference at Saratoga Spring, NY, in 2004. It will have a Astrobiology-related session.

Program Committee of NAI Annual meetings was served by Ohmoto (2001) and Arthur (2003).

E-4.3. Serving on Editorial Boards of Major Journals related to Astrobiology

Ohmoto: Associate Editors of *Geochimica et Cosmochimica Acta* (through '02) and *Chemical Geology* (through '02); Editorial boards of *Resources Geology*, and *Astrobiology*.

Arthur: Editor, *Paleoceanography* (through '02).

Brantley: Editor, *Chemical Geology* (through '01).

Castleman: Editor in Chief, new book series devoted to Cluster Sicences/Springer Verlag; Advisory Editorial boards of *Chemical Physics Letters*, *Advances in Chemical Physics*, *ResearchTrends*, and *Journal of Cluster Science*.

Freeman: Editorial Board of *Annual review of Earth and Planetary Sciences*.

Kasting: Editorial boards of *Origins of Life and Evolution of the Biosphere*, *Astrobiology*, and *Geobiology*.

Kump: Editor, *Geology* (through '00); Editorial boards of *Geochimica et Cosmochimica Acta* and *Chemical Geology*.

House: Editorial boards of *Astrobiology*, and *Geobiology*.

Schoonen: Editor, *Geochemical Transactions*; Editorial bords of *Chemical Geology*.

E-4.4. Serving on National Committees related to Astrobiology

Freeman: Member of the NRC Committee on the Origin and Evolution of Life (COEL).

Hedges: Member of the Astrobiology Roadmap Committee.

Kasting: Chair, the Exobiology review Panel; Member, NRC Committee on the Origin and Evolution of Life (COEL).

E-4.5. Participations in NAI Focus Groups

Missions to the Early Earth: Ohmoto, Arthur, Capo, House, Kump, and Stewart.
Mars: House, Ohmoto.
Evolutionary Genomics: Hedges (Co-chair).
Europa: Brenchley, House.
Ecogenomics: House, Kump.

E-4.6. Research Collaborations

The PSARC created five years ago the Associate Membership Program to enhance collaboration in research, education, and public outreach in Astrobiology with the (Co-)PIs. The monetary rewards for the Associate Members are very small, if any. Occasionally, PSARC has provided support for travel expenses to visit PSU to work with PSARC PIs, to attend workshops and seminars at PSU, to attend NAI meetings, or to conduct fieldwork with PSARC PIs. However, all associate members are registered at the NAI Central and receive almost the same privileges as PSARC graduate students, such as receiving mail/announcements/updates directly from the NAI Central and invitations to attend NAI meetings. Currently, PSARC has the following 29 Associate Members in Research and 4 Associate Members in Education and Public Outreach:

Associate Members of the
Penn State Astrobiology Research Center

For research,

Mark Barley (U. of Western Australia): Precambrian geology
Hubert L. Barnes (PSU): hydrothermal geochemistry
Nicolas Beukes (Rand Afrikaans Univ., South Africa): Precambrian geology
Paul Braterman (Northern Texas Univ.): atmospheric chemistry
Oliver A. Chadwick (UC Santa Barbara): pedology
Catherine Drennan (MIT): biochemistry
Charles Fisher (PSU): marine biology
Sorel T. Fitz-Gibbon (UCLA): geonomics
Tracy Frank (U. of Nebraska): Proterozoic geochemistry
Robert C. Graham (UC Riverside): soil geochemistry
Steven Holland (U. of Geogia): sequence stratigraphy
Clark Johnson (U. of Wisconsin, Madison): Fe isotope geochemistry
Takeshi Kakegawa (Tohoku Univ., Japan): sulfur isotope geochemistry
Sudir Kumar (Arizona State Univ.): molecular genetics
Donald R. Lowe (Stanford Univ.): Precambrian geology
Tim Lyons (U. of Missouri): Proterozoic geology
Gwendolyn L. Macpherson (U. of Kansas): geochemistry
Arnold Miller (U. of Cincinnati): paleontology
Robert Minard (PSU): atmospheric chemistry
Hiroshi Naraoka (Tokyo Metropolitan Univ., Japan): organic geochemistry
Munetomo Nedachi (Kagoshima Univ., Japan): paleosols, ore deposits
Thomas Olszewski (Museum of Nature History, Smithsonian Institute): paleontology

Victoria J. Orphan (NASA Ames Research Center): environmental microbiology
Anthony Prave (St. Andrews Univ., Scotland): Neoproterozoic geology
Douglas Rees (Cal Tech): biochemistry
Greg Retallack (U. of Oregon): soil geochemistry
David Schwartzman (Howard University): biology
Kerry Smith (Clemson Univ.): biochemistry
Jennifer Macoledy (Carlton College): geomicrobiology

For public education,
Lisa Brown, Director, Pennsylvania Space Grant Consortium
Kathleen M. O'Toole. Writer/Host, "What's in the News", WPSX
Judi Wakhungu, Director, WISE Institute

In addition to the above Associate Members, the PSARC (Co-)PIs have actively collaborating with many researchers of other NAI Teams and also outside of NAI.

RESEARCH FACILITIES & EQUIPMENT

All of the research outlined in the previous sections can be completed using the existing facilities and equipment except where permanent equipment was requested.

The Materials Characterization Laboratory, a multi-user facility within the College of Earth and Mineral Sciences at PSU provides AFM (Digital Instruments Digital Instruments AFM/LFM Scanning Probe Microscope and Digital Instrument Nanoscope IIIa Dimension 3100 microscope), EM (Cameca SX-50 electron microprobe), ICP-AES (Leeman Labs PS3000UV inductively coupled plasma emission spectrophotometer), ICP-MS (Finnigan Element high resolution inductively coupled plasma mass spectrometry), IR (several models of infrared spectrometers are available for both solution and solid state analysis), SEM (ISI SX 40 secondary electron microscope, ISI SX 40A with energy dispersive xray analysis capability, JEOL JSM-6300F with EDS detector and analysis), SIMS (Cameca IMS-3F secondary ion mass spectrometer/ion microscope), DTA and TGA (DuPont 2100 and Netzsch STA 429 thermal analyzers for thermogravimetric analysis and differential thermal analysis), TEM (Hitachi HF 2000 and Philips EM420ST transmission electron microscopes), XPS/AES (Kratos Analytical XSAM800 pci for x-ray photoelectron spectroscopy and Auger electron spectroscopy), and XRD (Rigaku Geigerflex x-ray diffraction analysis).

The Department of Geosciences provides rock preparation systems, stable isotopes facility, organic and isotopic facility, in-situ laser ablation BrF_5 system at Dr. Peter Deines' laboratory, and the hydrothermal laboratory of Dr. Hu Barnes. The rock preparation systems include rock saws, thin section makers, grinders, and polishers. The stable isotopes facility includes a Finnigan MAT 252 stable-isotope-ratio mass spectrometer equipped with a dual inlet and multiple collector assemblies, a Finnigan Delta-Plus XP mass spectrometer equipped with a GC, two elemental analyzers and micro-volume gas sampling systems for the analyses of C, N, S, O, and H isotopes (under the direction of K. Freeman), a second Finnigan 252 in the same facility (under the direction of M. A. Arthur) is equipped with common-acid-bath automated carbonate analyzer and a third Finnigan 252 (under the direction of T. Sowers) is dedicated to isotopic analyses of trace gases.

The organic and isotopic facility at includes a Rock-Eval pyrolysis unit, a coulometer and an elemental analyzer for the analysis of inorganic and organic carbon in waters, soils, and sediments. The laboratory is well supplied with routine equipment, including glassware, analytical balances, rotary evaporators, solvent-extraction systems, and two GC-FIDs, as well as a HP 5973 GC/MS (with autosampler, EI and CI capabilities), and an HPLC with absorbance detection.

The department of Biochemistry and Molecular Biology provides, as common use equipment: autoclaves, ice machines, ultracentrifuges, scintillation counters, walk-in incubation and cold rooms, etc. Penn State operates a Nucleic Acid Facility which will be used on a fee basis for synthesizing oligonucleotide primers and for nucleic acid sequencing.

The Chemistry Department provides access to a number of mass spectrometers for structure determination, including an HPLC- time of flight mass spectrometer with atmospheric pressure chemical ionization (LC-APCI-MS).

Michael Arthur's facility includes rock crushing and powdering equipment (SPEX mill), a UIC Coulometrics Automated Carbon Coulometer System (new 2002; CM 5200 Autofurnace, CM 5240 TIC device, CM 5014 Carbon Coulometer, and Printer) and computing equipment (HP 2250TN Workgroup printer,5 Sun 10 Workstations with access to MATLAB for modeling, Mac G4 and PCs).

Susan Brantley's laboratory is fully equipped for solution chemistry analysis, mineral preparation, and analysis of rates of dissolution of earth materials. Up to 12 chemical reactors can be run simultaneously. Standard equipment such as balances, pH meters, rotors, water baths, optical microscopes, and other such apparatus is available. For microbiological work, a Coy anaerobic glove chamber, two laminar flow hoods, and an autoclave are all available.

Jean Brenchley's laboratory is equipped for the microbial diversity portions of the proposal. Small laboratory equipment such as balances, pH meter, hot plates, magnetic stirrers, fraction collectors, microcentrifuges, spectrophotometers, high speed refrigerated centrifuge, water baths, incubators, power supplies, electrophoresis equipment for DNA analyses, thermocyclers for PCR reactions, automatic pipetters, general glassware, chemicals and other general supplies are available in the laboratory.

Greg Ferry's laboratory contains anaerobic glove bags and anaerobic fermentation equipment for small and large-scale growth (30 and 80-liter fermentors) of anaerobes. In addition, the laboratory maintains special equipment for anaerobic biochemistry and molecular biology research that includes: three inert atmosphere systems. The laboratory is well equipped with general equipment items. Most of the equipment in the laboratory has a computer dedicated for data analysis and manipulation. Five computers are also available for general use.

Katherine Freeman's laboratory and the Department of Geosciences maintain a large number of glass vacuum lines for the preparation of samples for analysis by dual-inlet stable-isotope mass spectrometry.

S. B. Hedges's bioinformatics laboratory is equipped with 20 personal computers (Pentium IV and Xeon 1.7-2.0 Gigahertz, operating Linux and Windows; also PowerMac), about half of which are workstations with dual processors and 1 Gigabyte of memory for computationally intensive work. There are two servers: one for internal laboratory use and the other for web

databases; each is a Dell Xeon dual processor PowerEdge 4600 with 144 Gigabyte SCSI storage and 1 Gigabyte memory, with RAID mirroring. All are interconnected on the Internet and protected by a firewall. Additional computing resources, including a 140 CPU Linux cluster, are available in the Penn State Center for Academic Computing.

Christopher House's laboratory is equipped with a Nikon E800 photomicroscope set up for brightfield, phase-contrast, epi-florescence (for FISH), or reflected light microscopy. The microscope is connected directly to a cooled high resolution CCD camera with direct real time computer interface (iMac).

The laboratory is also equipped for aerobic or anaerobic microbial culturing at any temperature between 4°C and 110°C with continuous shaking and with light between temperatures of 4°C and 60°C. Equipment includes an anaerobic chamber, a gas station, and six temperature controlled incubators/shakers. The laboratory is well supplied with routine equipment, including glassware, pH meter, analytical balances, and a Beckman J2-HS centrifuge, as well as a laminar flow hood for working in a sterile environment.

Equipment also includes gel electrophoresis chambers, microcentrifuges, a PTC-100 MJ Research PCR thermocycler, an Beckman UV/Vis Spectrometer, -80 freezer, UV transilluminator and gel visualization box with digital camera, and dark box that can be connected to a cooled CCD camera for chemiluminescence detection.

Computing equipment includes a G4 Macintosh computer, a G3 iMac computer, and a unix workstation with dual 933Mhz Pentium III processors. The laboratory has standard phylogenetic software including the following programs that may be of use in this project: Mac Software (PAUP for phylogenetic analysis, MACCLADE for phylogenetic analysis, AUTODECAY for phylogenetic analysis, MACPATTERN for prosite and blocks searches, OLIGO for primer design, DNASTAR package for sequence analysis and manipulation), Unix Software (FASTA3, BLAST (NCBI Toolbox), HMMer, tRNAScan, CLUSTALW for sequence comparisons, PERL for data manipulation, PHYLIP for phylogenetic analysis, ARB for phylogenetic analysis (programs from University of Munich))

James Kasting's laboratory has 3 Sun Spark 20 workstations, 4 PCs, and access to two Cray supercomputers run by the Environment Institute.

Lee Kump's laboratory maintains 6 Sun Ultra 10's with 768 Mbytes RAM and a Sun Ultra 60 Unix workstation with one processor and 512 Mbytes of RAM. These workstations are part of a network of Unix, PC, and Macintosh computers maintained by the Environment Institute (EI) and Department of Geosciences which are available to students, faculty, and postdoctoral associates affiliated with this project. EI's supercomputing facility is also at our disposal, which houses a Cray Research SV1 (16 CPUs, 4GB memory), and an IBM RS/6000 SP with 10 nodes and 4 CPUs per node, on which many of the model codes are already running. In addition, we will have shared use of one existing 2-processor 750-Mhz Sun server in the EI.

Nutrient analyses are performed on a Technicon AutoAnalyzer II according to an approved EPA quality assurance project plan in the laboratory. The laboratory is fully equipped for aqueous and sedimentary geochemical sample collection and analysis. The Department of Geosciences has also recently purchases a YSI multiprobe for water-column profiling of temperature, conductivity, chlorophyll, oxygen, Eh, pH, turbidity, and PAR light.

Hiroshi Ohmoto's laboratory has two mass spectrometers for isotope analyses of H, C, N, O, and S, an in-situ laser ablation system, an elemental analyzer for H, C, S, and N contents of solids and liquid samples, a continuous flow micro-analytical system for S, C, and N isotope analyses, a BrF_5 line for O and S isotope analyses, gas preparation lines for C, S, O, N, and H isotope analyses, including a SF_6 line recently built for the measurement of ^{32}S, ^{33}S, ^{34}S, and ^{36}S,

petrographic microscopes with digital camera, video camera, and TV monitor, a cthodoluminoscope, facilities for mineral-solutions-gas reaction systems, and a recently purchased x-ray chemical microscope (Horiba XGT-5000) for chemically mapping up to 30 elements simultaneously with a spatial resolution of 10 µm on hand specimen-sized samples.

Non-Penn State facilities:

Secondary ion mass spectrometry

The CAMECA ims 1270 ion microprobe at UCLA will be used for some of the proposed ion probe hours needed for this work. It is equipped with a multicollection array of detectors, as well as a normal incidence electron gun for charge compensation. This instrument will be used for carbon isotopic analyses on samples where multicollection is needed to help resolve small signals in carbon isotopic signatures or where large fluctuations in secondary ion yield is expected. Additionally, the instrument will be used for the development of other isotopic measurements where the larger magnet is desirable to achieve high transmission at high mass resolving power. A letter is included here indicating the enthusiastic agreement of the UCLA group for the collaborative research proposed. The additional ion probe time needed for the proposed research including the SIMS analysis of trace metals will be purchased from one of the other NAI groups that have a CAMECA 6f. These instruments are monocollection instruments smaller than the 1270, but also equipped with normal incidence electron guns for charge compensation.

University of Pittsburgh, Department of Geology and Planetary Science
Rosemary Capo & Brian Stewart

The University of Pittsburgh Geochemistry Facility includes a clean chemistry laboratory housed in a 490 sq. ft. space, and provided with positive pressure and filtration to a 95% level. It contains 16 lineal feet of hood space as well as ULPA filtered laminar flow stations, HEPA-filtered microbalance room and a Millipore Milli-Q reverse osmosis-filter water system for ultraclean chemical procedures. Nearby sample preparation and acid cleaning laboratories contain fume hoods, muffle furnace, ovens, ultrasonic bath, centrifuges and balances.

Major instruments are housed in a 500 sq. ft. lab filtered to the 95% level with positive pressure and fume hoods. Isotopic analyses are determined on a Finnigan MAT 262 thermal ionization mass spectrometer (TIMS) with eight faraday collectors (seven movable, including high mass cup fixed at ^{208}Pb-^{207}Pb position) and a RPQ+ high abundance sensitivity device. For elemental analysis of Fe and other metals, the laboratory also houses a Spectroflame Modula (FTM 08) axial inductively coupled plasma atomic emission spectrometer (ICP-AES), for major and trace element analysis. The instrument is equipped with an end-on plasma option, UV monochromator (160-480 nm) for sequential elemental analysis, and a seven-channel polychromator for simultaneous analysis of alkalis (Li, Na, K, Rb, Cs) and internal standard and background corrections (Sc, Ar).

The department's rock sample preparation facilities include mineral separation equipment (motorized sieves, Frantz magnetic separator, density separation equipment), jawcrusher, ball mill, and rock saws. Petrographic analysis facilities include thin section preparation equipment and high quality petrographic microscopes. Additional XRD and scanning electron microscope capabilities are available in the University's Materials Science Department, including a Philips

X'Pert powder (Cu Ka) and a. a Philips XL 30 field emission gun scanning electron microscope (SEM) equipped with an EDAX energy dispersive system.

A NASA-owned videoconference system (provided by NAI) is available for use by University of Pittsburgh Astrobiology faculty and graduate students. The equipment includes a Polycom videoconference system and monitor, VCR, video projector for simultaneous projection of remote graphics, and an electronic white board. The equipment is housed in a department conference room with full internet, cable and ISDN connections that is now being renovated (scheduled completion in late spring, 2003) specifically to accommodate the NASA equipment.

SETI Institute
Mountain View, CA

Planetary Biology, Evolution, and Intelligence

Principal Investigator:

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Co-Investigators/Collaborators:

At SETI Institute:
Peter Backus
Janice Bishop
Edna DeVore
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Emma Bakes, *NASA Ames Research Center*
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Max Bernstein, *NASA Ames Research Center*
Nathalie Cabrol, *NASA Ames Research Center*
Edmond Grin, *NASA Ames Research Center*
Bishun Khare, *NASA Ames Research Center*
Rocco Mancinelli, *NASA Ames Research Center*
Lori Marino, *Emory University*
Brenda McCowan, *University of California, Davis*
Daniel McShea, *Duke University*

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SETI INSTITUTE NAI PROPOSAL

EXECUTIVE SUMMARY

The Astrobiology Roadmap asks three fundamental questions: (1) How does life begin and evolve; (2) Does life exist elsewhere in the universe? and (3) What is the future of life on Earth and beyond? The SETI Institute will conduct a set of coupled research projects in the co-evolution of life and its planetary environment, beginning with fundamental ancient transitions that ultimately made complex life possible on Earth. We conclude with a project that brings together many of these investigations into an examination of the suitability of planets orbiting M stars for either single-celled or more complex life. Results will help the next generation scientific Search for Extraterrestrial Intelligence (SETI) choose the 10^5 to 10^6 target stars that it will survey for signs of technical civilizations using the new Allen Telescope Array (ATA) being built by the SETI Institute in partnership with the University of California, Berkeley.

SETI is a natural part of the continuum of research that comprises astrobiology. This is recognized explicitly in the Astrobiology Roadmap, which calls for a strategy "for recognizing novel biosignatures" that "ultimately should accommodate a diversity of habitable conditions, biota and technologies in the universe that probably exceeds the diversity observed on Earth." From the life detection point of view, the Roadmap notes that "although technology is probably much more rare than life in the universe, its associated biosignatures perhaps enjoy a much higher 'signal-to-noise' ratio. Accordingly, current methods should be further developed and novel methods should be identified for detecting electromagnetic radiation or other diagnostic artifacts that indicate remote technological civilizations."

Overview of Proposed Research

The research in the Institute's proposal intends to elucidate the co-evolution of life and its planetary environment, typically investigating global-scale processes that have shaped, and been shaped by, both. Throughout, we recognize the importance of pursuing the planetary evolution aspects of this research in the context of comparative planetology: since laboratory experiments are impossible over some of the time and spatial scales relevant to early Earth, we must supplement laboratory data with the insight as we can gain by exploring extraterrestrial environments that may provide partial analogs to the early Earth environment and its processes.

We begin by proposing two new investigations into the oxidation of early Earth's environment. While the biological aspects of this "oxygen transition" have been recently emphasized, both mechanisms to be explored here (peroxy in rocks [Drs. Friedemann Freund and Lynn Rothschild] and aerosol formation in the atmosphere [Dr. Emma Bakes], building on an analogy to processes now occurring in the atmosphere of Saturn's moon Titan) are non-biological. If such mechanisms were to be shown to be quantitatively significant, it would suggest that the oxygen transition on an Earth-like world could take place independently of the invention of any particular metabolic pathways (such as photosynthesis or methanogenesis) that have been proposed as driving this transition. Since Earth's oxygen transition ultimately set the stage for the oxygen-based metabolism evidently essential for metazoa, understanding this transition is crucial to elucidating both Earth's evolution and the evolution of complex (including intelligent) life. Our geological investigations are tightly coupled with microbiological experiments to understand the extent to which the proposed mechanism might have led to the evolutionary invention of oxidant protective strategies and even aerobic metabolism.

One of the major sinks for oxygen on early Earth would have been reduced iron. At the same time iron could have provided shielding against ultraviolet (UV) light that would have been reaching Earth's surface in the absence of the ozone shield generated by atmospheric oxygen. Nanophase ferric oxide minerals in solution

could provide a sunscreen against UV while allowing the transmission of visible light, in turn making the evolution of at least some photosynthesic organisms possible. We will test [Drs. Janice Bishop and Lynn Rothschild] this hypothesis through coupled mineralogical and microbiological work in both the lab and the field, and examine its implications not only for Earth but for Mars as well—with an emphasis on implications for upcoming spacecraft observations.

The survival of microorganisms in very high UV environments can also be tested empirically through the exploration of Earth's highest altitude lakes and ponds, in Bolivia and Chile. We propose [Drs. Nathalie Cabrol and Edmond Grin] a series of investigations of these lakes to examine the strategies employed by these microorganisms.

Just as global-scale changes in oxygen (or iron) were critical for the early biosphere, so too would have been global processes involving other key "biogenic" elements such as carbon [Dr. Bakes] or nitrogen [Drs. Rocco Mancinelli, Amos Banin, David Summers, and Bishun Khare] . We propose coupled laboratory and field research to understand the partitioning of nitrogen on early Earth—and on Mars—between different possible reservoirs, and the abiotic to biotic transition in this cycling.

The work described so far examines the evolution of planetary surface habitability. With the recognition that a subsurface ocean likely exists on Jupiter's moon Europa, we know that habitability in possibly entirely subsurface environments must also be explored. We propose spacecraft data analysis and modeling to examine the geology of Europa and its implications for the free energy sources that would be needed to power a europan biosphere [Drs. Cynthia Phillips and Christopher Chyba] . We will then couple these results with terrestrial analog work [Chyba] and direct low-temperature laboratory experiments [Dr. Max Bernstein] to make predictions about the possible abundance and survivability of any oceanic biomarkers that might reach Europa's surface through active geology. These results will have implications for astrobiological exploration of Europa from either an orbiter or a surface lander.

Finally, we suggest research (Drs. Peter Backus, Jill Tarter, and Chyba) to examine the prospects of planets orbiting dwarf M stars being habitable for either microscopic or complex life. The results of this work will directly influence the strategy employed in the next generation SETI search program to begin in 2005.

The SETI Institute

The SETI Institute was founded in 1984 as a nonprofit scientific research institution. From its beginning, its mission has been to explore the origin, nature and prevalence of life in the universe, and to explain this science to the public. The Institute may be best known for its ongoing Search for Extraterrestrial Intelligence (SETI) programs, but the study of virtually all other aspects of astrobiology has been part of our mandate from the beginning.

The Institute employs nearly 120 individuals. Sixty-four of these are employed through the Center for the Study of Life in the Universe, directed by Dr. Frank Drake. Another twenty-six scientists and engineers are employed within the Center for SETI Studies, directed by Dr. Jill Tarter, who is a Co-Investigator on this grant. The remainder are in administration and education. The Institute has a public/private partnership with the University of California Berkeley, with which it is currently building the Allen Telescope Array to conduct the next generation of SETI searches. Funding for the Institute's SETI projects is almost entirely through private funds, whereas research within the Center for the Study of Life in the Universe is nearly entirely funded through peer-reviewed grants. Several of the projects proposed here have so far been funded at the pilot project level through private resources made available by the Institute. This proposal therefore represents a private/public partnership with the opportunity for peer-reviewed funding to leverage previous substantial initial private investments into major research accomplishments.

Strengthening the Community

The SETI Institute's EPO programs have a long and successful history of engaging our scientists and educators with teachers and the general public. The Institute's proposed NAI-EPO program [Ms. Edna DeVore] will comprise four major activities: (1) conduct professional development for high school science teachers implementing *Voyages Through Time (VTT)*, an integrated astrobiology curriculum developed by the SETI Institute, and make VTT a resource available to the entire NAI by inviting other NAI teams to participate in the professional development program; (2) collaborate with the California Academy of Sciences to plan and develop new exhibits and to support and participate in education and outreach programs with CAS throughout the NAI membership period; (3) facilitate education outreach, media opportunities, and public event appearances for Institute NAI scientists; and (4) participate in the NAI EPO network and NASA OSS and Education Activities.

For much of the public, one of the topics of greatest interest in astrobiology is the scientific search for intelligent life. While our research and EPO activities span the range of astrobiology, the SETI Institute is unique in being able to bring forefront scientific research on this issue to the NASA Astrobiology Institute.

Strategic Planning and Management

Professor Christopher Chyba is the Principal Investigator for this proposal. Chyba holds the endowed Carl Sagan Chair for the Study of Life in the Universe at the SETI Institute. Chyba is also an associate professor (research) in the Department of Geological and Environmental Sciences at Stanford University. He teaches undergraduate and graduate courses in astrobiology at Stanford, and is currently the PhD thesis advisor for Mr. Kevin Hand.

Chyba has extensive service as chair or member of numerous NASA and other federal committees, and as a leader and integrator of diverse research projects at the SETI Institute. In 1996 he received a Presidential Early Career Award and in October 2001 was awarded a MacArthur Fellowship for his work in both astrobiology and international security.

Chyba, Mancinelli and DeVore will constitute an ongoing strategic planning and management “troika” for this proposal.

Over the past two years, the Center for the Study of Life in the Universe at the SETI Institute has carried out, using private funding, a strategic planning process that demonstrates its ability to bring together research scientists in interdisciplinary and successful ways. Co-chaired by Chyba and USC geomicrobiologist Ken Nealson, this Strategic Planning Group (SPG) brought together some two dozen scientists and engineers to range across the field of astrobiology and identify new areas of innovative research ripe for pursuit by the SETI Institute.

The projects proposed here will benefit from ongoing NAI-SETI workshops at the Institute that the PI will continue to host. The Co-Investigators for this proposal all have experience in being principal investigators in their own research. No “micromanagement” by the PI of this proposal will be necessary. Rather, the PI’s role will be to ensure communication, cooperation, and synergy among the various research projects.

1. Introduction to Research & Management Plan: Astrobiology and Intelligent Life

The three fundamental questions of astrobiology in the Astrobiology Roadmap are: (1) How does life begin and evolve; (2) Does life exist elsewhere in the universe? and (3) What is the future of life on Earth and beyond? This proposal pursues a unique approach to these questions that has not been previously represented within the NASA Astrobiology Institute (NAI). We propose a set of coupled research projects in the co-evolution of life and its planetary environment, beginning with fundamental ancient transitions that ultimately made complex life possible on Earth., Our proposal concludes with a project that will bring together our knowledge about the stellar and planetary evolution requirements for the origin of life through the evolution of advanced technical intelligence. Results will help the next generation scientific Search for Extraterrestrial Intelligence (SETI) choose the 10^5 to 10^6 target stars that it will survey for signs of technical civilizations using the new Allen Telescope Array (ATA) being built by the SETI Institute in partnership with the University of California, Berkeley.

SETI searches should be pursued in the context of an understanding of the origin and evolution of life, from the simplest single-celled organisms to complex communicating metazoa. Just as studies of the microbial deep biosphere on Earth have informed the search for extraterrestrial life in subsurface environments on Mars or Europa, so can the search for extraterrestrial intelligence be informed by studies of planetary habitability.

SETI is a natural part of the continuum of research that comprises astrobiology. This is recognized explicitly in the Astrobiology Roadmap, which calls for a strategy "for recognizing novel biosignatures" that "ultimately should accommodate a diversity of habitable conditions, biota and technologies in the universe that probably exceeds the diversity observed on Earth." From the life detection point of view, the Roadmap notes that "although technology is probably much more rare than life in the universe, its associated biosignatures perhaps enjoy a much higher ' signal-to-noise' ratio. Accordingly, current methods should be further developed and novel methods should be identified for detecting electromagnetic radiation or other diagnostic artifacts that indicate remote technological civilizations." As will be seen below, this is exactly what the SETI Institute has been doing, and will continue to do in new ways with support from this grant.

Consistent with the Astrobiology Roadmap, funding SETI-related activities is now embraced by NASA. As NASA Associate Administrator Ed Weiler told Congress in 2001, (Subcommittee on Space and Aeronautics 2001), "NASA is no longer prohibited by any congressional language from considering or funding SETI research, so SETI is currently eligible and considered fairly under peer review for NASA opportunities."

1.1 Overview of Proposed Research

The research in this proposal intends to elucidate the co-evolution of life and its planetary environment, typically investigating global-scale processes that have shaped, and been shaped by, both. Throughout, we recognize the importance of pursuing the planetary evolution aspects of this research in the context of comparative planetology: since laboratory experiments are impossible over some of the time and spatial scales relevant to early Earth, we must supplement laboratory data with the insight as we can gain by exploring extraterrestrial environments that may provide partial analogs to the early Earth environment and its processes. (In this overview, we leave out citations to the literature; full citations are provided throughout the text of the proposal.)

We begin by proposing two new investigations into the oxidation of early Earth's environment (Secs. 2 and 3). While the biological aspects of this "oxygen transition" have been recently emphasized, both mechanisms to be explored here (peroxy in rocks and aerosol formation in the atmosphere, building on an analogy to processes now occurring in the atmosphere of Saturn's moon Titan) are non-biological. If such mechanisms were to be shown to be quantitatively significant, it would suggest that the oxygen transition on an Earth-like world could take place independently of the invention of any particular metabolic pathways (such as photosynthesis or methanogenesis) that have been proposed as driving this transition. Since Earth's oxygen transition ultimately set the stage for the oxygen-based metabolism evidently essential for metazoa, understanding this transition is crucial to elu-

cidating both Earth's evolution and the evolution of complex (or even intelligent) life. Our geological investigations in Sec. 2 are tightly coupled with microbiological experiments to understand the extent to which the proposed mechanism might have led to the evolutionary invention of oxidant protective strategies and even aerobic metabolism.

One of the major sinks for oxygen on early Earth would have been reduced iron. At the same time iron could have provided shielding against ultraviolet (UV) light that would have been reaching Earth's surface in the absence of the ozone shield generated by atmospheric oxygen. Nanophase ferric oxide minerals in solution could provide a sunscreen against UV while allowing the transmission of visible light, in turn making the evolution of at least some photosynthesic organisms possible. We will test (Sec. 4) this hypothesis through coupled mineralogical and microbiological work in both the lab and the field, and examine its implications not only for Earth but for Mars as well—with an em phasis on implications for upcoming spacecraft observations. This work could provide insight into a key step in terrestrial biological evolution that also likely played an important role in the oxidation of Earth's surface environment.

The survival of microorganisms in very high UV environments can also be tested empirically through the exploration of Earth's highest altitude lakes and ponds, in Bolivia and Chile. We propose (Sec. 5) a series of investigations of these lakes to examine the strategies employed by these microorganisms. This work will also provide insight into conditions prevailing on lakes that may have existed in early Mars history, and therefore help inform Mars exploration.

Just as global-scale changes in oxygen (or iron) were critical for the early biosphere, so too would have been global processes involving other key "biogenic" elements such as carbon (Sec. 3) or nitrogen (Sec. 6 and 7). We propose coupled laboratory and field research to understand the partitioning of nitrogen on early Earth—and on Mars—between different possible reservoirs, and the abiotic to biotic transition in this cycling.

The work described so far examines interactions among a world's geology, atmosphere, oceans and biology in the evolution of that world's surface habitability. With the recognition that a subsurface ocean likely exists on Jupiter's moon Europa, we now know that habitability in possibly entirely subsurface environments must also be explored. We propose work to examine the geology of Europa and its implications for the free energy sources that would be needed to power a Europan biosphere (Secs. 8 and 9). This work will make use of both spacecraft data and modeling. We will then couple these results with terrestrial analog work (Sec. 9) and direct low-temperature laboratory experiments (Sec. 10) to make predictions about the possible abundance and survivability of any oceanic biomarkers that might reach Europa's surface through active geology. These results will have implications for astrobiological exploration of Europa from either an orbiter or a surface lander.

Finally, the proposal concludes (Sec. 11) with research (to include interdisciplinary workshops) to examine the prospects of planets orbiting dwarf M stars being habitable for either microscopic or complex life. The results of this work will directly influence the strategy to be employed in the next generation search for extraterrestrial intelligence, to begin in 2005.

1.2 The SETI Institute

The SETI Institute was founded in 1984 as a nonprofit scientific research institution. From its beginning, its mission has been to explore the origin, nature and prevalence of life in the universe, and to explain this science to the public. The Institute may be best known for its ongoing Search for Extraterrestrial Intelligence (SETI) programs, but the study of virtually all other aspects of astrobiology has been part of our mandate from the beginning. Indeed, Dr. Chuck Klein, former head of the Viking spacecraft biology team, joined the Institute in 1985 as our first "Life in the Universe" scientist. Since its founding, the SETI Institute has managed more than $136 million in private and government research funds. Our current annual budget is $21 million, of which about $5.4 million is in the form of funding for peer-reviewed grants.

The Institute employs nearly 120 individuals. Sixty-four of these are employed through the Center for the Study of Life in the Universe, directed by Dr. Christopher Chyba, PI on this grant. Another twenty-six scientists and engineers are employed within the Center

for SETI Studies, directed by Dr. Jill Tarter, who is a Co-Investigator on this grant. The remainder are in administration and education. The Institute has a public/private partnership with the University of California Berkeley, with which it is currently building the Allen Telescope Array to conduct the next generation of SETI searches. Funding for the Institute's SETI projects is now almost entirely through private funds, whereas research within the Center for the Study of Life in the Universe is nearly entirely funded through peer-reviewed grants. This proposal appropriately brings both research sides of the Institute together, as they naturally both fall within the study of astrobiology. Five of the thirteen projects proposed here have so far been funded at the pilot project level through private resources made available by the Institute, typically through funds from Chyba's Sagan Chair. This proposal therefore represents a true private/public partnership, with the opportunity for peer-reviewed funding to leverage previous substantial initial private investments into major research accomplishments.

The PI and two Co-Is on this proposal request no salary support, but other SETI Institute employees hold "soft-money" positions and therefore request some salary to perform the research proposed. However, SETI Institute overhead (which stands at 20%) is so low compared to other government or academic institutions (often 50% or higher) that our research/cost ratio remains extremely competitive.

1.3 Principal Investigator

Professor Christopher Chyba is the Principal Investigator for this proposal. Chyba holds the endowed Carl Sagan Chair for the Study of Life in the Universe at the SETI Institute. Chyba is also an associate professor (research) in the Department of Geological and Environmental Sciences at Stanford University. He teaches undergraduate and graduate courses on "The Origins of Life in the Solar System" at Stanford, and currently supervises three graduate students and two undergraduates in their research. Chyba serves as the PhD thesis advisor for Mr. Kevin Hand.

Chyba has extensive service on NASA and other federal committees (e.g. chair of the Science Definition Team for NASA's Europa Orbiter Mission, chair NASA's Solar System Exploration Subcommittee, member Executive Committee, NASA's Space Science Advisory Committee, member NASA's exobiology review panel, member National Academy of Science's Committee on International Security and Arms Control) and is a leader and integrator of diverse research projects at the SETI Institute. Chyba's own educational background demonstrates the kind of interdisciplinary science that characterizes astrobiology. His PhD is in astronomy (planetary science), from Cornell University where Carl Sagan was his thesis advisor. His undergraduate degrees are in physics and mathematics, and he also holds a graduate degree (MPhil) in the history and philosophy of science. His earliest peer-reviewed publications were in general relativity, but beginning in 1985 his research shifted to planetary science and, especially, exobiology (as it was then called). His biology training includes the intensive summer courses at MBL Woods Hole in Microbial Diversity (250 laboratory hours, 110 lecture hours) and Molecular Evolution (course run by Mitch Sogin). In 1996 he received a Presidential Early Career Award and in October 2001 was awarded a MacArthur Fellowship for his work in both astrobiology and international security.

1.4 Strategic Planning and Management

Over the past two years, the Center for the Study of Life in the Universe at the SETI Institute has carried out, using private funding, a strategic planning process that showcases its ability to bring together research scientists in interdisciplinary and successful ways. Co-chaired by Chyba and USC geomicrobiologist Ken Nealson, this Strategic Planning Group (SPG) brought together some two dozen scientists and engineers in both full plenary and breakout workshops to range across the field of astrobiology and identify new areas of innovative research ripe for pursuit by the SETI Institute. Members of the group included Barry Blumberg (NAI), Leslie Orgel (Salk), Simon Conway Morris (U. Cambridge), Bill Schopf (UCLA), Lori Marino (Emory), Dan McShea (Duke), Pascale Ehrenfreund (Leiden), Eric Mathur (Diversa Corporation), Jay Melosh (U. Arizona) and many others. The research proposed in section 11 of this proposal is a direct outcome of ideas discussed and pursued at these meetings, then funded at the pilot level by Chyba out of his privately funded Sagan Chair budget.

This SPG paralleled an analogous effort by the Institute to plan its next 20 years of SETI research. In this effort, the Institute brought together 40 scientists and engineers into a SETI Science and Technology Working Group (STWG) that ultimately proposed three major projects. The first of these, the Allen Telescope Array of over three hundred fifty six-meter dishes, is now being built with private funding. The STWG has published its strategic and technical studies in the 550-page book, *SETI 2020: A Roadmap for the Search for Extraterrestrial Intelligence* (Ekers et al. 2002). The projects proposed here will benefit from ongoing NAI-SETI workshops at the Institute that the PI will host, that all Co-Investigators will be expected to attend whenever possible, and that will be open to outside scientists. The Institute already hosts an ongoing colloquium series; these will be continued but focused on reports from proposal Co-Investigators (and associated graduate students) on their ongoing research. Such team group meetings have already been of great value in formulating this proposal, and it will be clear from the tight coupling of many of the sections of this proposal that the Co-Investigators have and will strongly benefit from ongoing collaborations among the leaders of different research tasks. Moreover, as part of Sec. 11 of this proposal, more specialized workshops on planetary habitability around M stars will be held at the Institute. These will necessarily draw on the leading Co-Investigators of several sections of this proposal.

Despite these coordination efforts, it should be emphasized that the Co-Investigators for this proposal all have experience, typically years to decades of experience, in being principal investigators in their own research. No "micromanagement" by the PI of this proposal will be necessary. Rather, the PI's role will be to ensure communication, cooperation, and synergy among the various research projects. The PI and the Institute have done this quite successfully before, and will ensure that that in this case as well the whole will be greater than the sum of the parts.

1.5 Building the Astrobiology Community

The goal of the proposed Education and Public Outreach (EPO) activities is to share the cutting-edge research and discoveries of astrobiology with educators and the public in order to inspire students to pursue careers in science, technology, engineering and mathematics, and to contribute to general science literacy. The proposed EPO activities will focus on these key audiences: high school science teachers at schools with underserved students in urban, rural and minority communities; science museum visitors--students, teachers, and families; and the general public via the media, lectures, and events. The SETI Institute's EPO programs have a long and successful history of engaging our scientists and educators with these audiences in formal and informal education as well as public outreach. The Institute's proposed NAI-EPO program will comprise five major activities: (1) conduct professional development for high school science teachers implementing *Voyages Through Time (VTT)*, an integrated astrobiology curriculum developed by the SETI Institute, and make VTT a resource available to the entire NAI by inviting other NAI teams to participate in the professional development program; (2) collaborate with the California Academy of Sciences to plan and develop new exhibits and to support and participate in education and outreach programs with CAS throughout the NAI membership period; (3) facilitate education outreach, media opportunities, and public event appearances for Institute NAI scientists as a part of our ongoing outreach activities; and (4) participate in the NAI EPO network and NASA OSS and Education Activities.

For much of the public, one of the topics of greatest interest in astrobiology is the scientific search for intelligent life—this is a natural first interpretation of the question "Are we alone?". While our research and EPO activities span the range of astrobiology, the SETI Institute is unique in being able to bring forefront scientific research on these issues to the NASA Astrobiology Institute.

2. Oxygen on Early Earth: A New Geological Hypothesis

Life originated early in Earth's history. At that time the atmosphere was anoxic, and the conditions were far more reducing than today. Over the period of the next two billion years or more, the Earth system became slowly oxidized (Holland 1984; Kasting 1993; Kasting 2001; Kump et al. 2001; Wiechert 2002). The reasons for this evolution towards an ever more oxidized state remain major topics of investigation. The answer is of major significance to astrobiology for a host of reasons,

ranging from the evolution of defensive mechanisms for life to protect itself against assault by oxidants, to the development of a UV-filtering ozone shield, to the availability of a number of electron acceptors for microbial metabolism, as well as the availability of molecular oxygen and its implications for the possibility of metazoa, and ultimately even intelligent life.

There are two important and related questions: (1) How was the oxygen produced that drove Earth's surface environment to a more oxidized state? (2) To what extent and how did early life forms participate in this transition?

On early Earth, the oxygen level of the atmosphere was buffered by ferrous iron (Fe^{2+}), dissolved in, and continuously washed into, the ocean through weathering of the highly reduced, mafic and ultramafic rocks that dominated the early land masses. Over ~10^9 yr the Fe^{2+} oxidized to ferric iron (Fe^{3+}), consuming an estimated ~10^{12} g O_2/yr in the process (Holland 1984). This led to the worldwide precipitation of banded iron formations (BIFs). During this time the O_2 partial pressure in the atmosphere rose slowly. Later, atmospheric O_2 rose to a level that is comparable to its present partial pressure.

Many physical or physico-chemical processes have been evaluated as possible causes for the slow oxidation of the early Earth system. These include photo-dissociation of H_2O in the uppermost atmosphere by solar hard ultraviolet radiation followed by H_2 escape to space (Hunten et al. 1989) or photo-oxidation of Fe^{2+} to Fe^{3+} in a thin surface layer of the ocean (Braterman et al. 1983). However, these reactions and related processes seem inadequate to account for the flux of O_2 needed (Holland 1984). Similarly, O_2 and H_2O_2 production due to ^{40}K decay in Earth's oceans (Draganic et al. 1987, 1991) seems quantitatively insufficient.

Currently, possible biological explanations seem more able to explain Earth's early surface oxidation. One is the hypothesis that microorganisms "invented" oxygenic photosynthesis at a very early stage in their evolution (e.g., (Pierson 1994; Sleep 2001). Based on the discovery of stromatolites in precambrian and even archaean rocks it has been suggested that such microorganisms might have been similar to present-day oxygenic cyanobacteria (Pierson 1994). Like cyanobacteria today, they might have formed stromatolites in shallow coastal waters, i.e., microbial colonies with layer-like textures suggesting phototaxy (e.g. (Copley 2001)). However, phototaxy does not *per se* prove oxygenic photosynthesis. A different hypothesis is that methanogens would have driven an irreversible oxidation of the surface through the production, and subsequent UV photolysis, of methane (CH_4)—leading to hydrogen escape to space (Catling et al. 2001). A sufficient source of CH_4 on early Earth would have led to UV-driven polymerization into an atmospheric haze (Sagan and Chyba 1997, Pavlov et al. 2000, 2001) and subsequent H_2 loss and resulting surface oxidation. This candidate mechanism has a partial analog in the contemporary atmosphere of Saturn's moon Titan, and we explore it in both the Earth and Titan contexts in Sec. 4 of this proposal. Here, we first examine a different, geological hypothesis and its biological implications: the possibility that simple weathering played a key role. If the research to be carried out under this proposal were to show this hypothesis to be correct, this would imply that the oxidation of an Earth-like planet's surface should be expected due to a mechanism that should be quite common, and does not depend on the evolution of a particular metabolic style (methanogenisis). This would suggest that planets with oxygen-rich atmospheres, and therefore the prospect for metazoa and possibly even intelligent life, could be greater than otherwise expected.

2.1 Motivation and Statement of Hypothesis

Previous discussions have had too little to say about the fact that oxygen and its activated species, collectively known as reactive oxygen species (ROS), are highly toxic to life (e.g., (Rothschild and Mancinelli 2001)). Organisms cannot survive unless they possess a biochemical machinery to enzymatically degrade or quench any ROS and to repair damaged cell components, especially DNA (Newcomb and Loeb 1998).

Yet it is hard to believe that the complex biochemical machinery required for oxygenic photosynthesis would have been present in the earliest organisms. This leads us to suspect another source of oxygen on early Earth that provided the necessary evolutionary stimulus, i.e., a "training ground" for microorganisms to "learn" how to respond to ROS. To exert the necessary selective pressure it would have

been sufficient if this other source expressed itself primarily in the microenvironment where the organisms lived. Therefore, while the "training" was going on in microenvironments, the rest of the Earth could have remained anoxic. Organisms in the right microenvironments would have been pre-adapted to life on a later, globally oxidized world.

There is another source of oxidative power beyond biology that has not been previously recognized. This source is contained in igneous and high-grade metamorphic rocks. It comprises a small but non-trivial fraction of oxygen anions in minerals of those rocks, which have converted from their usual 2- oxidation state to the higher 1- oxidation state, i.e., from O^{2-} to O^{-}. The O^{-} combines in pairs to form peroxy links, i.e., $O_3Si\text{-}OO\text{-}SiO_3$.

The processes that lead to this unusual change in valency on the O^{2-} are due to the incorporation of small amounts of H_2O in the form of hydroxyl anions, Si-OH, in the minerals of rocks that solidify from magma or recrystallize in an H_2O–laden metamorphic environment. During cooling Si-OH pairs undergo a redox conversion in the course of which the hydroxyl (OH) protons become reduced to H_2 molecules, while two oxygen anions act as electron donors and oxidize from the 2- to the 1- oxidation state. From that moment onward the rocks contain both molecular H_2 and oxidized oxygen anions in the form of peroxy links, $O_3Si\text{-}OO\text{-}SiO_3$ (Freund et al. 2002). The underlying reactions occur in rocks that make up the bulk of the crust and could therefore be of global importance. Techniques for studying these processes have been described in recent papers (Freund 2002, 2003).

To investigate this hypothesis, the main reaction to consider is the hydrolysis of peroxy links:

$$O_3Si\text{-}OO\text{-}SiO_3+2H_2O = 2O_3Si\text{-}OH+H_2O_2 \quad (2.1)$$

The hydrogen peroxide (H_2O_2) then decomposes, releasing oxygen via H_2O + O. Eq.(2.1) has potentially far-reaching consequences for the microenvironments in which some early organisms must have lived, *viz.* in the mud and at rock–water interfaces. Such microorganisms would have been exposed to a constant trickle of H_2O_2 liberated as minerals slowly dissolved under the combined action of water and organic acids, which microorganisms typically secrete as part of their metabolism. This scenario leads us to suspect that microenvironments existed in which organisms would have been under the constant challenge to fend off the ROS due to the liberation of H_2O_2 in their immediate surroundings.

Our biological hypothesis is directly connected with the geological hypothesis expressed by Eq.(2.1). We will test the hypothesis that, in spite of anaerobic conditions on early Earth, organisms living in intimate contact with rocks were nevertheless under evolutionary stress to develop the enzymatic defense mechanisms that helped them survive the threat from ROS. Gaining insight into these complex bio-geological relationships will open a new approach to understanding a key step in the evolution of early life, *viz.* the evolution of aerobic metabolism.

Eq.(2.1) has implications not only for the evolution of early life but also for the evolution of the early atmosphere and indeed the Earth system as a whole as it changed from overwhelmingly reduced to ever more oxidized conditions. The reason is that any amount of H_2O_2 liberated during weathering oxidizes reduced components in the environment, foremost ferrous iron, Fe^{2+}, in the early ocean. From the weathering rate of early landmasses and islands, depending on the concentration of peroxy links in average igneous and high-grade metamorphic rocks, we can estimate the flux of H_2O_2, and therefore O_2, into the surface environment.

2.2 Preparatory Work

During preparatory work (Rothschild and Freund 2001) we evaluated several methods to measure the amount of peroxy in rocks, using titration techniques and a new thermal technique (Freund et al. 2000; Rothschild et al. 2002). The latter, which appears to be most promising, is based on the disproportionation of peroxy upon heating, $O_3Si\text{-}OO\text{-}SiO_3 = O_3Si\text{-}O\text{-}SiO_3 + 1/2\ O_2$. We used a Y-doped ZrO_2 oxygen detector to measure the partial pressure of O_2 in a stream of N_2 to which we had added 100 ppm O_2. A typical O_2 evolution curve, obtained by heating a sample of gray granite from Barre, VT, as shown in Fig. 2.1 yielded a peroxy content of at least 500 ppm. Studies of selected single crystals yielded 10-15 times higher peroxy contents.

The preliminary results presented in Fig. 2.1 can be used to estimate the amount of O_2 that could have been liberated on early Earth through weathering. For a global weathering

rate of 3 km^3 yr^{-1} (the same as today; Goddé ris et al. 1999; Franck and Bounama 2001; Foley et al. 2003) and taking as a conservative value the average peroxy content in igneous rocks such as granite to be 500 ppm, the total amount of O_2 injected into the early Earth's surface environment would have been of the order of $5x10^{12}$ g yr^{-1}. This yearly O_2 flux is about an order of magnitude less than that suggested by Catling et al. (2001) to result from the biological methanogenesis mechanism, but it is about the same as the flux estimated to have been necessary over a period of ~10^9 years to precipitate the banded iron formations (Holland 1984).

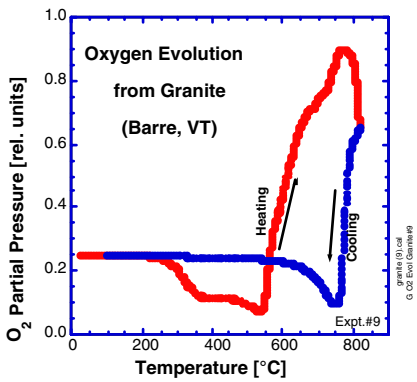


Fig. 2.1: O_2 evolution from crushed granite from the type location Barre, VT, during heating in a stream of N_2 with 100 ppm O_2. Between 250-550°C O_2 is consumed, but above 550°C excess O_2 is released. The amount of O_2 gives an estimate of the average peroxy content in this rock of at least 500 ppm.

Our preparatory work indicates that peroxy in rocks represent a potentially large source of oxygen on a tectonically active planet like Earth with liquid water at the surface and a powerful global weathering cycle. If confirmed by the work that we propose here, the presence of such a large inorganic source of O_2 has far-reaching consequences for our understanding of the evolution of the Earth. It would suggest that any other planet with active tectonics and Earth-like surface conditions is bound to evolve toward an ever more oxidized state—irrespective of the co-evolution of life that is capable of carrying out either methanogenesis or oxygenic photosynthesis.

With respect to the evolution of terrestrial life, the O_2 flux through the weathering of peroxy-bearing rocks provides for relentless environmental pressure forcing certain early microorganisms to adapt to the presence of ROS in their immediate surrounding. If the work proposed here continues to suggest that rocks are indeed an important source of ROS, it will help us understand the evolution of (some) early life from anaerobic to aerobic.

Astrobiologically, this would lead us to predict that life on other Earth-like planets will be under evolutionary pressure to overcome ROS, even under globally anaerobic conditions, and would therefore also be forced to evolve aerobic metabolism.

2.3 Work Plan

The lead Co-Investigators for this section, Drs. Friedemann Freund (SETI Institute) and Lynn Rothschild (NASA Ames) will exploit their complementary backgrounds to test the hypotheses proposed here. Under Dr. Freund, a physicist whose work has led to the discovery of the peroxy content of planetary materials, rock samples will be selected, analyzed for their peroxy content, and prepared for microbiological experiments under conditions that capture the ROS at the mineral surfaces. Dr. Rothschild, an evolutionary microbiologist with expertise in oxidative damage, will determine whether the ROS levels would be sufficient to provoke a selective pressure to evolve detoxification systems.

Physical Studies: The peroxy analysis of minerals and rocks needs to be further refined. Heating, crushing or mechanical deformation of rocks are all methods that take advantage of the inherent weakness of the peroxy bond and its tendency to break apart. These methods can be used to give us information on the concentration of peroxy links in common minerals and rocks susceptible to liberating H_2O_2 during weathering or in contact with acidic secretions from microbes living in intimate contact with mineral surfaces. Selected minerals and rocks will be crushed in inert and O_2-bearing atmospheres. The powders will be heated, varying the heating rate and other parameters. The O_2 evolved will be assessed quantitatively with an O_2-sensitive detector or by GC analysis.

During other crushing experiments we plan to measure the H_2 gas released by GC analysis, as described in Freund et al. (2002). The evolution of H_2 gas will be studied as a function of time and temperature. This is important in the context of the out-diffusion of H_2 over geological time and the accumulation of "excess"

peroxy in minerals and rocks. On a prebiotic Earth, much of this H_2 will escape into the atmosphere and subsequently to space. After primitive life became established, this H_2 may have served as an energy source for microorganisms (see Freund et al. 2002).

Following our previously published laboratory practice (e.g. Freund et al. 2002), for controls we will use silicate glass that does not contain measurable amounts of peroxy. We shall replicate every step of the experiments with both rocks and peroxy-free controls. Previously published control experiments indicate that the oxidation state of the atmosphere has little effect on the production of peroxy deep inside the rock mineral matrix (Freund and Wengeler 1982, Freund et al. 1993, 2002).

Our data will allow us to estimate the amount of excess oxygen in minerals and rocks. These values will be used with models for the weathering rate of early Earth to estimate Earth's oxidation rate oxidized over its first 1-2 Gyr. The data will also be used for evaluating such processes on Mars, where igneous rocks may also be peroxy-laden. This aspect of Martian petrology will be of value in understanding the oxidation of Mars and the potential of Mars as a haven for subsurface life.

Biochemical Studies: The mechanism proposed here for the generation of ROS on an anaerobic Earth could provide the impetus for the origin of detoxification mechanisms, and ultimately lead to anaerobic metabolism. However, the only way to test this is to try to recreate this scenario using modern organisms. Therefore, we will use two types of approaches: we will assess damage from oxidants by measuring DNA damage, and we will use fluorescence microscopy to assess the presence of ROS within cells.

To test for oxidative damage in naked DNA, we expose commercially obtained DNA to crushed rocks, digest and de-phosphorylate the DNA, and determine the ratio of the oxidized base 8 hydroxy-2'-deoxyguanosine to the un-oxidized 2-deoxyguanosine, using HPLC with a COULCHEM II electrochemical detector. The HPLC is done in conjunction with the certified Analytical Laboratory at NASA Ames.

The results obtained so far are comparable to those in the literature using both a similar technique as well as immunological techniques (e.g. Mitchell et al. 2002). Briefly, either naked DNA or cells are exposed to the presumptive oxidizing agent. We plan to expand on these findings and to use a wider variety of microorganisms, including anaerobic microorganisms, which are highly susceptible to oxidative damage. We have developed a new experimental method that does not involve rock crushing but does activate ROS.

At the same time as ROS are damaging DNA, repair takes place. Thus, if we want to determine the damage, we need to inhibit the repair mechanisms. The simplest method to achieve this goal with organisms that are meso- or thermophilic is to keep the cells cold during damage. For example, *Cyanidium*, a red alga that in nature is usually found at 40-55°C (e.g. Rothschild 2001)), was exposed to H_2O_2 for one hour both on ice at 0°C and at 20°C. The algae kept on ice showed a >1.5 x higher ratio of 8-hydroxyguanosine to deoxyguanosine. This work will be expanded under this grant, using suitably prepared rock samples as a source of ROS.

If the amount of ROS generated is too little to cause damage to even naked DNA, it is unlikely to have been an impetus for detoxification mechanisms. However, if the levels are so high as to be lethal to even modern aerobic organisms, once again the hypothesis may be flawed

It is possible that ROS are affecting the microbes, but that enzymatic detoxification mechanisms are detoxifying the ROS before they damage the DNA. To test this, we will use a fluorescent stain, 2'7'-dichlorodihydrofluorescein, to determine the presence of ROS within cells. Cells will be stained after exposure to rocks with the suitable controls, stained, and analyzed in Rothschild's lab on a Zeiss Axioskope with a digital camera and image analysis software (all already acquired) that allows the integrated imaging and quantification of intensity of staining, greatly improving prospects for using fluorescent imaging to quantify ROS exposure to individual cells.

We would also like to test whether exposure to the rocks may have a lethal effect. We will use anaerobic and aerobic species, as well as some aerobic species deficient in DNA damage repair. Currently Rothschild has *E. coli*, yeast and *Chlamydomonas* wild-type cells and strains deficient in various types of DNA dam-

age repair. To determine whether the cells are alive, we will use the Live/Dead stain kit from Molecular Probes.

The results of these experiments will tell us: (1) Whether enough ROS are produced to damage DNA; (2) Whether the ROS are entering the cells; (3) Whether there are sufficient ROS to kill modern organisms that do or do not have DNA damage repair mechanisms. In sum, is the "right amount" of ROS produced by the rocks to make a difference to organisms present on early Earth?

2.4 Timeline

Physical studies: Year 1: Set up and test laboratory procedures to measure excess oxygen and lattice-bound hydrogen in minerals and rocks. The procedures will include (i) physical techniques to be applied as a function of temperature, time and grain size of the samples (thermal gas evolution followed by dedicated O_2 and H_2 analysis) and (ii) wet-chemical methods (1-10-phenantroline photometric titration). Year 2 and following: Systematic survey of rocks that are characteristic of the continental crust, both igneous and metamorphic rocks and sedimentary rocks, from different localities worldwide. Survey of individual mineral phases from such rocks. Use laboratory data to model the global O_2 flux on the basis of weathering rates and continental growth rates on the early Earth. Eventually this will permit models for the evolution of any planet with surface or subsurface weathering. Year 3 and following: Systematic study of selected mineral systems that will be crystallized from melt under various water partial pressures, followed by a systematic study of the amount of excess oxygen in such synthetic rocks. At first this study will be conducted at 1 bar pressure, later at elevated pressures up to several kilobar (planned collaboration with Geophysical Laboratory, Carnegie Institution, Washington).

Biological studies: Year 1: Standardization and calibration of the three types of protocols that will be conducted (DNA damage, ROS in cells, and Live/Dead analysis). Continues into year 2 as needed. Years 2-5: Biological tests performed in conjunction with Freund on a time schedule that depends on his measurements of oxygen release.

The work proposed here addresses Goal 4, Objectives 4.1 and 4.2,and Goal 5 of the Astrobiology Roadmap.

3. Titan, Photochemical Haze, and the Oxidation of Early Earth

An important tool of astrobiology is comparative planetology. In particular, since experiments with spatial or temporal scales comparable to those "experiments" in the origin of life that occurred on early Earth cannot be conducted in the laboratory, it is important to explore and contrast conditions on the other worlds of our Solar System with those that may have existed on Earth. In this spirit, we may use Jupiter's moon Europa as an analog to a possible ice-covered early terrestrial ocean (Bada et al. 1994), carbonaceous meteorites as an analog to scenarios for the origin of life at depth (Chyba and McDonald 1995), high-altitude lake environments on Earth as analogs to early Mars or ozone-less Earth (Sec. 5) and the organic chemistry of Saturn's moon Titan as an analog to both organic aerosol production on the early Earth (Sagan and Thompson 1984; Coustenis and Taylor 2000) and hydrogen-loss mechanisms that may help account for the net oxidation of early Earth's surface. The latter two possibilities will be examined in the research proposed in this section, and the relevance and limitations of these Titan-Earth analogs explicitly probed. Slow oxidation of Earth's surface by hydrogen loss due to aerosol formation will supplement the mechanisms considered in Sec. 2 of this proposal, emphasizing that there were evidently a suite of geological, atmospheric, and perhaps biological mechanisms acting on early Earth—and probably on many Earth-like worlds—that would have driven the environment towards more oxidizing conditions, with the implications for metabolism and, ultimately, aerobic life previously discussed in Sec. 2.

3.1 Titan Overview

Models of the accretion of Titan indicate that it formed from an ammonia-methane rich circumplanetary nebula that went on to condense into Saturn and its other satellites (Prinn and Fegley 1981). As a result, Titan is expected to have developed a layered structure containing mixed ices and silicates in the core, with an overlying silicate carapace and a deep ocean of ammonia-water liquid (Sagan and Thompson 1984). Titan's surface, most probably composed of water ice coated in accumlated hydrocarbons and nitriles, is seen by many as a prebiotic chemical laboratory (see

Lunine and McKay 1995). The action of ionizing radiation (Sagan and Thompson 1984), of lightning (Borucki et al. 1984), and of meteorite impacts (Jones and Lewis 1987; Thompson et al. 1992) is likely to have driven an exotic prebiological chemistry, perhaps as far as the amino acid or nucleotide base stage (Fortes 1999). The absence of water provides a serious stumbling block to the formation of biological molecules. However, impact ejecta are expected to contain large quantities of water. Thompson et al. (1992) calculated that around 70% of Titan's organic inventory has been exposed to impact melted water for mean periods of around a century. This may have yielded a range of organic compounds by hydrolysis of tholins (radiation-processed organic solids), although the products would inevitably freeze again, so that potential biological activity on Titan's surface is likely to be trapped in a permanent stasis. On early Titan, hydrothermal reactions between the ocean and the underlying rock layer likely reprocessed a portion of the ocean, plus infalling cometary and chondritic material, into more complex organic compounds (Shock and McKinnon 1993). Significantly, Titan is unique as the only moon in our Solar System that formed or retained a thick atmosphere. This atmosphere contains mainly methane and nitrogen components that have been energetically processed via UV radiation and cosmic ray particles into a complex organic haze in its stratosphere.

3.2 Titan Haze Synthesis and Prebiology

Titan's haze dominates its temperature, atmospheric circulation and climate control. Photochemistry plays a key role in the structure and evolution of the haze. A detailed knowledge of Titan's photochemistry therefore provides a deeper understanding of its global properties. A comprehensive study of organic chemistry and the resulting hydrocarbon haze in a theoretical simulation of Titan's atmosphere provides vital information on similar processes that occurred on early Earth (Sagan and Chyba 1997, Miller et al. 1998, Pavlov et al. 2001a,b). Titan also provides a partial early Earth analog with its reducing atmosphere resembling primitive terrestrial atmospheric chemistry. We have made significant inroads into bridging the longstanding gap in knowledge of Titan's haze photochemistry between simple molecules and complex polymers (Ricca, Bauschlicher and Bakes 2001; Bakes, McKay and Bauschlicher 2002; Lebonnois et al. 2002a,b;2003; Bakes et al 2003). We have also constructed the first ever Titan aerosol charging model to account for photoelectric charging. Bakes et al. (2002a) have shown it is essential to consider this mechanism, highlighting how daytime and nighttime (or Summer and Winter) haze chemistries are quite different. Knowing the charge on macromolecules in the haze forms a solid foundation for a truly representative chemical network. Our charging model has also produced spinoffs resulting in the revision of seminal models of charging (Borucki et al. 1987) and aerosol growth and albedo (Toon et al. 1992) for the Titan haze. In addition, we have included the effects of aerosol charging and macromolecular catalysis of H_2 formation (Bakes et al. 2002b) and inserted this treatment into the Titan photochemical model of Lebonnois et al. (2002a,b). The results of our models are applicable to the hydrocarbon haze postulated to exist in the atmosphere of early Earth (Pavlov et al. 2002a,b) and the hydrogen catalysis is directly relevant, producing H_2 efficiently to fuel methanogenic and anoxygenic photosynthetic bacteria and to provide a physical mechanism by which to efficiently transport unreactive H_2 out of the stratosphere to facilitate the formation of a primitive oxygenated atmosphere.

The applications of this kind of chemistry are therefore wide-ranging and inform: (1) early terrestrial prebiology; (2) impact of icy bodies on planetary or satellite surfaces' (3) distant past or far future scenarios concerning the chemical evolution of Titan's atmosphere and how the physical conditions under which the aerosols formed and reacted with water and ice affect the variety of amino acids synthesized; and (4) evolution of the atmosphere of the early Earth.

3.3 Terrestrial Prebiotic Chemistry

What comparisons can we draw between a prebiotic Earth and present day Titan? If we make the assumption that significant liquid reservoirs are present on Titan, we can draw comparisons between the land-air-sea interactions on both planets (Fortes 1999). On prebiotic Earth, the dominant radiative gas was likely (but not certainly) carbon dioxide (Kasting, 1984), which provided most of the atmospheric opacity. On present-day Titan, nitrogen serves this purpose. Both of these gases are

also somewhat soluble in their respective oceans: carbon dioxide in water and nitrogen in methane. Both planets had large liquid seas at the surface, which were intimately related to their atmospheres. Both water on prebiotic earth and methane on present-day Titan are not insignificant species in the atmosphere, having an influence on the chemistry and ultimately the evolution of the planet (Lunine and McKay, 1995). Titan's haze would have a significant interaction with potential liquid reservoirs on the surface. The aromatic structures in the Titan haze can be hydrolysed to form amino acids (Khare et al. 1986). In essence, this prebiological chemistry to some extent parallels early terrestrial chemistry in a reducing or neutral atmosphere. We suspect that the aerosols in early Earth's atmosphere would form via similar chemical mechanisms as those in the Titan haze, incorporating nitrogen into a carbon based aromatic structure. The presence of water on Earth would facilitate hydrolysis of these aerosols to form amino acids and could provide a significant source of building blocks for the origin of terrestrial life. In summary, as a result of defining the quantum chemical mechanisms by which macromolecules and aerosols form on Titan, we can extend this chemistry to parallel that of the early Earth by introducing the crucial step of hydrolysis and subsequent amino acid formation.

3.4 Icy Body Impact Chemistry

The hydrolysis of nitrogenated aromatic macromolecules in both water and an icy matrix could occur by impact-driven, shock-heated chemistry to produce amino acids. Pierazzo & Chyba (1999) have recently shown that the survival of cometary amino acids is plausible for large cometary impacts. Blank (2000?, and private communication) has found that when two icy bodies impact one another, that if one or both bodies contain amino acids, peptides are formed. If an icy body impacted Titan, it would already contain aromatics of the sort that form via atmospheric gas phase chemistry in the Titan haze (reference to comet/meteor composition needed). When impact occured, the formation by shock-heated chemistry of both amino acids and peptides may result, providing a source of prebiological molecules. Since Pierazzo and Chyba (1999) agree that amino acids may plausibly survive large cometary impacts, then the complex organic chemistry which could occur via this kind of impact may be highly relevant to present day Titan as well as the Titan of the past.

3.5 Distant Past and Far Future Scenarios

During the first one ~10^8 yr of Titan's history, there was a warm (300K) aqueous environment with abundant energy sources at Titan's surface (Thompson et al. 1984; McKay et al. 1993; Lunine & McKay 1995). This may be the closest Titan is ever likely to have come to the primitive terrestrial environment in which life is thought to have formed (Fortes 1999). In the absence of evidence to the contrary, we may assume that if there ever was life on Titan, it originated in the same way as it did on Earth, plausibly through a period of abiological chemogeny (Fortes 1999), yielding the building blocks of proteins and DNA (Laczano and Miller 1996; Lifson 1997; Freeland et al. 1999). The main change in Titan's atmosphere over time is most likely to have been due to changes in temperature and the reduction in the amount of methane CH_4 (McKay et al. 1993). This would affect both the rate of formation and the subsequent charge and agglomeration rate of the aerosols. Since we expect aerosols to have formed in this period of Titan's history, we can predict synthesised products via hydrolysis of these aromatics in a potentially warm aqueous environment. The abundance and type of amino acid synthesized may well vary over time due to the changing abundance of CH_4, which alters the aerosol formation rate and the temperature and UV radiation field transmitted to the lower atmosphere. We are well placed to quantify this effect via the modification of the photochemical model of Lebonnois & Toublanc (2000) into a time dependent mode.

Turning to the far future scenario, Khare et al. (1986) point out that while life on Titan is hardly to be expected at its current ambient surface temperature of 95 °C, a few billion years from now, the Sun will enter its red giant phase and for~10^8 yr, the surface temperature of Titan will be above the freezing point of water and chemical evolution with go further towards prebiology. We can take the present day haze composition, containing macromolecules and tholins and model the effects of an emerging liquid environment to see whether the rate of amino acid formation would be significant. The acceleration of prebiology in Titan is an exciting theoretical concept and

would further our understanding of precisely how mixing carbon-based aerosols, nitrogenated aromatic hydrocarbons and water may have kick-started terrestrial prebiology.

Moreover, we can make a detailed theoretical analysis (using quantum chemical models and the state of the art Titan photochemical model of Lebonnois & Toublanc 2001) of plausible aerosol hydrolysis chemistry. In addition, the model of Lebonnois & Toublanc may be enhanced by the addition of a time-dependent component that allows us to track the chemical evolution of Titan's atmosphere as its composition changes with time.

In summary, the building blocks of life may change in their compositon and abundance with time, affecting the evolution of prebiology and the eventual emergence of biology. This time-dependent evolution of life at the molecular level may have considerable ramifications concerning the probability of the origins of life on a particular planetary body or satellite. We have all the capabilities right now to investigate this time dependence using a synergy of laboratory experiments and quantum photochemical models of Titan's haze.

3.6 Oxidizing Earth's Surface

Our current research combines the photochemical models of Lebonnois et al. (2002a,b) with the ab initio photoelectric charging model of Bakes et al. (2002a), the hydrogen formation model of Bakes et al. (2002b), the quantum chemical studies of Baushlicher and Ricca (2000) and Ricca et al. (2001) with the laboratory analyses of Khare et al. (2001; 2002). Our results are directly applicable to haze chemistry involving large hydrocarbon molecules. The chemical and thermodynamic processes fuelled by these hydrocarbon molecules is strongly relevant to the reducing or neutral atmosphere of the early Earth (Pavlov et al. 2001a,b). A terrestrial atmospheric organic haze layer is an effective source of gaseous heating via the UV-powered photoelectric effect (Bakes et al. 1994; Bakes et al. 2002a). Bakes et al. (2002a,b) also find that aerosol surface catalysis is an efficient producer of H_2. It is logical that any physical mechanism facilitating the efficient escape of H_2 from the atmosphere of the early Earth would accelerate the oxygenation of the early atmosphere. This is precisely the effect of both aerosol surface catalysis and charged macromolecule catalysis of H_2 formation, be it for Titan's haze or a putative haze on early Earth (Bakes et al. 2002b).

Our models can shed light on the chemical transition from a reducing to an oxygenated atmosphere and they can predict how significant the photoelectric heating from a terrestrial haze would be relative to other sources of heating on the early Earth (Bakes et al. 1994; Bakes et al. 2002a). Our initial studies would concern the charging, growth and albedo of a haze layer in the atmosphere of the early Earth. This will be done for a range of plausible early solar radiation fields to probe how critical the strength of the UV radiation would be in affecting the thermodynamic processes and possible photoelectric heating in the haze. Once a foundation is formed for the charging and heating via haze macromolecules and submicron aerosols, we can make broad predictions about the dominant gas phase and aerosol chemistry. We will estimate the rate of escape of H_2 from the early terrestrial atmosphere via heterogeneous and macromolecular catalysis of H_2 formation. We can gauge its relative importance by comparing our H_2 production and escape rates with those estimated for the transition period between a reducing and an oxygenated early terrestrial atmosphere.

It is straightforward to adapt our existing models (Bakes et al. 2002a,b) to conditions more appropriate to the early Earth in order to study primitive terrestrial haze chemistry, charging and thermodynamics. This will be done by simply altering the altitudinal variation of the UV radiation field, electron and positively charged particle density and gas temperature to match estimates for the early Earth (Canuto et al. 1983; Sagan and Chyba 1997, Heap et al. 2001). Sagan and Chyba (1997) (due to abiotic sources) and Pavlov et al. (2001a,b) (due to biotic sources) predict the presence of a hydrocarbon haze in the early terrestrial atmosphere that may be similar to Titan's haze. We predict that as with the Titan haze (Bakes et al. 2002a,b; Lebonnois et al. 2002a,b), the photoelectric charging of aerosols and the effects of aerosols on H_2 formation will have a significant effect on the haze chemistry and thermodynamics.

3.7 Goals of this Study

Support under this proposal will allow us to adapt the current state-of-the-art photochemical model of Lebonnois and Toublanc by add-

ing: (1) the aerosol charge balance model of Bakes, McKay & Bauschlicher (2002); (2) a time dependent mode involving the Gear Routine to solve stiff differential chemical rate equations; and (3) quantum-chemical calculations involving Titan haze macromolecules on icy surfaces. This would enable us to predict the likelihood of amino acid formation coupled to the chemical composition and time dependent evolution of the haze, from Titan's distant past, through the present epoch, to a far future scenario (time permitting).

Work on the formation of the Titan photochemical haze can be seen as a parallel to aerosol formation in the atmosphere of early Earth. Not only does the formation of the Titan photochemical haze shield the lower layers of the atmosphere from ionizing UV radiation, but synthesized aerosols can also provide the raw materials for the synthesis of the building blocks of life. Furthermore, icy body impacts with Titan may help synthesise peptides, further increasing the complexity of the potential prebiological chemistry. By investigating the formation of amino acids and the chemistry of nitrogenated aromatics such as those formed in the Titan haze in aqueous and icy environments, we can derive broad principles for the general formation of prebiology that we can and will relate to early terrestrial chemistry. Because chemistry runs by the laws of physics, we hope to thereby derive generalized formation mechanisms that can be applied to a diverse array of planetary environments.

3.9 Investigative Timeline

Dr. Emma Bakes is the Co-Investigator primarily responsible for this research, and will devote 25% of her time over the next five years to this effort. Year 1: Implement a quantum chemical investigation of the addition of successive aromatic rings to neutral and charged two-ring molecular structures to confirm a growth trend requiring successively less energy with each additional ring. This chemical pathway will be added to an existing photochemical model of the Titan haze to study its effects on the gas phase chemistry and molecular abundances. A detailed analysis of the photochemical evolution of our large molecules and aerosols with altitude in Titan's atmosphere will be performed. Year 2: Adapt our infrared spectral models to predict the macromolecule emission at relevant altitudes for Titan in order to predict the spectral profile between 2 and 100 μm and to explain data at relevant wavelengths yielded by the Huygens probe. We will also make a user friendly version of our charging routines and our infrared spectral model available for analysis of Cassini/Huygen data. Year 3: Apply the results of the previous two years to the early Earth scenario. Haze chemistry, aerosol growth, agglomeration, corresponding changes in albedo, photoelectric heating (which affects the thermodynamics of a gaseous system) and catalytic H_2 production mechanisms will be investigated for possible thermal and chemical impact on a primitive terrestrial atmosphere and its evolution towards a progressively less reducing environment. Year 4: The implications of the results for the early earth will be analyzed, interpreted and modified to fit the evolutionary timeline of the early terrestrial atmosphere. We will work on modifying the codes to fit a time-dependent Gear routine computational analysis. This will allow us to tweak results over time as well as with respect to atmospheric composition and solar UV field. Year 5: Writing and publication of the results yielded from our previous analysis of the early terrestrial atmosphere, and the incorporation of relevant results from other groups working on the early Earth into our computational models.

The work proposed here will address Goals 2 and 6 of the Astrobiology Roadmap.

4. Iron, the Oxygen Transition, UV Shielding, and Photosynthesis

Iron is geologically abundant, has multiple oxidation states, and is a key metal for life because of its role in many metabolic processes often involving oxygen. Banded iron formations (BIFs), alternating layers of iron-rich and iron-poor minerals, are found on the early Earth and include those dominated by oxides, siderite (iron carbonate), silicates, and sulfides (James 1954; James and Sims 1973; James and Trendall 1982). The bulk of these formed fairly rapidly around 2.5-2.0 Gyr ago, although many formed earlier than 3 Gyr ago (Holland 1984).

One of the unanswered questions about the early Earth is why atmospheric O_2 first rose to higher levels ~2.3 Ga when oxygenic photosynthesis existed much earlier, 2.5 to possibly even 3.8 Ga (Des Marais 2000; Olson 2001; Pace, presentation at the NAI biennial meet-

ing, Feb. 2003). Recent studies have suggested that methane (Catling et al. 2001), sulfur (Kasting et al. 1989) or iron (Konhauser et al. 2000) could have influenced biogeochemical cycles on the early Earth; Secs. 2 and 3 of this proposal consider other possible mechanisms for surface oxidation. Reduced sulfur and/or iron are thought to be the first electron donors available for early organisms and may have been involved in early photosynthesis (Olson 2001). Some or all of these processes may help explain why the oxygenic photosynthesis arose so much earlier than the rise in abundance of atmospheric O_2. The oxygen produced by early photosynthesis (and other mechanisms) may have been used up oxidizing localized deposits of reduced sulfur and/or iron associated with the organisms, thus requiring more time to reach levels sufficient to impact the atmosphere.

Before the buildup of O_2 in the atmosphere, the UV flux to the early Earth was at least as high as it is today, and most likely substantially higher. This is because while the Sun was younger, solar UV radiation from 200 to 320 nm was able to penetrate the ozone-poor early atmosphere. The UV flux from the early Sun (before 3.5 Gyr ago) at these wavelengths would have been only a factor ~2 lower than the solar UV flux today (Chyba and Sagan 1992). Since the peak absorption for DNA is 260 nm, and that of proteins 280 nm, an increase in solar radiation in the 200 to 320 nm range would have been extremely hazardous to early life, especially forms that would have to access solar energy such as photosynthetic organisms (e.g., Rothschild 1999).

We *hypothesize* that evolution of some early photosynthetic organisms is related to the presence of nanophase ferric oxide minerals and BIF deposition during this time period because of the sunscreening capabilities of ferric oxides/oxyhydroxides. *We will test this hypothesis* in the laboratory and in the field by: (1) determining whether these minerals can provide a favorable environment for photosynthetic organisms by supplying the necessary protection at UV wavelengths, while allowing sufficient transmittance at visible wavelengths for photosynthesis; (2) determining which ferric oxides/oxyhydroxides/ oxyhydroxysulfates are active *in situ* where organisms grow; (3) evaluating the performance of imported ferric oxide minerals as a UV radiation shield; and (4) determining the potential for ferric oxides/oxyhydroxides/oxyhydroxysulfates to screen UV where it occurs in nature today.

We will use these data to inform the search for life in other high radiation environments where iron could act as a sunscreen, most importantly on Mars. As described in Sec. 5, we will be able to compare these results for those implicated in the UV strategies employed by microorganisms in high UV environments on contemporary Earth. Perhaps some of the early photosynthetic organisms synthesized their own iron oxide sunscreen, or utilized the iron oxides already present as a sunscreen. Both the solar energy filtration and the oxygen-scavenging ability of these minerals may have been beneficial to these organisms. Our results should provide data for modeling the early reducing and oxidizing chemistry on planets and may help explain whether or not links exist between early phototrophs, BIFs and the oxidizing atmosphere on Earth. Additionally, the project may provide information about the role of such minerals in the protection of modern aquatic organisms from UV radiation or oxidative damage, an area of particular interest to the near-term future of life on planet Earth.

4.1 Significance

This project is inherently interdisciplinary, and involves a novel approach to evaluating the relationship between organisms and their environment. It requires a combination of physics (reflection and absorption of radiation as a function of composition and abundance), geology (mineral identification and formation processes), chemistry (pH, temperature and presence of ions/salts in the environment), and biology (organisms' survival needs).

The fundamental question of this project focuses on how early organisms evolved in a harsh physical environment. This study is expected to lead to a better understanding of how organisms on Earth were able to live in a world bathed in UV radiation, while still accessing the visible radiation (400 to 750 nm) so critical for photosynthesis. Specifically, it will examine the relationship between the formation of nanophase ferric oxide minerals on the early Earth and their impact on the evolution of life at the critical juncture of the transition from anoxygenic to oxygenic photosynthesis. If these iron oxide minerals can provide adequate coverage for primitive photo-

synthetic organisms, then a link between the survival of these organisms and the formation of nanophase ferric oxides/oxyhydroxides/ oxyhydroxysulfates may exist.

This may also inform future Mars missions. Water on Mars is thought to have played a prominent role in the formation of the hematite deposit in Sinus Meridiani (Christensen et al. 2000, 2001) and in the formation of gullies through seepage and surface runoff (Malin and Edgett 2000), and where evidence of subsurface water ice has been observed recently by the GRS on Mars Odyssey (Boynton et al. 2002; Feldman et al. 2002). Mars Express will soon be orbiting the planet and the Omega spectrometer will be collecting visible-infrared spectra (0.5-5.2 µm) at surface resolutions as low as 300 m across. The CRISM spectrometer (0.4-4 µm at 100-200 m or better spatial resolution) will be flying on the Mars Reconnaissance Orbiter (MRO) scheduled to launch in 2005. These instruments are expected to provide evidence of clays, carbonates, silicates and iron oxides if they are present near the surface. Perhaps some of the smaller, pixel-sized, hematite deposits identified by TES on Mars also contain other minerals that are commonly associated with BIFs. Understanding the visible/infrared spectral character of natural and synthetic ferric oxide-bearing systems capable of screening UV radiation for phototrophs will be important as we evaluate the new spectral data returned from Mars Express and the MRO.

The work proposed here addresses Goal 4, Objectives 4.1 and 4.2,and Goals 2, 5 and 6 of the Astrobiology Roadmap.

4.2 Extending Current State of Knowledge

In addition to its sunscreening capability, iron is an integral component in the evolution of life. Although iron is the fourth most abundant element on the Earth's crust, it is frequently a limiting factor in biological systems (Falkowski et al. 1998) because the bioavailability of iron depends on its redox state and solubility (Martin 1992). The iron balance between organisms and their environment can be viewed as a web, where multiple processes and systems are interlinked and interdependent. The traditionally accepted geologically controlled anoxic-oxic transition (e.g. Des Marais 1992) may be sufficient to explain the processes shaping the evolution of early Earth. However, if the possibility exists that biologically mediated processes also played a role in the rise of oxygen, it could be that a combination of geological and biological processes is crucial in the evolution of habitable worlds.

Photosynthesis was a critical evolutionary breakthrough; however, photosynthetic organisms faced a dilemma. On the one hand, they needed access to solar radiation for energy; on the other, the UV rays were extremely damaging. Despite our current atmosphere, which attenuates most radiation below 300 nm, ultra violet radiation (UVR) causes DNA damage to life. Without the ozone layer, UVR would have posed a substantial burden to early life. Photosynthesizers were forced to protect themselves either through UV damage repair or by finding a shield from harmful UVR that still transmits photosynthetic radiation (~400-900 nm). Of primary importance is a filter at ~250-290 nm, where DNA and protein absorptions occur (e.g., Rothschild 1999). Many kinds of materials could have blocked UVR on the early Earth including iron (Olson and Pierson 1986; Pierson et al. 1987), ferric oxide minerals (our suggestion), sulfur vapor in the atmosphere (Kasting et al. 1989), a photochemical haze (Sagan and Chyba 1997, Miller et al. 1998; see also Sec. 3), and a combination of inorganic and prebiotic organic compounds in the oceans (Cleaves and Miller 1998; Cockell 2000). Sagan and Pollack (1974) suggested that a depth of ~1 cm below the surface on Mars would be a possible photosynthetic niche, allowing visible light to penetrate while reducing the UVR to acceptable levels.

Ferric oxides are unique among the range of possibilities in that they transmit light through much of the visible region, it is universally accepted that they were available in early environments on the Earth, and they are synthesized by many organisms. Thus, *we suggest here that ferric oxides may be critical in the early evolution of a life-supporting planet as a sunscreen that allows the evolution of photosynthesis prior to the accumulation of sufficient stratospheric ozone.* We propose several experiments to test our hypothesis that the evolution of some early photosynthetic organisms is related to the presence of nanophase ferric oxide minerals and BIF deposition. Specific objectives of these experiments include determining the influence of selected nanophase iron oxide minerals on living photosynthetic organisms through controlled UV ab-

sorption experiments in the lab, and identifying the nanophase iron oxide minerals forming in natural environments such as Iron Springs near Nymph Creek, Yellowstone National Park, where we believe the iron oxide minerals are assisting the organisms.

BIFs have been associated on Earth with a major transformation in the redox environment of the oceans and atmosphere, the kinds of organisms present, and the surface mineralogy (e.g. Holland 1984; Beukes and Klein 1992; Des Marais 1997; Holland 1999; Catling and Moore 2000). Ehrenreich and Widdel (1994) recently summarized three likely mechanisms of BIF deposition. These include (1) chemical oxidation of dissolved Fe(II) by oxygenic phototrophs (e.g. cyanobacteria) in oxygenated ocean water, (2) abiotic UV light-driven aqueous oxidation of Fe(II) giving off H_2, and (3) biological oxidation of Fe(II) by anoxygenic photosynthetic organisms. Localized, ancient iron formations are associated with hydrothermal activity in many locations documented by Gross (1983), as well as small, current sites in Yellowstone (Pierson et al. 1999) and Iceland (Bishop and Murad 2002). Such sites on the early Earth may have provided a convenient habitat for early photosynthesizers. Holland (1984) supports volcanism as a source of many smaller BIFs, especially those associated with volcanic features, but argues that volcanic processes are unlikely to be responsible for many of the large BIFs because of the enormous volume of iron-rich material and relatively short formation time for the bulk of the iron formations.

Perhaps the ferric oxide/oxyhydroxide minerals that formed the smaller BIFs enabled the then new photosynthesis mechanism to become more widespread. It is likely that oxygenic photosynthesis occurred at least 600 Myr prior to the time of high atmospheric O_2 levels (Des Marais et al. 1992). The negative influence of solar radiation may have initially hindered the evolution of photosynthesis, until appropriate ferric oxide sunscreening niches were found. If our hypothesis is correct, then the presence of nanophase iron oxides could have significantly enhanced survival rates of photosynthetic organisms, thereby linking iron oxides to an increase in atmospheric O_2 levels.

The Thermal Emission Spectrometer (TES) on board the Mars Global Surveyor (MGS) mission has provided spectral data that enabled Christensen et al. (2000, 2001) to identify a large deposit of crystalline, gray hematite on Mars in Sinus Meridiani. They evaluate possible formation mechanisms for this deposit and suggest formation via precipitation of Fe-rich hydrothermal fluids as the most likely process. Controversial evidence for large bodies of water on Mars in the past (Baker et al. 1991; Head et al. 1999), plus recent evidence for surface water on Mars (Malin and Edgett 2000) support the possibility that BIFs might have formed on Mars, as well as Earth. Additional smaller regions have been identified on Mars where crystalline, gray hematite is present at 20% or more abundance levels (Christensen et al. 2001). If these aqueous iron oxide deposits on Mars are associated with BIFs and/or redox changes in the atmosphere on Mars, it would be interesting to consider the possibility of photosynthesis as well. The idea that Mars has experienced more recent hydrological processes than previously thought was inspired by Malin and Edgett (2000).

Burns (1993) first suggested the possibility of abiotic BIFs on Mars in the form of dessicated iron oxide/silica deposits. Schaefer (1996) discussed the potential formation of BIFs on Mars through several abiotic mechanisms including oxidation of ferrous iron by free oxygen, photooxidation of ferrous iron, oxidation of ferrous iron by H_2O_2 and anoxic precipitation of ferrous iron by changes in pH. More recently, Calvin (1998) suggested that BIFs on Mars may have precipitated ferrous clays and other dark ferrous minerals and provided a model to explain the dark regions on Mars through a BIF-like mechanism.

Iron has been linked to the evolution of photosynthesis and the origin of BIFs (Cloud 1973; Pierson and Parenteau 2000). Cloud (1973) asserts that ferrous iron in solution served as a sponge for the biologically generated oxygen that was formed as a waste product of photosynthesis and which would have been toxic to life if it accumulated. According to Cloud's model, the formation of ferric oxides kept O_2 levels sufficiently moderate for photosynthesizers to live and grow. Recent studies have shown that some forms of iron stimulate photosynthesis (Pierson 1994; Pierson et al. 1999; Pierson and Parenteau 2000) and earlier studies showed that iron could be important in protecting the organisms from UV radiation (Olson and Pierson 1986; Pier-

son and Olson 1989). Related studies (Emerson and Revsbech 1994a,b; Pierson et al. 1999; Pierson and Parenteau 2000) have shown that organisms are intimately clustered with amorphous or nanophase iron oxides on the scale of a few µm. Ferric oxide-bearing minerals that are produced via biologically mediated processes are typically nanophase in scale (~1-10^2 nm in size). This small size would have facilitated the formation of niches for microorganisms because of the high surface area. If nanophase ferric oxides did provide a protective niche for photosynthetic organisms, this could have allowed for a much more rapid evolution of these organisms than otherwise possible and would have implications for the anoxic-oxic transition on Earth.

In a pre-Viking assessment of the possibility of life on Mars, Sagan and Lederberg (1976) argue that liquid water is the most stringent limiting factor. They suggested that the low oxygen partial pressure would be a negligible impediment to life and that the UV flux could have been countered by living below the surface or by developing a UV-absorbing exoskeleton or shield (that might also be able to conserve water). Such speculation has been largely forgotten since the lack of strong evidence for life by the Viking biology experiments (e.g. Klein 1978). Weiss et al. (2000) recently voiced the availability of liquid water, rather than energy or other factors, as the most important limiting factor for organisms on Mars. With the recent geomorphologic evidence supporting frozen aquifers and some liquid water on Mars (Malin and Edgett 2000; Christensen 2003), it may be timely to re-evaluate the possibility of life on Mars, and the possible niches that may be available now (or in the past) for life on Mars-like planets. In a recent summary of a variety of chemical energy sources for bacteria, Nealson (1997) described ecosystems on Earth that may have been possible on Mars (or other planets) at one time in the past. These include Antarctic endoliths or dry valley lakes, hydrothermal regions, and deep subsurface ecosystems. If our hypothesis that nanophase ferric oxides could protect photosynthetic organisms is correct, then Mars-like planets with abundant ferric oxides and some liquid water may be able to support life.

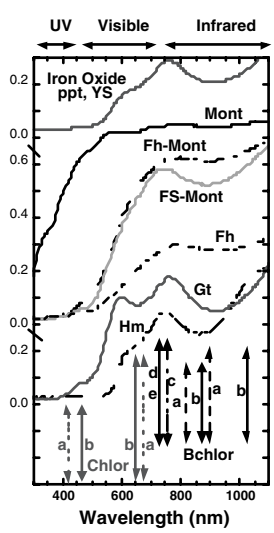


Fig. 4.1 Optical properties of a Yellowstone precipitate, clays and iron oxides in relation to chlorophyll absorptions. Spectra are shown for montmorillonite (mont), ferrihydrite-montmorillonite aggregates (Fh-mont), ferrihydrite-schwertmannite-montmorillonite aggregates (FS-mont), ferrihydrite (Fh), goethite (Gt), and hematite (Hm). Approximate pigment absorption energies are indicated for chlorophyll a and b, and bacteriochlorophyll a, b, c, d, e.

The optical properties of iron oxide minerals vary substantially depending on the mineral structure (e.g., Bishop et al. 1995; Bishop and Murad 1996). The nanophase minerals ferrihydrite and schwertmannite exhibit UV-visible spectral properties that allow transmittance at chlorophyll absorption energies, while blocking the damaging UV rays. These minerals are also frequently formed in the presence of the *Thiobacillus ferrooxidans* (for schwertmannite, pH 2-3.5) and *Gallionella ferruginea* (for ferrihydrite, pH ~7-8), although inorganic formation mechanisms also exist (e.g., Murad et al. 1994; Bigham et al. 1996; Cornell and Schwertmann 1996). Other minerals, such as hematite and goethite, may also serve as effective sunscreens; however, these typically form in larger crystalline grains and are not as frequently associated with soils and microorganisms. Ferrihydrite is the third most prevalent biogenically produced mineral on Earth (Lowenstam 1981).

At Yellowstone National Park iron oxide precipitates, or sinters, are frequently found in the water or on the shores at hydrothermal sites in conjunction with photosynthetic organisms. Extended visible region reflectance spectra are shown in Fig. 4.1 for an orange precipitate collected from the banks of Nymph Creek, where UV radiation studies have been performed (Rothschild et al., work in progress). As shown through the low reflectance (high absorbance) at short wavelengths in Fig.

4.1, radiation is blocked by the precipitate at wavelengths shorter than ~500 nm, but allowed to penetrate at longer wavelengths. For comparison, the extended visible region reflectance spectra of selected iron oxide/oxyhydroxide/ oxyhydroxysulfate minerals are shown as well (from Bishop et al. 1995; Bishop and Murad 1996). Hematite and goethite are crystalline minerals, while ferrihydrite (ferric oxyhydroxide) and schwertmannite (ferric oxyhydroxysulfate) are nanophase minerals and exhibit broader spectral bands. Schwertmannite contains sulfate, whereas the others contain only Fe, O, OH and/or H_2O. The absorption bands for chlorophyll *a* and *b* (Salisbury and Ross, 1992) and the chlorophyll found in green and purple bacteria (Stanier et al. 1986) are indicated in Fig. 4.1 for comparison. A recent study of organisms at Yellowstone by Wilson et al. (2000) has shown that Fe(II)-Fe(III) cycling is linked to H_2O_2 concentration and both may be important environmental factors in pigmentary grains.

Preliminary spectral measurements of suspensions of nanophase ferrihydrite-clay-aggregates (Fh) and ferrihydrite-schwertmannite-clay-aggregates (Fh/Sw) have been performed and the spectral absorbance of variable concentrations of these pigmenting agents are shown in Fig. 4.2. This shows that these suspensions should mask some of the harmful UV rays while allowing some visible light to pass through for photosynthesis. Growth experiments with organisms are required in order to determine if sufficient light at critical energies penetrates through the organism growth.

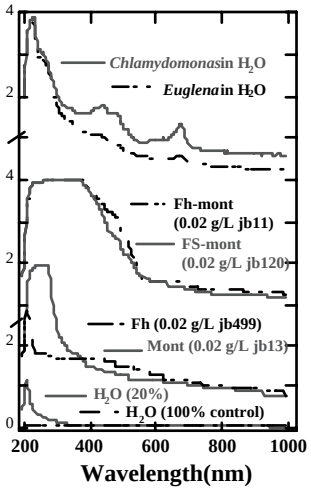


Fig. 4.2 UV-VIS absorption spectra of nanophase iron oxide suspensions with variable concentrations.

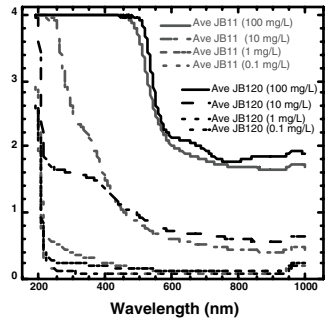


Fig. 4.3 Absorbance of Euglena and Chlamydonomonas in suspensions. The spectral absorbance due to chlorophyll in these organisms is clearly observed near 450 and 670 nm.

Preliminary tests of the toxicity of the Fe performed on active cultures of two photosynthetic protests, *Chlamydomonas* and *Euglena*, showed that this is not a problem. Test measurements were also performed to ensure that the chlorophyll spectral features can be observed and tested for organisms grown in iron oxide suspensions. Shown in Fig. 4.3 are absorbance spectra of several suspensions of *Chlamydomonas* and *Euglena* in media plus H_2O and the nanophase iron oxides/clay aggregates from Fig. 4.1. These spectra show that the chlorophyll pigments can be clearly seen above the spectra of the iron oxides suspensions.

4.3 Approach and Methodology

This project sounds fairly intuitive, leading to the suspicion that this work must have been done before. However, extensive review of the literature has shown that others have been primarily concerned with the abundance of iron (Pierson and Olson 1989; Pierson 1994; Wilson et al. 2000) and morphology of iron oxide-organic clusters (e.g. Emerson and Revsbech 1994a,b; Pierson et al. 1999; Pierson and Parentau 2000). Previous investigations (Olson and Pierson 1986; Pierson et al. 1987; Pierson and Olson 1989; Pierson 1994) have evaluated the transmittance of light through microbial mats and organic sediments in order to determine that some of these natural materials were effective in blocking the damaging UV rays with as little as 1 mm depth and that the spectral properties of the mats are an im-

portant aspect of the organisms' environment. However, little consideration has been given so far to the mineral structure of the iron oxides, or the impact of these minerals on the spectral environment of the organism. This study is thus critical in its ability to link studies of early evolution on the Earth with the search for life elsewhere.

Our proposed projects include UV transmission experiments in the lab and in the field. Some of the lab experiments will require synthesis and characterization of fresh iron oxide/oxyhydroxide minerals. The field experiments will require collection of the *in situ* iron oxide/oxyhydroxide minerals for characterization.

4.4 Outline of Proposed Tasks

Task 1: Determine whether these minerals can provide a favorable environment for photosynthetic organisms. We will test the iron oxide/oxyhydroxide minerals ferrihydrite and schwertmannite (in a clay matrix) and freshly prepared iron oxide/oxyhydroxide minerals as UV radiation shields for photosynthetic organisms. This will involve whole organisms and DNA dosimeters and take place in the lab and in a special environment on the roof of the lab building. Task 2: Determine which ferric oxides/ oxyhydroxides/ oxyhydroxysulfates are active *in situ* where organisms grow. We will run UV transmission experiments in naturally occurring bodies of water high in iron. First among these is in Nymph Creek and adjacent iron springs, Yellowstone National Park. Other likely sites include Rio Tinto (Spain), Iron Mountain (California), and Landmannalaugar (Iceland). We will test the already present iron oxide/oxyhydroxide minerals as UV radiation shield for *Cyanidium* and other photosynthetic organisms, collect samples of the iron oxide surface layers for characterization, and compare growth rates using fresh iron oxide/oxyhydroxide minerals and *in situ* iron oxides. Task 3: Analyze spectral, chemical and biological properties of iron oxides, organisms and their environments. Compare spectral character in UV, visible and near-IR regions of synthetic iron oxides and iron-bearing components of mats. Evaluate differences in spectral and chemical factors with respect to the organisms used.

4.5 Detailed Descriptions of Proposed Tasks

Task 1**.** Growth experiments will be performed using representatives of an ancient photosynthetic taxa, including the cyanobacterium *Synechococcus*, and eukaryotes including the red alga *Cyanidium*, the green alga *Chlamydomonas*, and the mixotrophic protist *Euglena*. Cyanobacteria are ancient organisms that first invented oxygenic photosynthesis, so are likely to have been prominent prior to the formation of the banded iron formations. The other three organisms are already in culture in Dr. Rothschild's lab. The *Cyanidium* is critical because it is found in and around the Iron Spring in Yellowstone. Dr. Rothschild has 10 years experience working with the primitive alga *Cyanidium* both in the lab and in the field. *Chlamydomonas* and *Euglena* are well known, easily grown, large and so can be easily seen in the microscope as distinct from the iron grains. Additionally, various mutants including those defective in DNA damage repair, are available in Rothschild's lab. *Euglena* is a mixotroph, in other words, it can either function as a heterotroph or as an autotroph.

Solar radiation experiments will be conducted by adding aliquots of freshly prepared media culture to several iron oxide/clay sample suspensions, letting them grow in the indoor incubator to mature cultures, then exposing them to natural solar radiation. Note that because the media used contain very minor amounts of organics (e.g., vitamin supplements) they themselves do not attenuate UV.) Samples will be enclosed in water-tight plastic bags, and floated in a constant temperature water bath cooled to 25 °C for several hours under direct sunlight. The total UV-A (mJ/cm2, 320 to 400 nm) and UV-B (µJ/cm2, 290 to 320 nm) exposure of the samples will be measured during these experiments using UV meters. Following the solar treatment the sample bags will be wrapped in opaque foil (to prevent further exposure to UV, visible or IR radiation) and returned to the incubator until the following exposure period. A temperature-controlled water bath is operating on the roof of our building under the direction of Dr. Rothschild. This system is designed for growing microbes in a constant temperature/solar radiation environment, and will be used for these experiments. Absorbance spectra will be measured periodically in order to monitor the growth of the organisms in the incubator. Samples will be collected following UV exposure period for measurement of spectral absorbance and de-

termination of cell counts under the microscope.

These organisms will be grown first in suspensions of the iron oxide minerals imbedded in clay matrices available from previous studies (e.g. Bishop et al. 1995). Additional growth experiments will be performed using the freshly-prepared ferric oxide minerals and the organisms described above. In these experiments the organisms will be grown in the presence of solar radiation and suspensions of the iron oxide minerals without clays or other silicates.

Synthetic ferric oxide/ oxyhydroxide/ oxyhydroxysulfate minerals will be prepared in the lab using techniques similar to those outlined in Schwertmann and Cornell (2000) and Cornell and Schwertmann (1996). These reactions will be performed by Dr. Bishop and the student/lab tech as in prior studies (Bishop et al. 1993, 1995). Synthesis of ferrihydrite, schwertmannite, goethite and hematite are planned initially. Depending on which minerals are identified in the field studies of Task 2, additional ferric oxide-bearing minerals will be synthesized.

The iron oxide/oxyhydroxide minerals will be characterized and their purity will be verified using visible/ infrared reflectance spectroscopy, bulk chemistry, and other techniques. The samples will be sent to the NASA-supported RELAB facility at Brown University under the direction of Carle Pieters for reflectance spectroscopy. These synthetic minerals will be sent out for chemical (X-ray Fluorescence) and mineralogical (X-ray Diffraction) analysis as necessary. As fine-grained samples of these minerals age with time, it is important to synthesize fresh minerals for experiments where the mineral structure and crystallinity are factors. Suspensions will be prepared with these freshly prepared iron oxides and the UV, visible and NIR absorbance spectra will be measured with a scanning spectrometer.

Task 2. Growth experiments will be performed *in situ.* The first site tested will be Iron Springs at Nymph Creek, Yellowstone, where iron oxide-bearing minerals are present in the water (Rothschild and Mancinelli 2001). The objective of these experiments is to determine how effective the local ferric oxides are in shielding UV radiation for the *Cyanidium*. These experiments will entail growing the local *Cyanidium* in their local water and nutrient environment in UV transparent containers: one set of containers will be solar UV-screened while the other will be exposed to the normal solar UV radiation. Visual inspection at the experiment site will provide qualitative analysis of the organism growth.

Several other sites that are known to be high in iron are being considered for further studies. One is the Rio Tinto river in southwestern Spain (e.g., Davis et al. 2000). Discussions with Juan Mercador of the Centro de Astrobiologia in Spain have begun that will lead to our analysis of the iron in Rio Tinto in collaboration with the Spanish affiliates of the NAI. The other is Iron Mountain in northern California, a site that has been studied extensively with respect to its microbiology (e.g., Edwards et al. 2000). Bishop is currently funded to study alteration processes of volcanic material in Iceland (through MFRP), and could cost-effectively perform field experiments at the Landmannalaugar hydrothermal site (e.g. Bishop and Murad 2002) for this project as well.

Aliquots of each experiment will be collected for UV-VIS-NIR absorbance measurements with the field spectrometer in order to monitor activity and determine the mineralogy of the iron species. Based on these measurements, samples will be selected for quantitative analysis in the lab via cell counts and mineral characterization. These samples will be sent out for chemical analysis and to the NASA-supported RELAB facility at Brown University for visible/infrared reflectance spectroscopy. It is likely that a mixture of ferric oxide /oxyhydroxide/ oxyhydroxysulfate minerals is present in the water in Nymph Creek and the adjacent Iron Spring. If the experiments show that the local ferric oxide-bearing minerals are highly effective in shielding UV radiation, while allowing penetration of the necessary visible region light, then the combination of minerals identified in this natural system will be employed in the latter experiments in Task 1.

Additional growth experiments will be performed *in situ* at Nymph Creek, Yellowstone (and selected other sites) using combinations of ferric oxide / oxyhydroxide / oxyhydroxysulfate minerals that are successful in the experiments in Task 1. The objective of these experiments is to determine the relative suc-

cess of imported ferric oxides (from the lab studies) vs. the local ferric oxide minerals in shielding UV radiation for the *Cyanidium* in their local water and nutrient environment. These experiments entail setting up one batch of experiments with the *Cyanidium* in a closed environment and another set of experiments by removing the local ferric oxides and replacing them with the imported ferric oxides. Both sets of experiments will be exposed to the normal solar UV radiation. Visual inspection and spectral absorbance measurements using the field spectrometer at the experiment site will provide qualitative analysis of the organism growth. Aliquots of each experiment will be collected for quantitative analysis in the lab via cell counts and detailed spectral measurements.

Task 3. The spectral character of the synthetic iron oxides and iron-bearing components of mats from Nymph Creek, Yellowstone, will be compared in the UV, visible and near-infrared regions for the spectral absorbance in solution. Spectroscopic analyses will also be performed on the reflectance spectra of bulk powders for the successful sunscreening agents from the lab and field studies in order to develop methods for remote detection of these on other planets including Mars. Differences in the spectral and chemical factors of the experiments run in Tasks 1 and 2 will be evaluated with respect to the organisms included in these experiments and the evolution of photosynthesis. Determining the optimal spectral and chemical environments for two different photosynthetic organisms will help us understanding the likelihood of a connection between photosynthesis, banded iron formations and oxygen.

4.6 Investigative Work Plan

Co-Investigator Janice Bishop will be the leader for the proposed projects in this section. This includes sample preparation and characterization, spectroscopic analysis, lab and field experiments, data interpretation and publication of results. The intended work commitment for Dr. Bishop is 0.2 per year for Years 1-5.

Collaborator Lynn Rothschild is a senior research scientist at NASA-Ames Research Center whose expertise is in algae and the influence of UV radiation on microorganisms. Because Dr. Rothschild is a NASA employee no cost is assigned for her salary on this project. The intended work commitment for Dr. Rothschild is 0.05 per year for Years 1-5.

A staff technician at the SETI Institute, Dana Rogoff, will work with Drs. Bishop and Rothschild at NASA-Ames Research Center on this project. The intended work commitment for the student/technician is 0.3 per year for Years 1-5.

4.7 Timeline

Task 1 is primarily lab work and will be initiated first. Task 2 is field work that will begin in year two. Task 3 involves analysis of the results of Tasks 1 and 2 and increases towards the end of the project. The combined tasks in this project will be carried out over a five-year period. Depending on the results of each task, some iterations in the experimental details may be necessary. Tasks 1 and 2 contain the bulk of the work and Task 3 will produce the results of the study.

5. Effect of High UV on Life in High-Altitude Lakes: Analogs to Mars

Data returned from the Mars Global Surveyor (MGS) spacecraft support the hypothesis that liquid water and geothermal energy may still be present on Mars. Fresh gullies (Malin and Edgett 2000), and lava flows could be less than 10^6 Myr old (Hartmann et al. 2000). Mars Odyssey Gamma-Ray spectrometer shows the presence of abundant hydrogen that could be related to up to 50% per weight of water-ice in the subsurface depending on latitude. Recent ice-covered lakes (Cabrol and Grin 2001, Moore and Wilhems 2001), rock glaciers and possibly debris-covered glaciers (Cabrol et al. 2002, Baker et al. 2001, Baker 2001, Kargel 2001) form a dense population of pristine landforms covering Mars from -30° to -60° latitude and $+27^o$ to $+50^o$ latitude (Cabrol et al. 2001a, 2002). If life arose on Mars in the past, the presence of both energy and water in recent times increases the odds of finding oases (McKay et al. 1992a,b, Farmer and Des Marais 1994, Farmer 1995, Farmer and Des Marais 1999, Wade et al. 1999, Farmer 2000,) However, in order to survive on Mars, life (if any) needed to adapt constantly almost from the very beginning to increasing environmental stress. Critical changes included the thinning atmosphere and loss of surface water escaping into space, and migrating and freezing into the subsurface.

At this early stage in our understanding of how life originated, the search for evidence that life might have evolved on Mars is necessarily guided by our knowledge of the range of environmental niches that can be successfully occupied by terrestrial life forms. Geologic and atmospheric models suggest that Mars was more habitable during the first 500 Ma of its history, (Kasting 1991, Haberle 1986, 1998, Jakosky et al. 1993, Squyres and Kasting 1994, Carr 1996, Baker 2001). Rivers and lakes (Carr 1996, Cabrol and Grin 1999, 2001), possibly an ocean (Parker et al. 1993, Head et al. 1999, Parker et al. 2001, Baker 2001) which had formed earlier started to decline around 3.5 Ga. There is evidence that they may have occurred again in later geological periods (Cabrol and Grin 2001) but only episodically and as lower magnitude events, their formation possibly driven by magmatic pulses (Dohm et al. 1999) and/or oliquity changes (Jakosky et al. 1995, Haberle et al. 2001, Cabrol and Grin 2001).

This project focuses on environmental conditions for life in terrestrial lakes located at extreme altitudes providing a unique analogy to Martian paleolakes of this transitioning period 3.5 Ga ago. Paleolakes have been identified on Mars in topographic lows (De Hon 1992), impact craters (Newsom et al. 1996, Cabrol and Grin 1999, 2001) and in volcanic regions (Cabrol and Grin 2002). The environment at the time of their formation included: episodic water supply; increasing evaporation and sublimation due to the thinning of the atmosphere which led to the reduction in size and depth of the lakes, hence generating chemical, pH, and salinity changes; high-UV radiation; cooling temperatures accompanied by seasonal, then perennial, ice-cover on the lakes; and short to long-term hydrothermal processes for lakes associated with impact craters and volcanic activity (Brakenridge et al. 1995, Gulick and Baker 1990). Understanding how this set of conditions affected putative living microorganisms is of paramount importance to assess their chances of surviving a changing Mars (Chang 1988, Clark 1998). Determining what type of microorganisms and defense strategies they could have developed is also critical to better prepare future instruments, experiments, technology, and landing site selection for future astrobiological missions (Des Marais et al. 1995, DeVincenzi et al. 1998) Moreover, it will help us understand better how life survived on early Earth as for two billion years after it emerged, the atmosphere contained very little oxygen (Kump et al. 1991; Kasting 2001) thus little ozone. The mechanism used by these organisms may be very ancient and could shed light on early Earth biology.

5.1 Previous Work

Because of their astrobiological potential, various aspects of extreme lake environments have been studied as Mars analogs, such as: the physical and biological constraints on the structure and function of dry valley ecosystems in the Dry Valleys of Antarctica (Doran et al. 1998, 2000), the nature of lake sedimentary environment (Benison 2002), the geomorphology of high latitude lakes (Rice 2001), the formation of tufa (Stoker et al. 1996) the conditions of evaporite formation (Wentworth and Morris, 2001) and their remote sensing signatures (Baldridge et al. 2001), the biology of impact crater lakes in the High-Arctic (Cockell and Lee 2001, Cockell et al. 2001), and the physical environment of subglacial lakes in Antarctica (Oswald and Robin 1973, Siegert et al. 1996). Significant work has also been performed on the effects of UV in lakes and models of UV flux over time (Cockell 2000). Sunlight penetrating lake water is absorbed in different ways by particles and natural chemicals in the water: water molecules absorb red and infrared wavelengths; pigments (chlorophyll) in microscopic algae that float in the water absorb blue and red light for use in photosynthesis; and UV light is absorbed by organic material dissolved in water. Lakes showing a high content of dissolved organic material (DOM) shield organisms from UV (McKenzie et al. 1999, Rae et al., 2000). DOM acts as a natural sunscreen as it influences the water transparency and so is a determinant of photic zone depth (Reche et al. 2001). In sparsely vegetated alpine and tundra areas, lakes tend to be clearer and offer less protection from UV to organisms living in the water (Vinebrooke and Leavitt 1998). Transparent water, combined with high UV irradiance may maximize the penetration and effect of UV radiation as shown for organisms in alpine lakes (e.g., Vincent et al. 1984, Vinebrook and Leavitt, 1996). Shallow-water benthic communities in alpine lakes are particularly sensitive to UV radiation. Periphyton, which de-

fines communities of microorganisms in bodies of water, can live on various substrates. While on rocks, they include immobile species that cannot seek low UV refuge unlike sediment-dwelling periphyton (Happey-Wood 1988, Vincent et al., 1993) or alpine phytoflagellates (Rodhe et al. 1966, Rott 1988) which both undergo vertical migration. Inhibition of algal photosynthesis by UV radiation has been documented in laboratory (Hä der 1993, Vincent and Roy 1993) and shown that phytoplankton production is reduced by formation of nucleic acid lesions (Karentz et al. 1991) or production of peroxides and free oxygen radicals (Cooper et al., 1989). Most of the experiments that have demonstrated in situ suppression of algal growth by UV radiation have either used artificially enhanced UV irradiance (Worrest et al. 1978) or shallow systems (< 1 cm) that lack significant natural attenuation of UV radiation (Bothwell et al., 1993, 1994).

5.2 Unique Analogs

We propose to investigate the short and long-term effects of Ultraviolet (UV) radiation in the highest lakes and ponds on Earth located in Bolivia and Chile as analogs to Martian paleolakes. Their elevation ranges from 6,046 m to 4,300 m. Two complementary experiments will test for the effects of UV radiation on periphyton: (1) the monitoring of biomass accrual, chlorophyll *a*, and species abundance on the underside of acrylit*e* plate stations; and (2) the positioning of UV dosimeters that will integrate the level of UVA (315-400 nm), UVB (280-315 nm), Photoactive Radiation PAR (400-700 nm), and temperature in the lakes. Environmental and physical data will be collected through long-term data logging. Core samples will be extracted to characterize the geologic and sedimentologic environment. Biological samples will undergo DNA extraction, chlorophyll *a* determination FISH analysis, DGGE analysis of bacteria and Archea 16S rDNA fragments. The environmental conditions (elevation, latitude, temperature, evaporation) and volcano-tectonic hydrothermal setting at these lakes provide a unique analog to Martian paleolakes 3.5 Ga ago at the time when the atmosphere became thinner on Mars and life, if present, would have been subjected to increased environmental and survival stress. This research, never performed before at such altitude, will allow to: (a) characterize life in high-altitude lakes; (b) identify the defense strategy developed by organisms living in shallow lakes against UV radiation; (c) advance our understanding of both the habitability potential of Mars and the limits of life on Earth. Results will provide critical clues to search and identify potential sites for life (extinct and/or extant) on Mars.

We will investigate the crater lakes and ponds at the summit of the Licancabur (6,017 m), Acamarachi (6,046 m), Aquas Calientes (5,924 m) dormant volcanoes as well as "laguna Verde-Blanca" (4,300 m). These lakes are located in Chile in the Atacama desert, at the border of Chile and Bolivia in volcanic and desert environments. Their geographical location (see Table 5.1) combined with their exceptional elevation provides a unique UV radiation environment. Moreover, the conditions of low oxygen, low atmospheric pressure (450 to 640 mb), low temperatures, aridity, volcano-tectonic environment, and hydrothermal activity make these lakes the closest terrestrial analogs to what we believe conditions were in early Martian lakes. Life is thriving in these lakes.

Table 5.1 Lake Characteristics

Name	Location		Elevation (m)	Temp. (°c)		Lake dimensions (l•w•d)	Environment	Status Volcano
	Lat°S	Long° W		Max.	Min			
Licancabur	22.50	67.53	6,017	+4	-40	(100 x 70 x 10) m	Volcanic Hydrothermal?	Dormant
Acamarachi	23¼18	67⅓37	6,046	+4	-40	(~20 x 50 x ?) m	Volcanic	Dormant
Aquas Calientes	23½22'	67¼41'	5,924	+5	-35	(~80 x 80 x ?) m	Volcanic Hydrothermal	Dormant
Laguna Verde	22.49	67.47	4,300	+17	-25	(7 x 1) km x 40 m	Hydrothermal	N/A

5.3 Preliminary Investigation

In 2001, Co-I Nathalie Cabrol was awarded a NASA Ames Directorate Discretionary Funds (DDF) as seed money to perform a reconnaissance at the Licancabur summit lake to assess its astrobiological potential. During the expedition at 6,017 m, the team was able to successfully deploy one acrylite plate station (see below) and data loggers (Cabrol et al., 2002). This was the first time that this experiment was taken to such high elevation. This preliminary experiment needs to be now expanded and completed (a) by the positioning of ELDONET dosimeters that will record the level of UV A, UVB, PAR, temperature at Licancabur and (b) by the deployment of acrylite stations, ELDONET dosimeters and data loggers to the other surrounding lakes in order to complete our vision of life and UV defense strategies at increasing altitudes. Their investigation will provide a unprecedented transect of shallow lakes, UV, and life between 4,300 m and 6,046m. Performing these experiments over long periods of time is critical to observe changes in the population leaving in the lakes. The approach to this is described below.

5.4 Plate Station Experiments

These experiments will test the effect of UV radiation on immobile periphyton located on shallow lake sediments. What is of primary interest for this study are organisms that live and are immobile in the water zone where the influence of UV radiation is the most severe (surface to 1 m). Upon selection of biologically promising sites in the lakes and following the technique successfully used in Licancabur in 2002, we will construct a gazer chamber formed by acrylite [Cyro indutries] submersed sheets in shallow water. The sheets will be suspended on four acrylite 30-cm long rods at ~10 cm above sediment. Each sheet will be surrounded by a mosquito nylon screen fixed on the upper part of the rods and closed by heavy rocks on the sediment. The sheets will be anchored to heavy rocks by four nylon ropes. Each station will be composed of one (60 x 60) cm x 3.4 mm OP-3 UV filtering and one(60 x 60) cm x 3.4 mm OP-4 UV transmitting sheet. The acrylite OP-3 absorbs 98% of the incident UV. The OP-4 transmits much of the radiation in the range from 260-370 nm and light between 395-1000 nm. It is specifically formulated to resist the degradation caused by continuous exposure to the high-intensity ultraviolet radiation. We will leave the stations over long periods of time and harvest their underside regularly to study the evolution of microorganisms that have been exposed and those that have been protected from UV. Harvest will happen after 1, 2, 3, and 4 years of exposure. The sheets are large enough (3600 cm^2) to allow 4 x 900 cm^2 harvest over this period for each station. The harvest will be performed using a brush and a scraper system (Aloi 1990). The results of this experiment will be directly compared to those obtained by Vinebrook and Leavitt (1996)—with whom the team is in direct contact—using the same method that they used on a small alpine lake in Canada (2,320 m). Samples of water and biomass will be collected in amber bottles and analyzed by the biologists on the team.

5.5 Physical Environment

The physical environment will be characterized by a) Mapping: Data will be collected using GPS units to determine the lakes' location, size, and altitude. We will determine their size; and will observe changes in annual water levels to extract some information about the lakes' water budget. Additionally, lake sur-

face area is an important factor in energy budget (e.g. evaporative flux and the effect of insulation; b) Geophysics. We directly measure the air temperature, soil and water temperature as a function of depth, relative humidity, and UV flux at the lake surface and as a function of water depth. A lightweight, handheld probe will be used to record conductive heat flux from the lake bottom. We will install dataloggers (e.g. Onset Computing HOBOs) to monitor air temperature and relative humidity, and water temperature as a function of depth throughout one year. The prime focus of this task, however, is characterizing the UV environment at the lake surface and at the depth of the acrylite UV plate experiment. For this, a 3-channel (UVB: 280-315 nm, UVA: 315-400 nm, PAR: 400-700 nm) submersible, logging ultraviolet dosimeter will be installed at each site. Results from mapping (lake surface area, approximate volume) will be paired with data on geothermal flux and evaporative flux (from temperature and relative humidity) and used among other information to build an energy balance model. This model will be applied to investigate the role of geothermal heating in the lakes' existence and stability, and to better understand the relationship between the physical environment and the endemic biology. Because the optical properties of freshwater vary greatly in the UV, the deeper parts of these lakes (depths greater than ~1-2m) may receive only nominal UV dosages—sim ilar to lower altitude surface environments. The shallow portions, however, are truly unique because they receive far greater incident UV flux than other freshwater environments on Earth and remain scientifically unexplored. Therefore, some of the most important results from the geophysical data collection effort will be the time-dependent flux of ultraviolet radiation at the shallow mounting depth of the UV sheet experiment. The dosimeter units will continuously monitor automatically the UV radiation on all three data channels as well as external temperature and pressure (depth); the former will be used to calculate total UV flux and the UV index. Optical properties in the UV for the lake water will be extracted from direct measurements of UV flux as a function of water depth. Data will be downloaded every year. Power is provided to the dosimeters through solar panels; c) Chemistry. Water samples will be taken from each site for laboratory analysis of fluid chemistry. Inorganic anion (e.g. sulfate, chloride, others) analysis will follow standard protocol (EPA Method 300.1) using Ion Chromatography (IC). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Emission Spectrometry (ICP-AES) will be employed (also following standard protocol (EPA method 200.7, 200.8) to determine trace element concentrations. Some data will be collected *in situ* using commercially available kits, which will be used to direct laboratory analysis and detect any effects of sample storage and transportation. Results from the water chemistry study (*in situ* and lab-based) will be employed to characterize the presence of geothermal fluid input to the lakes and allow us to build a more complete model of energy/hydrologic balance. Specifically, ICP methods will allow us to analyze the main rock-derived elements in volcanic lake fluids (e.g. Na, Ca, K, Mg, Fe, and Al from andesitic volcanoes). This will be paired with IC analysis of the principle anions derived from magmatic gas-hydrothermal fluid interaction (e.g. Cl, SO_4, and F) to characterize the presence, type, and extent of any hydrothermal activity in the lakes.

5.6 Characterization of Organisms

Shallow water and sediment samples will be taken from the lakes and ponds and will undergo a series of analyses: a) Organic Geochemical and Microbiological Characterization: samples will be analyzed using Gas Chromatograph-Mass spectrometry (GC-MS) to document the structure of lipids and photosynthetic/UV-screening compounds such as chlorophyll and carotenoids. This will identify specific adaptations in the structure of these molecules to high the UV environment. Genetic analyses will be conducted on DNA extracted from samples, leading to the sequencing of genes encoding from the 16S rRNA of organisms present in the samples. Fluorescent-In-Situ-Hybridization (FISH) will also be applied to identify quantitatively the communities present in the samples. Additionally, arsenic/sulphur enrichment media will be developed in order to cultivate arsenic reducing bacteria and sulphur reducing bacteria from the arsenic/sulphur sediment. Microorganisms will also be characterized by microscopy. b) Taxonomy: Abundant samples of the microorganic, bacterial, and algal communities in surface water and 1-m water will be collected and

analyzed to identify the various species living in these environments. Slides will be attached to a line on a float at each lake. The slides will be separated at regular space interval down to 2-m depth. They will collect microorganisms at various depths and provide information on the distribution of the biological population.

5.7 Anticipated Results

These lakes combine many of the environmental characteristics suggested for early Martian lakes and thus constitute possibly the closest analogs ever studied. Learning how the organisms in these lakes protect themselves from UV may help us understand how life survived on early Earth - and perhaps on early Mars as well. For as long as two billion years after life emerged on Earth, the atmosphere contained very little oxygen and therefore little ozone (Kump et al. 1991, Kasting 2001). Somehow, life on Earth started without a protective atmosphere present. By studying the organisms that live in these lakes, we hope to discover clues about how early life on Earth managed to cope with high UV. The survival mechanisms employed in this environment may be very ancient. Results will provide not only clues for the search of life on Mars, but may also provide important information about the limits of life on Earth.

5.8 Expedition Safety and Risk Mitigation

This project involves high mountaineering and its associated risks, including Acute Mountain Sickness (AMS), and accidents associated with climbing volcanoes. For this reason, via the NASA Ames Research Center DDF program in 2001, the Licancabur expedition underwent a thorough safety and risk evaluation. Risks and mitigation were reviewed by an official safety panel at NASA Ames.. The review led to the production of official technical and safety reports showing risks closure and satisfactory risk mitigation. They can be provided upon request from Robert Navarro and Alexis Flippen (NASA Ames/Code Q). All team members listed in this project are veterans of the 2002 Licancabur expedition and have proven high-mountaineering achievements.

5.9 Logistics and local support

This project will benefit from the logistics set up for the ‘ 02 Licancabur expedition, including team transportation, a familiar and experienced team of mountain guides and porters, lodging, food, and permits. This includes support from the Chilean (Universidad Catolicà de Norte) and Bolivian (Sergeomin) Institutions. Letters and documents of support can be provided upon request. This project also builds upon a successful history of scientific collaboration between UCN and the Co-I started during the Nomad field experiment

5.10 Project Milestones

Year 1: Position dosimeter on Laguna Verde (can be reached by car). First harvest of acrylite positioned in 2002. Harvest Licancabur plate positioned in 2002. Position dosimeter. Collect samples of water at various depth and set data loggers; Climb Acamarachi and position one acrylite Station and one UV dosimeter. Perform geophysical tasks and sample water and sediment for chemical, geological and biological analysis. Year 02: Climb Aquas Calientes set one acrylite Station and one dosimeter. Perform geophysics, chemistry and biology task. Return to Licancabur, Laguna Verde and Acamarachi for acrylite harvest and retrieve data from the dosimeters. Ongoing sample analysis. Year 03 to Year 05: Return to all sites for harvests, plates and dosimeters maintenance, data retrieval, sample collection and completion of geophysical, chemical and biological tasks.

5.11 Team Member Responsibilities

Nathalie Cabrol is a planetary geologist and the project and expedition lead. She will be responsible for meeting deadlines and for the completion of the project, for its astrobiological implications and Mars analogy aspects. She was the Project PI and lead of the 2002 Licancabur expedition. Edmond A. Grin, a planetary geologist, is responsible for the UV acrylite Experiment. Andrew Hock, a Ph-D student at UCLA, will be responsible for the geophysics task. David Fike is a Ph-D student and responsible for GC-MS, DGGE, and FISH analyses; Cecilia Demergasso, a Collaborator at the Universidad Catolica del Norte, Antofagasta (Chile), will be responsible for the biology task. Guillermo Chong, Collaborator at the Universidad Catolica del Norte, Antofagasta (Chile), will be responsible for assisting in geology and logistics.

This research will address Goals 2, 4, and 5 of the Astrobiology Roadmap.

6. Planetary-Scale Transition from Abiotic to Biotic Nitrogen Cycle

Nitrogen is an essential element for living organisms, and is found in most biologically important molecules including proteins, DNA, and RNA. As a consequence, understanding the planetary nitrogen cycle is critical to understanding the origin and evolution of life on a planet. In fact, an insufficient endowment of nitrogen on a planet will inhibit the origin and evolution of life (Mancinelli and McKay, 1988). Of particular importance to prebiotic and biotic chemistry is "fixed N" (NH_3, NH_4^+, NOx, organic-N). Without fixed nitrogen the origin of life would not have occurred on Earth and would most likely not occur on any other planet. It is the chemistry of nitrogen and how it chemically reacts and combines with carbon that makes it so unique.

The transition from abiotic to biotic planetary N cycling will affect profoundly N distribution among the planetary N reservoirs—the atmosphere, lithosphere, hydrosphere and, if present, biosphere. The chemical speciation of nitrogen in each of the reservoirs or "spheres" will also change dramatically as biology takes hold. This project aims to improve our understanding of these changes in the chemical speciation and the distribution of nitrogen on a planet's surface and suggest methods for their assessment. The project will focus, specifically as a test case, on the nitrogen evolution on Mars and its present-day availability and distribution.

Besides N, the other essential major biogenic elements, H, C, O, S, P, and the bioessential trace elements are present on Mars at concentration similar to those of the Earth and their availability is not considered to be limiting for the development of life (Mancinelli and Banin, 1995: Banin and Mancinelli, 1995). Furthermore, recent observations by the Mars Global Surveyor (MGS) and the Odyssey Missions have shown that Mars is relatively rich in water and that liquid water has likely been intermittently present and active in shaping the surface up to the present (Malin and Edgett, 2000a,b; Boynton et al., 2002). This leaves the issue of nitrogen availability on the surface of Mars as a major unknown in the assessment of Mars ability to support biotic evolution and its future habitability.

According to current hypotheses, the atmospheres of planets considered habitable (i.e., in habitable zones) formed in a manner similar to that of Earth, that is, from the release of gases originally trapped in the solid interior of the planet during its final stages of accretion (Hunten, 1993). For example, Earth and Mars formed as hot, molten, and geologically differentiated objects. Geochemical and geological evidence suggest that the oxidation-reduction state and hence, the chemical composition of outgassed volatiles is determined by the composition and structure of a planet's interior (Hunten, 1993). These volatiles include water vapor, carbon dioxide, and dinitrogen. Based on data and models of the development of Earth and Mars CO_2 and N_2 were, and are, the major atmospheric constituents of planets formed in this manner. This makes N_2 from outgassing the primary source of N for terrestrial type planets in habitable zones. Additional sources of N on this type of planet include comets and meteorites that would provide reduced N (e.g. Chyba 1991). These sources and mechanisms should result in similar initial forms of N on habitable zone planets.

Nitrogen cycling occurs abiotically and, for those planets that harbor life, biotically. This results in two fundamentally different fates for N on inhabited vs. uninhabited planets. On those planets that harbor life organic-N is the dominant form, whereas, abiotic cycling favors inorganic forms, specifically HNO and N_2O in the atmosphere, if no liquid water is present on the planet's surface and NO_3^- and NO_2^- if water is present. (Although comets and moons of gas giant planets [e.g., Titan] can possess high levels of organic-N, they possess essentially no appreciable amounts of inorganic-N salts.) If a planet is geologically active an abiotic N-cycle such as that described by Mancinelli and McKay (1988) can occur. If geologic activity ceases, as appears to have happened on Mars, cycling does not occur, and the volatiles, including N_2, in the atmosphere can be depleted by impact erosion and hydrodynamic escape to space. In a planet that once had oceans on its surface, N may remain in the regolith as NH_4^+ attached to clays, and/or buried in the regolith as NO_3^- and NO_2^- salts.

6.1 Abiotic Nitrogen Cycling

Atmosphere. The most predominant series of non-biological N-transformation reactions in an N_2/CO_2 containing atmosphere occur as a result of thermal shock waves from either meteoritic/cometary in-fall, or lightning that

breaks the dinitrogen triple bond. In such an atmosphere, the thermal shock produces NO (Borucki and Chameides 1984), that readily forms nitrosyl hydride (HNO), the primary end product of the reaction (Kasting and Walker 1981). Mancinelli and McKay (1988) defined the fate of HNO. Briefly, HNO is a reactive N^{+1} species. In the atmosphere it dimerizes and dehydrates to form N_2O and H_2O (Fig. 6.1a.).

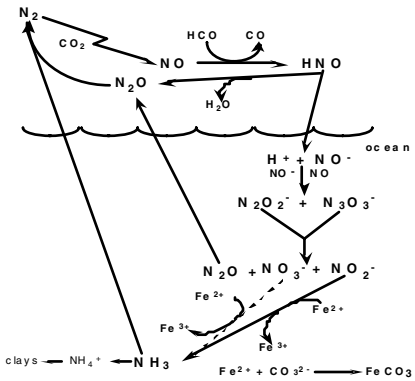


Fig. 6.1a . Summary schematic of the major pathways of the abiotic N cycle. Dashed line indicates that this reaction is less likely to occur.

Impact shock formation of nitrogen species other than NO (e.g., HCN, NH_3) have been considered (Fegley et al. 1986, Zahnle 1986). However, in a predominantly CO_2 atmosphere as thought to have existed on early Mars, early Earth and other planets in habitable zones, HCN is not produced, and any NH_3 produced photolyzes rapidly to N_2. Another potentially important form of fixed nitrogen on early Mars may have been organic-N. In a CO_2-dominated atmosphere, the formation of organic compounds is extremely difficult (Folsome, et al. 1981). Comets and meteorites, however, could have imported organic nitrogen compounds to early Mars, early Earth and possibly other planets in habitable zones (Chyba and Sagan 1992, Pierazzo and Chyba 1999).

Hydrosphere. Because the rate of dimerization of HNO is slow and it is readily soluble in water nearly all of the HNO formed falls into the ocean (on those planets that have oceans). It first dissociates into H^+ and NO^-. Once in aqueous solution NO^- reacts with itself and/or solubilized NO to form $N_2O_2^-$ and $N_3O_3^-$ and their conjugate acids. These species decay rapidly into N_2O, NO_2^- and NO_3^- (Mancinelli and McKay, 1988 and references therein). The N_2O is released to the atmosphere and is readily photolyzed to N_2. Any HNO_2 formed as a side product falls into the ocean and forms NO and HNO_3. The HNO_3 dissociates into H^+ and NO_3^-. So, if a planet did possess water on its surface then it is clear that the final product of thermal shock through the early atmosphere would produce NO_3^- and NO_2^- in the oceans.

Nitrite, but not NO_3^-, in oceans containing Fe^{2+} could be reduced to NH_3 (Zohner and Broda, 1979; Summers and Chang, 1993). These results suggest that NO_3^- would accumulate faster than NO_2^-. Most of the NH_3 that formed would be volatilized and escape to the atmosphere where it would be photolyzed to N_2. Some of the NH_3 would form NH_4^+ where it could be mineralogically fixed and stabilized by inclusion as a structural cation in the crystal lattices of certain phylosillicates replacing K^+ (reviewed by Mancinelli, 1996). The chemistry of NH^3 is the focus of section 5 of this proposal. The abiotic nitrogen cycle is summarized in Figure 6.1a.

6.2 Biological Nitrogen Cycling

The different types of fixed nitrogen, primarily organic-N, NH_3, NH_4^+, and NO_3^- are biologically useful and readily assimilated by organisms. Nitrogen is found most commonly in pools consisting of N_2, N_2O, NH_4^+/NH_3, NO_3^-, NO_{2-}, or organic-N. These pools are found throughout the various reservoirs. Reservoirs of nitrogen include the lithosphere, atmosphere, hydrosphere and biosphere. The biological and non-biological transfer of N among the various reservoirs constitutes the global nitrogen cycle (reviewed by Mancinelli, 1996; 1992) (summarized in Figure 6.1b).

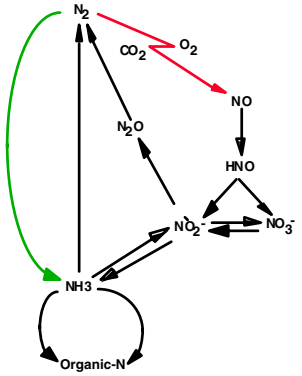


Fig. 6.1b. Diagrammatic representation of the biological and non-biological (abiotic) nitrogen cycle. The heavier arrows indicate the predominate pathways.

The biological nitrogen cycle consists of the following: Biological nitrogen fixation, the transformation of N_2 into NH_3; Ammonification, the enzymatic process of organic-N conversion to NH_4^+; Nitrogen assimilation, the conversion of NH_3 to organic-N and biomass; Nitrification, the oxidation of NH_4^+ to NO_2^- and NO_2^- to NO_3^-; Denitrification, the dissimilatory reduction of NO_3^- to N_2O or N_2.

If H_2O was on the surface of a habitable planet for extended periods, then fixed-N (especially NO_3^-) that are at least hundreds of thousands of years old. These desert areas essentially lack biological activity and leaching, which have been suggested as the reason for the NO_3^- stability (Claridge and Campbell, 1968).

6.3 Research Approach

Overview: The overall approach is to divide the research into two tasks that relate to the transition of an abiotic N-cycle to a biotic N-cycle. Task 1 concentrates on the abiotic portions of the N-cycle whereas task 2 adds the biological component. Task 1 involves understanding issues related to the chemistry, mineralogy and geology of N on a planetary basis. This specifically includes understanding and studying factors influencing the binding of N as NH_4^+ to poorly crystallized silicate minerals that are believed to be present on the surface of Mars, and the stability of nitrate mineral salts in different environments. Occurrences of nitrate accumulation on a regional scale, such as in the Atacama Desert and Antarctic soils, will be the subject of specific studies and the relevance of such accumulation of nitrate to N distribution in the Mars environment will be specifically studied using N transport simulation experiments and regional nitrogen balance modeling. In task 2, using the Atacama Desert as a study site, we will explore the biological reasons behind the stability of NO_3^- salts through geologic time on Earth. Although hypotheses have been presented for their stability, i.e., low rainfall and little biological activity (e.g., Claridge and Campbell 1968), these hypotheses remains untested. We will use the Atacama Desert in Chile as a model system. In addition, we will use the N-cycle in the microbial mat *Lyngbya aestuari* from the pacific intertidal, to model microbe-environment interactions with regard to the N-cycle as a function of organic-N:inorganic-N ratios, C:N ratios, as well as denitrification, nitrification, nitrogen fixation rates and photosynthesis and C-fixation rates (i.e., feedback effects of changing environment, including release of N_2O into the atmosphere). The results of the field, laboratory and regional modeling studies (including the results of Sec. 7 below) will be integrated into a unified framework that quantifies the partition of nitrogen into, and fluxes between, the various compartments (soils, lithosphere, hydrosphere and, if present, biosphere) in an extremely arid environment on the surface of a terrestrial, potentially habitable planet. The Atacama Desert will be our terrestrial field test site and Mars' shallow surface layers will be the target planetary site.

Task 1. Surface chemistry mineralogy and partitioning of planetary N (Dr. Amos Banin will take the lead). Nitrogen interactions with surface materials on solid-bodied planets and their distribution and accumulation in surface soils and regoliths will be studied in the following 2 ways.

The fate of N fixed and bonded as NH_4^+ in the crystal lattices of various minerals including zeolites, micas, phyllosilicates (clays) and poorly crystallized silicates (amorphous) minerals, as proposed to be present on the surface of Mars (Banin et al, 1997). We will determine the capacity of representative minerals to fix NH_4^+ under competitive conditions of high crustal K^+ (e.g. Earth), or low crustal K^+ (e.g. Mars), and the energetics of its binding. The availability of the fixed NH_4^+ to living organisms will be studied under non-oxidizing and mildly oxidizing conditions and the importance of this N-storage mechanism as a planetary sink-source to an emerging biosphere will be assessed. The ion-exchange and analytical methods to be used are described in Banin (1973) and Banin et al. (1993), respectively.

The environmental conditions and chemical-mineralogical mechanisms leading to the accumulation of soluble nitrogen as nitrate, as observed on Earth in the Atacama Desert and in Antarctic soils, will be explored. This will be done by reassessing existing observations and hypotheses on the causes and mechanisms of regional-scale accumulation of nitrates and testing the hypotheses elaborated below that such accumulation was primarily due to lack of rapid denitrification processes. The role of terrestrial plants in controlling the regional budget of nitrate/nitrogen will be quantified.

We will measure and evaluate the rates of accumulation of nitrates in soils receiving organic and ammoniacal forms of nitrogen, but where denitrification has been stopped or slowed due to high redox potential. The results will supply the basic information and data that will enable us to calculate the regional nitrate salt balance (using the approach presented by Banin and Fish, 1995) under extremely arid/high redox conditions. This sub task will be conducted in conjunction with Task 2, below.

Task 2. The Biosphere (Dr. Rocco Mancinelli will take the lead). We will focus on two systems, the Atacama desert and a microbial mat from the Pacific intertidal dominated by *Lyngbya aestuari*. (described by Rothschild and Mancinelli, 1990).

The Atacama desert is the exception rather than the rule on a wet inhabited planet such as Earth in that it is essentially a large NO_3^- salt deposit that is stable through geologic time. The surprising lack of denitrification under these conditions, apparently leading to the local nitrate accumulation, is of particular interest as an analog and model for planetary surfaces, or niches of limited biological activity. As part of a past study we have assembled a complete meteorological station in the desert that will provide pertinent data (e.g., humidity, rainfall, temperature, etc.). Preliminary denitrification data collected at one site on 3 samples during a field trip for another study in 1999 using the adetylene reduction method suggests that if denitrification occurs it is probably at a low rate. We propose to expand this preliminary data and test several sites to determine the potential for denitrification, activity in the field using standard techniques (e.g. acetylene reduction and $^{15}NO_3^-$ tracer studies). Further, to determine if denitrifiers occur in the area we will collect samples and, using probes specific for denitrifiers, probe the samples as described by Ward and Cockroft (1993) and Ward et al., (1993). Results of these tests will reveal the presence and activity, if any, of denitrifiers. These data will be correlated with the meteorological data. If denitrifiers are present and show little or no activity, this will help to determine if the lack of denitrification is due to lack of rainfall. If denitrifiers are not present (a doubtful result given their ubiquity and the broad range of prokaryotes possessing this ability) a systematic search for their inability to occupy a niche in this environment will be explored.

We have chosen an intertidal mat dominated by the cyanobacterium *Lyngbya aestuari*. This mat was chosen because cyanobacteria are important primary producers in microbially dominated ecosystems, and they likely formed the earliest recorded ecosystem of Earth (reviewed by Rothschild and Mancinelli, 2001). This mat inhabits the intertidal, so that in nature it undergoes daily drying and wetting, thus making not only a good candidate for study for varying moisture regimes, but it is easy to store in the lab, easy to grow on the roof of the laboratory building, and ideal for experiments for the terrestrial or aquatic environment. Previous work has shown that the N-cycle and the C-cycle within the mat are linked together and each exhibits a diurnal cycle (Rothschild and Mancinelli, 1990, Rothschild, 1991). N-cycling (N-fixation, denitrification, nitrification and assimilation rates) in the microbial mat will be determined using standard techniques (acetylene reduction for N-fixation and denitrification, acetylene blockage for nitrification and as well as ^{15}N tracer studies). Photosynthesis will be determined as a function of C-fixation using $^{14}C\text{-}HCO_3^-$ as described by Rothschild and Mancinelli, (1990). *Lyngbya* mat will be collected and transported to the laboratory roof artificial intertidal pool for experimentation. The effects of changes in concentrations of NO_3^- and NH_4^+ will be determined by adding various different amounts of each individually and separately to the system while monitoring rates of the various reactions of the N-cycle as well as C-fixation. Changes in rates of photosynthesis as a function of C-fixation will be correlated with changes in NO_3^- and NH_4^+ levels within the mat. Rates of N-fixation, denitrification, nitrification and assimilation will be studied as a function of changes in mat concentration of NO_3^-, NH_4^+ and rates of C-fixation. Concentrations of NO_3^- will be determined using an Ingold nitrate electrode. NO_2^- and NH_4^+ will be determined colorimetrically. Temperatures will be determined using an Omega Engineering portable temperature meter attached to a probe or a thermometer. The pH will be measured with a pH meter.

6.4 Work Schedule

Year 1: Begin laboratory nitrogen mineralogy experiments. Determine the capacity of

representative minerals to fix NH_4^+ under competitive conditions of high crustal K^+, or low crustal K^+. Collect samples from the Atacama and conduct preliminary denitrification field studies. Collect microbial mat samples and set up roof experiment. Year 2: Continue laboratory nitrogen mineralogy experiments. Initiate experiments to measure the rates of accumulation of nitrates in soils where denitrification has been stopped or slowed. Continue field experiments on the Atacama; collect samples for denitrification gene probe analysis. Continue microbial mat N-cycle studies. Year 3: Complete laboratory nitrogen mineralogy experiments. Continue studies of nitrate accumulation rates in soils. Continue microbial mat N-cycle studies. Continue gene probe analyses on samples form the Atacama. Year 4: Continue microbial mat studies. Model the environmental conditions and chemical-mineralogical mechanisms leading to the accumulation of soluble nitrogen as nitrate on small scale and on regional scale. Year 5: Complete all studies and integrate the data into a unified framework and a conceptual model describing the partitioning of N on inhabited vs. uninhabited planets.

The research proposed here and in Sec. 7 addresses Goal 4 of the Astrobiology Roadmap.

7. Mars Nitrogen Simulation

Nitrogen plays an essential role in determining the habitability of a planet such as Mars. As described in Sec. 6, a prebiotic source of nitrogen is essential to the origin and early evolution of life (before the development of biotic nitrogen fixation). Nitrogen compounds also will have a significant effect on planetary habitability through its affect on such factors as climate. For example, N_2O and NH_3 both serve as greenhouse gases, N_2O is a catalyst for the destruction of ozone, and loss of N_2 from an atmosphere (and the resulting loss of atmospheric pressure) can have a catastrophic effect on a planet's climate leading to the inability to support liquid water, another essential element of life. The loss of atmospheric nitrogen on Mars may have been by fixation and sequestration in the regolith (Grady et al. 1995; Mancinelli 1996) or through loss by impact erosion, although nitrogen in the regolith would have been protected (Chyba 1990; Melosh and Vickery 1989) or by both processes.

Understanding the planetary nitrogen cycle is critical to understanding the origin and evolution of life on a planet. One planet whose habitability may have been significantly affected by the cycling of nitrogen is Mars. We propose to study directly the reactions of nitrogen that would have occurred in the early Martian atmosphere, seas, and crust by modeling the abiotic reactions experimentally. Experiments will examine the chemistry and photochemistry of a variety of proposed atmospheres, with and without water and model Martian minerals, and measure the products formed.

The predominant series of known N-transformation reactions in an N_2/CO_2 containing atmosphere occur as a result of thermal shock waves that produce NO. NO is thought to be converted (by photochemical and thermal reactions) to HNO and N_2O. In presence of water further conversion (through NO^-, $N_2O_2^-$, and N_3O_3) into non-volatile (or "fixed nitrogen") species such as N_2O, NO_2^- and NO_3^- can occur. In presence of aqueous solutions or suspensions of Fe(II) these species can be reduced to ammonia (Summers 2003; Summers and Chang 1993). It isn't known if such reactions can be support by thin layers of adsorbed or condensed water or if standing liquid water is required. The direct reduction, by Fe(II), of species such as HNO, NO, and N_2O has also not been studied. Some or most of the NH_3 that formed would be volatilized and escape to the atmosphere where it would be photolyzed to N_2. (The presence of water will keep some of the ammonia dissolved and reduce this process) Some of the NH_3 would be in the form NH_4^+ where it could be mineralogically fixed and stabilized by inclusion in certain clays. Nitrogen in the regolith (NO_2^-, NO_3^-, and NH_4^+ in clays) will protected from impact erosion & hydrodynamic escape but, if no processes exist to revolatilize it, may become permanently locked in the crust.

Our current understanding of the conversions between the product of shock heating (NO) and other forms of nitrogen is based on theoretical models (Kasting and Walker 1981; Levine and Augustsson 1985; Levine et al. 1982; Mancinelli and McKay 1988; McConnel and McElroy 1973; Yung and McElroy 1979). This has several unavoidable limitations. One

is that a model can only include those chemical pathways which an investigator is aware of. For example, early models neglected the reaction between nitric oxide and hydrogen radicals (McConnel and McElroy 1973; Yung and McElroy 1979). Also, comprehensive models must take rate data from wide variety of sources. Most of the experiments that produced these data were conducted for other reasons and under variety of different experimental conditions. Often data must be extrapolated and sometimes good data is lacking and assumptions need to be made. These models can only provide estimates based on chemistry that one would expect to see and the data available.

These models also only considered the reactions that take place in the atmosphere, while separate experimental work considered reactions that may have occurred in condensed phases (Summers 2003; Summers and Chang 1993). Some of the questions remaining to be addressed in this area are particularly relevant to Mars. Could nitrogen have been fixed only in periods where bulk liquid water was present, or could the adsorption/condensation of thin layers of water allowed nitrogen fixation and/or ammonia formation? Would ammonia in clays be protected from UV photolysis? Lastly, how these reactions are coupled to atmospheric reactions is not completely understood. It has been considered by only one steady-state model (Summers 1999) and by no experimental studies.

We propose to study directly the reactions of nitrogen in an early Martian atmosphere, ocean, and crust by modeling abiotic reactions experimentally. Experiments will examine the chemistry and photochemistry of an atmosphere with a variety of compositions, with and without water and model Martian minerals, and measure the products formed. We will study the reactions of nitrogen from the initial shock heating product, NO, to the formation of nitrite, nitrate, and/or ammonia in the aqueous phases. Work will start with a simple model and experimental situation, and move to more complex situations. Work will investigate if a thin layer of water absorbed or condensed onto the surface of various minerals is sufficient to promote the formation of nitrates, nitrites, and (with ferrous minerals) ammonia. Direct illumination of clays will be conducted to study how well ammonium is protected from photolysis.

This work will increase our understanding of issues related to the chemistry, mineralogy and geology of N on a planetary basis and how these factors contribute to, or hinder, a planet's habitability. It will help us understand the habitability of Mars in the past and how the cycling of nitrogen contributed to its current state of habitability. It will help us understand how the presence/absence of reduced nitrogen may have set the stage for, or inhibited, the formation and evolution of life on early Mars. It will provide us a better understanding of how these reactions may have lead to loss of atmospheric nitrogen through sequestration in the crust or, conversely, how sequestration maybe have helped protect nitrogen from impact erosion and/or hydrodynamic loss. It will help us understand how these processes changed over various stages of Martian history with different amounts of liquid water. Nitrogen is an essential element to life, both directly and indirectly, and if we are to understand a planet's habitability, we must be able to understand the cycling of nitrogen on that planet. These results can be combined with other data from observations of Mars and modeling of its processes to give a broader understanding of the planet's history and how these factors affect the habitability of planets in general.

7.1 Methodology

We will look at atmospheres made up of variable amounts of N_2, CO_2, H_2O and minor species such as H_2, CO, H_2S and SO_2 at a variety of pressures. Wall effects can be studied by looking at variations in the size of the flask. Initial work will be with a "one box model" situation (two counting the aqueous phase) where the gas is irradiated at one temperature and pressure. A portion of the gas will be passed through water to allow species to "rain out" and react in the "sea". . We will look at what effect added species in water phases, like those that might be been present in a prebiotic sea, may have had (see; Summers and Chang 1993; Summers 2003) and at variable amounts of Fe(II) species. Analysis of gas samples will be by IR spectroscopy or by GC/MS. Analysis of aqueous samples will use nitrite/nitrate and ammonia sensitive electrodes supplemented by ion chromatography and colorimetric methods (Verdouw et al. 1978). If necessary, water will

be trapped out of the return gas to simulate a cold trap on the atmosphere.

Depending upon initial results, subsequent work may begin to look at more complex simulations. For example, a second "box" where gases are irradiated at two different temperatures and pressures, such as those present in the stratosphere and the troposphere. The results from the first setup will allow us to make an informed decision as to whether the optimal use of time would involve a more complex experimental apparatus or whether spending more time running experiments with a simpler setup.

To understand whether nitrogen may be fixed in the absence of water, we will investigate reactions where the water is replaced with minerals that are typically found in the basalt and/or andesite lithologies identified on Mars (Bandfield et al. 2000; Christensen et al. 2000; McSween Jr. et al. 1999). Work will also investigate if a thin layer of water absorbed or condensed onto the surfaces of minerals is sufficient to promote the fixation/reduction reactions. . (The mineral surfaces may help keep thin layers of adsorbed water from "freezing"). Direct illumination of clays will be conducted to study how well they protect ammonium from photolysis. Mineral surfaces will be analyzed be washing with water (after reacting gases have been removed) and the washes will be analyzed (with appropriate controls to check for reaction during washing). Work can be later expanded to consider interaction of abiotic cycling with biotic sources by adding the appropriate inputs of biogenic species and/or trapping out specific species. Other areas of later study are to look at the input of amounts of organic nitrogen such a may have been delivered by comets.

This work will provide us with a better understanding of the reactions of nitrogen (and of the conditions associated with it, such as habitability of planets). It will provide us with a new understanding of abiotic cycling, contribute to our understanding of how abiotic reactions set the stage for the origin of life &early life and impact biotic cycling. It will tell us about the processes that would have gone on in the early atmosphere of Mars and what species (both nitrogen species and others) would have been present in various stages of its development.

7.2 Investigative Work Plan

Year 1: Build and test apparatus. Establish and verify optimum procedures for analysis of products. Begin to collect data under a variety of basic conditions (N_2, CO_2, CO, & water, pressure, temperature, etc). Year 2: Continue to run experiments under a variety of conditions. Test for wall effects. Evaluate where experimental apparatus needs to be improved (two box setup, etc.). Begin looking at additional sets of conditions (such as the presence/absence of water). Year 3: Expand and improve experimental set up as necessary. Test new procedures. Continue to expand range of conditions considered (such as the presence/absence of water, minerals and thin films of water) and begin to consider others (such as the photolysis of ammonia in clays). Year 4: Continue collecting data under wide variety of conditions including; photolysis of ammonium in clays and added species in water. Consider the effect of trace species such as H_2, H_2S, and SO_2. Year 5: Wrap up examination of any conditions not yet considered. Extend work to such areas as: mimicking biogenic inputs; looking at inputs of cometary type material. Compare data against theoretical models and apply data toward an understanding of the cycling of nitrogen on Mars over different eras.

This work will be conducted by Co-Investigator Dr. David P. Summers. He has been working on the prebiotic fixation of nitrogen and carbon for the last nine years and has been able to establish the reduction of nitrate and nitrite as a source of reduced nitrogen on early prebiotic planets (Summers 1999; 2003; Summers and Chang 1993; Summers and Lerner 1998). Co-Investigator Dr. Bishun Khare will also contribute expertise from many years of experience in experimentally modeling photochemical processes in reducing atmospheres.

8. Europa Geology and Astrobiology

The work proposed in Sec. 2-7 examines interactions among a world's geology, atmosphere, oceans and biology in the evolution of that world's surface habitability. With the recognition that Jupiter's moon Europa likely harbors a subsurface liquid water ocean whose volume is about twice that of Earth's oceans (e.g. Pappalardo et al. 1999), we now know that habitability in possibly entirely subsurface environments must also be examined (e.g. Sa-

gan 1996, Chyba 1997). In the following sections, we propose work to examine the geology of Europa and its implications for the free energy sources that would be needed to power a Europan biosphere (Secs. 8 and 9). We then couple these results with terrestrial analog work (Sec. 9) and low-temperature laboratory experiments (Sec. 10) to make predictions about the possible abundance and survivability of any oceanic biomarkers that might reach Europa's surface through active geology. These results will have implications for the astrobiological exploration of Europa from either an orbiter or a surface lander.

8.1 Europa Astrobiological Overview

Jupiter's satellite Europa is a fully differentiated body with a rocky interior covered with a layer of material with the density of water. This layer is approximately 100 km thick as measured gravitationally, but such measurements do not have sufficient accuracy to distinguish between liquid water and solid water ice (Anderson et al. 1998). However, magnetic field results (Kivelson et al. 2000) strongly suggest the existence of an ocean of liquid water beneath Europa's icy surface. Liquid water is possible because Europa has a significant heat input due to tidal flexing, caused by a forced eccentricity from its resonance with Io and Ganymede (Cassen et al. 1979, 1980; McKinnon, 1999). Other lines of evidence are also consistent with a liquid water ocean (Pappalardo et al. 1999). Such water, if present, could provide an abode for life provided sufficient energy sources were available (Chyba and Phillips 2001, 2002).

A biosphere on Europa would require liquid water, appropriate biogenic elements (Pierazzo and Chyba 2002), and useful sources of free energy (Chyba 2000a,b, Chyba and Phillips 2001, 2002; Chyba and Hand 2001). Liquid water now seems a possibility, and spectral evidence suggests the existence of organic compounds (McCord *et al*., 1999). Our work focuses on free energy sources that are independent of photosynthesis, which has long been argued to be unlikely at Europa (Reynolds et al. 1983) but remains under active investigation (Greenberg et al. 2000, 2002). Charged-particle interactions with materials at Europa's surface can produce biologically useful oxidants such as molecular oxygen and hydrogen peroxide. Irradiation of carbon-containing materials at the surface of Europa should also produce simple organics (Chyba, 2000a,b; Cooper et al. 2001; Chyba and Phillips, 2001). Oxidants and hydrogen can also be produced by processing within the ice shell due to radioactive decay of incorporated potassium-40 (Chyba and Hand, 2001). If transported downward through the ice shell to a liquid water layer, these could provide a significant amount of energy to sustain a biosphere (Chyba and Phillips 2002). However, transport mechanisms remain uncertain due to the uncertainty in formation models for Europa's surface features. That is, Europa's biosphere depends in part on the style and frequency of Europa's geological surface activity. In this research, we aim to elucidate this connection.

The work proposed in this section will examine possible environments for life on Europa. It will have two main components: (1) We will look for changes on the surface of Europa during the Galileo mission through a careful examination of the Galileo dataset; and (2) We will study various models of geological activity on Europa to determine their implications for the formation or transport of biologically useful material from the surface to the ocean, or vice versa.

8.2 Change Detection

The first aspect of our work, a search for changes on Europa's surface due to current geological activity, will follow the methods described in Phillips et al. (2000) to compare images of Europa's surface using systematic image processing techniques. Our previous study (Phillips et al. 2000) focused on comparing images of Europa's surface taken by the Voyager spacecraft with those taken 20 years later by the Galileo spacecraft. This comparison allowed a long baseline in time over which changes could occur, but was hampered by the inherently low resolution of the Voyager images of the surface of Europa. In the research proposed here, we will instead compare images of Europa's surface taken during Galileo's 5.5 years of observations in a search for current geological activity. We will have a shorter baseline in time than for the Voyager/Galileo comparisons, but we will be able to compare images at much higher resolution to allow the detection of smaller changes on Europa. Studies of the morphology of various features on Europa's surface (Greenberg et al. 1999) suggest that, given the observed size

distribution of features such as lenticulae, changes are more likely to be observed in high resolution images where smaller spatial scales can be examined, even with a timeline shorter by a factor 4.5.

Any changes on Europa's surface due to current geologic activity would indicate the location and style of that activity, and would have important implications for the presence of liquid water beneath Europa's icy surface. A null result, in which no changes attributable to surface geologic activity are detected, would allow us to place a lower limit on the surface age of Europa, and an upper limit on the global resurfacing rate. These limits will depend on the details of the images compared, their resolution, and the percentage of Europa's surface that they cover.

8.3 Astrobiological Implications of Physical Models

Coupled with this search for changes on Europa's surface, we propose to evaluate the myriad models now in the literature (e.g. Pappalardo et al. 1999, Gaidos and Nimmo 2000, Greenberg et al. 2002) for the formation and evolution of various geological surface features on Europa to examine their astrobiological consequences. The questions we wish to consider for each mechanism include: (1) Does the process itself form or bury compounds of astrobiological interest? (2) Does it result in the transfer of material, formed by this or other processes, from the surface or near-surface to a subsurface ocean (i.e., as plausible electron donors or acceptors for possible life)? (3) Does the process result in the transfer of material from the subsurface / ocean layer to the surface? (i.e., does it bring up material, perhaps containing biomarkers, from the ocean to the surface, making it more accessible to spacecraft exploration?) (4) If material is transferred from the ocean to the surface, or the surface to the ocean, by any of these mechanisms, can we estimate the physical and chemical processing done on that material during the trip? Could biosignatures arrive intact?

We will examine these questions for the various mechanisms proposed for the formation of surface features on Europa, including ridges, bands, chaotic terrain, impact craters, and others. We will then consider two classes of broad results from this work: 1) What feature type, given all the different formation mechanisms, is of greatest astrobiological interest for a future lander? Which has the best overall chances of having material brought from the subsurface up to the surface intact? 2) What is the overall budget of oxidants and fuels available for a possible subsurface biosphere? (This question also connects directly with some of the simulations of radioactive decay in salty ocean models, discussed in Sec. 9.) We should be able to put upper and lower bounds on the total amount of material transferred from the surface to the ocean by considering the highest and lowest cases for each feature type.

The combination of these two lines of inquiry will allow a characterization of the possible environments for photosynthesis-independent life on Europa. If we are successful in finding surface changes due to geologic activity, we will be able to combine them with the analysis of the second part of our study to examine the consequences of this activity for the presence of a biosphere. If, for example, we were to find evidence that a domed feature on Europa's surface has increased in uplift and diameter, we would be able to focus our attention on models of the formation of these features. We would study these varying models, which range from solid-state convection to liquid water intrusions near the surface, and attempt to determine which model is best fit by the observed changes. We would also determine, from the amount of uplift, the volume of subsurface material that could have been brought closer to the surface, and from studies of the energy considerations of this process, determine what sorts of chemical processing could have taken place. These results will help us place the change detection results into an astrobiological context, to determine what implications they have for the formation of biologically useful molecules whether such materials could have been transported to the subsurface, and what quantities of subsurface material could have been transported to the surface in the process.

8.4 Preliminary Work

We have performed a preliminary analysis of the Galileo dataset to begin the search for appropriate image pairs for comparison. Ideally, such images should be matched as closely as possible in resolution, filter, viewing geometry, and lighting conditions such as phase angle. Given the sparse nature of the Galileo dataset, such "redundant" images were obvi-

ously avoided in most cases, but we have identified a number of image pairs that are sufficiently similar to perform useful comparisons, and are continuing to study the dataset as a whole to identify more possible image comparisons. There are 95 separate observations of Europa that were taken by the Galileo spacecraft over its 5.5 years of observation, and each observation consists of a number of individual frames.

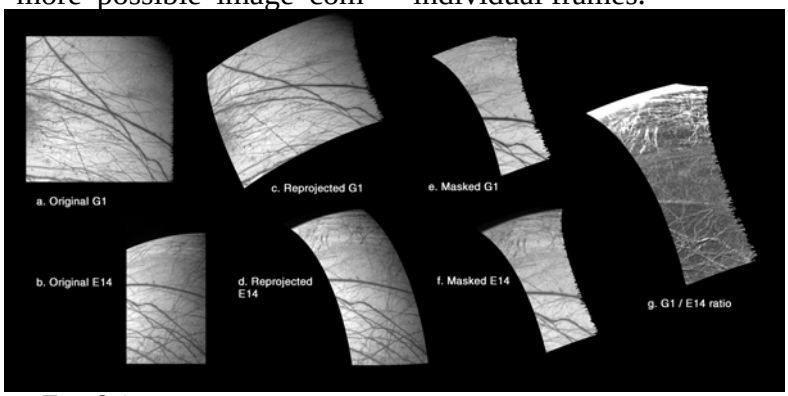


Fig. 8.1: Comparison of Galileo images taken about 2 years apart

One preliminary change detection analysis can be made between images taken on Galileo orbits G1 and E14. Fig. 8.1 illustrates the change detection procedure, with figures a) and b) showing the original images, c) and d) showing the images reprojected to the same viewing geometry, e) and f) showing the two images masked to show only the overlapping regions, and g) showing the ratio of the two images once an iterative subpixel registration procedure has been performed. The ratio image does not, however, indicate the presence of any new or changed features on Europa's surface over this 2-year period. Instead, all of the structure in the ratio image can be attributed to the difference in viewing geometry between the two images, which were taken with a difference in phase angle of almost 40 degrees. The phase angle difference, coupled with the non-linear photometric function of Europa's surface, results in apparent brightness changes of various features, which are visible in the ratio image.

Due to the non-optimized nature of the Galileo dataset, we will be forced to use non-optimal cases like this one to search for changes on Europa's surface. We are confident, based on work done with similar ratio images of Jupiter's satellite Io (Phillips 2000), that if any actual surface changes were present on Europa's surface, we could detect them. While lack of matching phase angles will make it difficult to discern changes in brightness of surface features, it will not hinder our detection of features which have changed in size or shape, and will certainly allow for the detection of new surface features. These real changes will have a completely different signature in the ratio images, and can easily be distinguished from mere brightness variations. We will also produce a new photometric function which we can use to photometrically correct the images of Europa's surface to account for the differing appearance at various phase angles. This will allow us to compensate somewhat for the changes in viewing geometry.

We have also begun the consideration of the astrobiological consequences of one particular surface process, impact gardening (the churning of the surface due to micrometeorite impacts; Phillips and Chyba 2000, 2001; Chyba and Phillips 2001). Irradiation of Europa's surface can create oxidants and organics there, but the same process also breaks down such materials if they remain exposed (Varnes and Jakosky, 1999). Material can also be removed from Europa's surface through sputtering erosion (the removal of material from the surface due to charged particle impacts). However, impact gardening will serve to mix the upper layers of the surface, and can potentially preserve surface material by moving it down below the sputtering and radiation processing depths. Sputtering erosion and surface mixing through impact gardening act together to change the preservation depth. Depending on the sputtering rate, the system may be in one of two regimes, sputtering-dominated or gardening-dominated. If sputtering dominates,

oxidants and organics produced at the surface are lost before they have a chance to be preserved for future transport to a subsurface ocean layer. However, if gardening dominates, then material may be buried faster than most of it can be removed through sputtering or destroyed by further radiation processing.

Using a model of impact gardening based on studies of the lunar regolith (Phillips and Chyba 2001), coupled with new studies of the impactor population in the outer solar system (Zahnle *et al.* 1998, 1999), we have previously estimated a gardening depth on Europa of about 1 meter over a surface age of about 10 million years. It appears that, on Europa, gardening rates dominate sputtering rates, so material formed at or delivered to (Pierazzo and Chyba 2002) the surface will thereby be preserved. We are working on revising those estimates to take into account new studies of the numbers of very small craters on Europa (Bierhaus *et al.,* 2001).

Once we have a reliable, global estimate of the gardening rate on Europa, we can use this estimate, coupled with estimates of the sputtering erosion rate (Cooper *et al.* 2001), to study how material created at the surface of Europa (Chyba and Phillips 2001) through radiolytic processing is preserved and mechanically mixed beneath the surface through gardening, and to what depth this process is effective. Since gardening will have approximately the same effectiveness on different terrain types on Europa, understanding the gardening rate is a necessary first step to considering the global transport of oxidants, organics, and other compounds of exobiological interest to and from a subsurface ocean layer. Thus, once our consideration of gardening rates is completed, we can incorporate our results into the analysis of other surface feature types and mechanisms.

8.5 Work Schedule

Year 1: Image selection and analysis, including careful analysis of the full Galileo Europa dataset and elucidation of all overlapping image pairs to be used for comparison, (Phillips). Continue work on gardening (Phillips). Also full literature review (Phillips and Chyba) of all proposed mechanisms for formation of surface features. Year 2: Finish gardening model, including small-crater slope and the effect of secondaries (Chyba and Phillips). Classify feature formation mechanisms into categories based on mechanism and process (Chyba and Phillips). Determine or develop photometric function to be used for image comparisons (Phillips). Process and reproject all appropriate images (Phillips). Year 3: Begin modeling of feature formation characteristics, including creation and transport of materials of astrobiological relevance (Phillips and Chyba). Create ratio images of appropriate comparison pairs (Phillips). Year 4: Continue work on astrobiological implications of each feature formation model (Phillips and Chyba). Begin analysis of ratio images (Phillips). Year 5: Continue analysis of ratio images, focusing on careful comparison of any potential changes with the initial raw images to rule out false detections (Phillips). Use changes, or lack thereof, to compute global resurfacing rate and surface age (Phillips). Use results of feature formation analysis to determine the overall budget of oxidants and organics available for a subsurface biosphere (Chyba and Phillips). If any surface changes are detected, focus on the mechanisms for producing that surface feature type and determine the astrobiological implications (Phillips and Chyba).

This research will address Goals 1 and 2 of the Astrobiology Roadmap.

9. Salts, Radiation and the Habitability of Europa's Ocean

In Sec. 8, we briefly reviewed the evidence for a liquid water ocean under Europa's ice crust. In this section, we propose research that would improve our understanding of the chemistry and habitability of this putative ocean. Within this context we will also begin to examine the influence of Europa's extreme surface radiation environment on potential biomarkers that may be delivered to the surface via resurfacing of the ice shell—i. e., via the mechanisms that the research in Sec. 8 intends to categorize. In Sec. 10, we propose a way to move the initial modeling of biomarker survivability described here to the next level, *viz.* direct laboratory simulation.

To assess the habitability of the Europan subsurface, we must improve our understanding of the available chemical pathways and redox gradients in the ocean (Chyba & Phillips 2001; Delitsky & Lane 1997; Gaidos et al. 1999; Kargel et al. 2000). The results of these models could inform experiments and instrumentation design for future Europa missions (Chyba and Phillips 2001, Chyba 2002).

9.1 Electron Donors and Acceptors

We will begin by expanding, via a far more detailed model, our previous initial work. Chyba and Hand (2001) made order-of-magnitude estimates of the delivery of electron donors and acceptors (fuels and oxidants) to Europa's ocean; these donors and acceptors could then fuel a Europan biosphere, allowing global biomass estimates to be made (Chyba 2000a,b, Chyba and Phillips 2001, 2002). We examined the transport of radiolytic products from the surface and the production of oxidants by radioactive decay of ^{40}K isotopes in the ocean. With a half-life of 1.25 Gyr, ^{40}K decays via alpha- or beta-particle emissions that then decompose water and produce molecular oxygen and hydrogen (Draganic et al. 1987, 1991).

These results can be done far more rigorously using the CHEMSIMUL software package (Kirkegaard & Bjergbakke, 2002), for which we hold a license. CHEMSIMUL allows simulations of aqueous chemical kinetics and enables the user to incorporate the decay of isotopes and resulting radiation chemistry into the model. This software served as the basis for the analysis of terrestrial and cometary radiated aqueous systems (Draganic *et al.*, 1984; Draganic *et al.*, 1987; Draganic *et al.*, 1991; Garzon & Garzon, 2001) and it is well suited to the Europan system. Using CHEMSIMUL we will examine the steady-state redox environment of Europa's salty ocean, based on over seventy-five aqueous chemical reactions influenced by the radiation environment of decaying potassium-40 and the influx of constituents from the Europan ice shell. (Appropriate models for the latter will be further informed by the work to be done in Sec. 9.2.) The rate constants and G-values for the simulation are adjustable to the low temperature environment expected for Europa (Elliot, 1994). Our model will assume a chondritic origin (Fanale et al. 1977; Fanale et al. 2001; Kargel 1991; Kargel et al. 2000) for Europa and will incorporate available Galileo results (see Sec. 9.2) to generate the best chemical model possible to date, allowing a new estimate of the oceanic biomass that could be supported on Europa.

9.2 Salinity of the Europan Ocean

The presence of salts in aqueous solutions is known to have important consequences for the origin of life (Monnard et al. 2002). Even small concentrations of sodium chloride have been shown to substantially reduce RNA oligomerization and impede membrane self-assembly (Monnard et al. 2002). Combine this with models that indicate a salty Europan ocean (Kargel et al. 2000; Zimmer et al. 2000; Zolotov & Shock 2001) and the prospects for the origin of life on Europa—a question that is distinct from the habitability of its ocean for extant life—becomes intimately tied to the type and concentration of salts on Europa. Rather than relying on models, here we propose using the conductivity data measured by the Galileo spacecraft to calculate possible concentration levels for plausible oceanic salts. Surprisingly, this has not yet been done. Our results will serve as checks on published models, probe the prospects for the origin of life on Europa, and establish initial conditions for chemical and biological simulations of Europa's ocean.

Jupiter's time-varying magnetic field induces an electric field within Europa's ocean (Colburn & Reynolds 1985; Colburn & Reynolds 1986). Based on Galileo magnetometer measurements (Khurana et al. 1998; Kivelson et al. 2000; Kivelson et al. 1997), the conductivity of Europa's ocean has been calculated to be between 58 mS m^{-1} and 2.75 S m^{-1} (Zimmer et al. 2000). The conductivity of aqueous solutions depends upon the quantity and nature of the salt content, as well as temperature (Calvert et al. 1958; Poisson 1980; Washburn & Klemenc 1936). Most of the work done in the terrestrial context has concerned sodium chloride (NaCl). This, however, is not compatible with chondritic models for Europa (Kargel 1991; Kargel et al. 2000; McCord et al. 1999; McCord et al. 2002; Zolotov & Shock 2001). The dominant salt expected for these systems is magnesium sulfate ($MgSO_4$). As a result, it could be the case that the magnesium sulfate concentration within the water is the primary factor controlling the observed conductivity. Using data available in the literature (Washburn & Klemenc, 1936, Pehybridge & Taba, 1978), we will derive $MgSO_4$ concentration estimates from the Galileo conductivity measurements, compare them with estimates from theoretical work (Kargel et al. 2000; Zolotov & Shock 2001) and use these results to constrain our ocean chemistry models. For comparison, we will also determine concentra-

tion ranges for the less realistic case where the dominant Europan ocean salt was NaCl.

9.3 Clathrate Hydrates

In addition to analyzing the composition of Europa's ocean, it is also important to examine the form and phase that constituents—in particular oxidants—may take. We will therefore also examine the role of hydrate clathrates in the ice shell and ocean. We have studied clathrates in the context of Antarctic subglacial lakes (McKay et al., submitted). This research has focused on the stability and density of clathrates in Lake Vostok, Antarctica. Here we propose extending our work on clathrates to the case of Europa.

Given the pressure and temperature environment of Europa's subsurface, the formation of various hydrate species may be expected in both the ice shell and in the ocean (Crawford & Stevenson 1988; Seigert et al. 2001; Sloan 1998). In the shell, clathrates may play a role in determining both the physical and electromagnetic properties of the ice. Structure I and II clathrates—those known to form with CO_2, O_2 and short hydrocarbons—have dielectric constants and bulk modulus parameters considerably different than those of pure water ice (Sloan, 1998). In addition, it is important to consider the fate of hydrate crystals in the ocean. Models typically predict that the Europan ice-water interface will be at roughly 270 K and 10-15 MPa (Crawford & Stevenson 1988; Kargel et al. 2000). At these conditions, clathrates of oxygen gas could be stable. Therefore, ice sheet processes such as basal melting and fracturing may introduce oxygen in the form of clathrates that then remain in crystal form in the ocean. Depending on the density of these clathrates relative to the salty ocean (which in turn depends on plausible salt concentrations, which we will work to constrain via the work in Sec. 9.2), they may form a hydrate layer at the base of the ice shell or sink to the ocean floor and form a layer of hydrate sediment. Furthermore, as a result of ^{40}K radiolysis, O_2 will form at depth in the ocean. Here the pressures are considerably larger than at the ice-water interface (>35-40 MPa), so hydrate formation is expected. It is possible than an oxygen-rich layer will form at the base of Europa's ocean, with implications for the metabolic styles that would be permitted in that environment.

9.4 Surface Biomarkers on Europa

No direct analysis of Europa's ocean is likely in the near term (Chyba 2002; Cooper et al. 2002; Space Studies Board 1999). The coming decades, however, should see orbiters and possibly landers capable of analysis of the surface ice down to several meters in depth (NASA 1999; Space Studies Board 1999). This emphasizes the importance of evaluating the survivability and detectability of biomarkers in the Europa's near-surface ice. Were life to exist in Europa's ocean, it is possible (see Sec. 8) that geological activity would sometimes deliver microorganisms to the surface of the ice shell. Once there, the organisms would be subject to the extreme radiation environment resulting from Jupiter's magnetic field (Cooper et al. 2001; Delitsky & Lane 1997, 1998; Paranicas et al. 2001). Consequently, surface evidence for sub-surface life would depend strongly on the extent to which biomarkers would be destroyed by this radiation. Understanding this process and being able to predict the potential steady-state population of biomarkers on the surface of Europa has critical implications for future missions that may search, from orbit or *in situ*, for signs of life (Space Studies Board, 2002).

In this section we propose an empirical study of the survivability of plausible biomarkers on Europa, extrapolating from the survivability of biomarkers on Earth. In Sec. 10, we will complement this work with, and test against it, the results of laboratory simulations.

Summons et al. (1999) and Brocks et al. (2001) have shown that hydrocarbon evidence of biological lipid membranes can be used to date terrestrial life back to 2.5-2.7 Gyr. These hydrocarbons, mostly hopanes and steranes, are the preserved molecular remnants of cyanobacteria and early eukaryotes. We will use these results to estimate survivability of these molecules against radiative degradation, and hence their lifetimes in the Europan surface environment.

A lower limit of these molecules' radiation survivability can be obtained by integrating the estimated radiation background from the time of their formation to the present. The resulting total radiation dose can then be compared to that of the surface environment of Europa and a limit for the survival time of biomarkers on the surface can be calculated. A radiation vs. depth profile for the ice can be used to esti-

mate the survivability of these biomarkers with ice depth (Cooper et al. 2001; Kminek et al. 2002; Space Studies Board, 1999). We will then incorporate gardening rates predicted for the surface of Europa (Chyba & Phillips, 2001), updated by the work in Sec. 8 above, and determine how long it would take for potential biomarkers delivered to the surface to be buried to a given depth. In addition to informing searchers for possible Europan surface biomarkers, these results will be germane to issues of planetary protection (Space Studies Board, 2000).

9.5 Personnel and Timeline

The work described in this section will form a component of the Ph.D. thesis work of Mr. Kevin Hand, now a first-year graduate student in Geological and Environmental Sciences at Stanford University, being advised by Dr. Christopher Chyba, the PI on this proposal. Dr. Chyba will work closely with Mr. Hand as he pursues the following investigations: (1) Analysis of relationship between Europan ocean conductivity and magnesium sulfate, fall 2003; (2) Calculations for extrapolating terrestrial biomarker survivability to Europan surface conditions, spring 2004; (3) Analysis of clathrate hydrate production and distribution in the Europan ice shell and ocean, fall 2004 (Hand hopes to take an intensive geobiology course in summer 2004); (4) Modeling of the Europan ocean chemistry using CHEMSIMUL, spring 2005; (5) Thereafter, continued work, still being defined, toward the completion of Hand's Ph.D. in astrobiology will be pursued.

This section and Sec. 10 address Goals 1, 2 and 7 of the Astrobiology Roadmap.

10. Irradiation of Biomarkers on Europa—Experimental Investigations

In this segment of the proposal we focus on the formation, detection, and fate of organic molecules similar to those seen in living things, but arising abiotically on icy surfaces of planets, comets, and the interstellar grains from which all Solar Systems form. We are interested in these important species under these unusual circumstances for two reasons, both central to astrobiology: First, it is important to identify and understand the chemistry of organic materials on the surface of planets in order to assess their pre-biotic potential. Second, it is imperative to characterize such life-like organic compounds that arise *non*-biotically so as not to mistake them for biomarkers when they are found, for example, on Mars or satellites of Jupiter and Saturn. Through this work we will advance NASA's Astrobiology goals of improving our knowledge of prebiotic chemistry and signs of life elsewhere in our Solar System (Objectives 2.1 and 2.2) and to recognize signatures of life on other worlds (Objective 7.1).

10.1 Organics on Europa

Astronomical observations demonstrate that organic materials are common throughout the universe (Ehrenfreund et al 2001). The appearance of molecules in space almost always differs greatly from that on Earth so, the detection and identification of compounds in space depends on them having been studied in the lab under conditions germane to the extraterrestrial environment in question. In the past we have successfully performed such work on interstellar ices (Hudgins et al 1993; Bernstein et al 1997, Bernstein & Sandford 1999). To advance the remote detection of pre-biological (or biological) molecules in the Solar System we intend to collect laboratory data on relevant compounds and conditions for comparison to astronomical spectra. We propose to reproduce ices of the potentially pre-biologically interesting Europa in the lab, measure infrared (IR) spectra at 50-100 K. From this we will generate refractive indices (n, k) that can be used to fit reflection spectra of Solar System ices. We will include molecules known to be present, such as CO_2 (Carlson 2001), and those we predict to be present, such as small cometary carbon compounds (CH_3OH, H_2CO, CO, and CH_4). Of course, many beautiful *mid* IR spectra of H_2O ice(s) have been recorded at lower (interstellar) temperatures, and *near* IR spectra of some ices have been measured at higher (Solar System) temperatures. However, our coverage of near- and mid-IR and emphasis on mixtures and temperatures that are germane to the Galilean satellites, means our measurements will complement and extend, not repeat, previous work. To our knowledge, few lab spectra exist for H_2O ices with carbon-containing molecules under these conditions and they will be well suited to fit reflection spectra of outer Solar System objects from ground based observatories that now extend out to 5 μm, and data from planned space borne infrared observatories.

We also contend that Solar System ices must contain larger, exogenously delivered, molecules as well, for example polycyclic aromatic hydrocarbons (PAHs). PAHs and closely related carbon-rich materials are abundant in our Solar System and are delivered to planet surfaces by carbon-rich meteorites and cometary and asteroidal dust. Our previous *interstellar* PAH-ice experiments produced prebiotically significant species, such as quinones, with structures similar to important biochemical intermediates (Bernstein et al. 1999, 2001). We propose to extend this work on the reactions of PAHs to Solar System conditions and radiation types.

One type of PAH central to the origin of life that remains largely overlooked in are PAHs with nitrogen atoms in the rings: aromatic nitrogen heterocycles (ANHs). From key metabolic agents to the nucleobases in the backbone of DNA, ANHs play a prominent role in modern biochemistry (Figure 10.1, below).

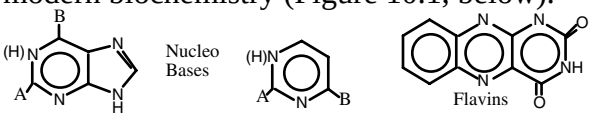


Figure 10.1, generalized structures of the purine and pyrimidine bases in DNA and RNA, and flavinoids, all aromatic nitrogen containing hydrocarbons (ANHs). Members of these classes of compounds have been extracted from meteorites.

Moreover, while the familiar nucleobases of modern biochemistry—adenine, uracil, guanine, cytosine, and thymine—are certainly of interest, they are not alone. It is plausible (if not likely) that alternative bases might have played a role in the origin and early evolution of life. Although there have been many suggestions of prebiotic alternative bases (Robertson & Miller 1995), all are ANHs. Interestingly, many of these ANHs are known to be present in meteorites. For example, xanthine, guanine, hypoxanthine, (alternative nucleic acid bases) and substituted pyrimidines were detected in Murchison (van der Velden, and Schwartz 1977) as have purines and triazines (Hayatsu et al 1975). Purine and pyrimidines have also been detected in the Jilin meteorite (Shi 1978). However, almost no work has been done on exogenous synthesis and modification of these molecules, or the impact they may have had on the evolution of life.

In addition to nucleobases, other, non-nucleic acid N-heterocycles are fundamental to biochemistry. These include folate, flavins, nicotinamides, porophyrins, and the amino acids tryptophan and histidine as well as the amino acid functional groups imidazole and indole, the latter two for which there is currently no compelling prebiotic synthesis (Keefe, Lazcano, and Miller 1995). In view of the central role these species play in modern biochemistry, and the fact that they are exogenously delivered, we propose to study the spectra and chemistry of these molecules under both interstellar and outer Solar System conditions, as we have done with PAHs (Bernstein et al. 1999, 2001, 2002a, 2003).

10.2 Pseudo Biomarkers

Over the next several years there will be numerous missions (e.g. Cassini, Rosetta, Mars NetLander) to explore Mars, Europa, Titan, and other bodies. One of most interesting objectives of these probes is the search for biomarkers–chemical evidence of extant or extinct life produced by biology. However, as we have mentioned of meteorites, extraterrestrial samples often contain organic compounds from abiotic processes that look like those from biology. It could be possible to be fooled by molecules that we think of as typically biological if one did not know that they could arise non-biologically under these unusual conditions.

For example, we have recently demonstrated the formation (under extraterrestrial conditions) of amino acids (the "building blocks" of proteins; Bernstein et al. 2002b) amphiphiles (molecules that form membrane-like structures; Dworkin, et al. 2001) quinones (ubiquitous co-enzymes; Bernstein et al. 2001) and a range of substituted aromatics, including some of the same class as those invoked as potential biomarkers in a Martian meteorite (McKay et al 1996). All of these compounds have been detected in meteorites.

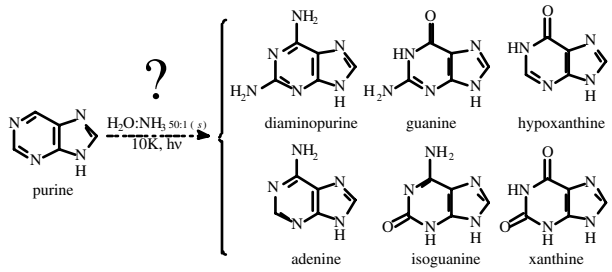


Fig. 10.2 by analogy with our previous work (Bernstein et al. 1999, 2002a, 2003) we predict the reactions of meteoritic compounds in Europan ice will lead to many biologically interesting molecules. For example, shown here are probable alternative nucleobases that would form from purine, a component of the Murchison and Jilin meteorites.

But molecules we will encounter in our exploration of the Solar System will not simply be those seen in meteorites but rather will depend on the specific conditions there. Would "biomarkers" even survive on the surface of Europa, and for how long? Our work on amino acids (Ehrenfreund et al 2001) suggests that they would be rapidly destroyed on exposure to the UV and ionizing radiation. Very little work has been done on assessing the stability and fate of larger molecules of the type that would be delivered to the Europan surface (Pierazzo and Chyba 2002). We expect based on our work on the oxidation of PAHs, however, (Bernstein et al. 2002; 2003) that the processing of meteoritic compounds such as purines in ice would yield other biologically important compounds, including alternative bases (see preceding Fig. 10.2).

10.3 Investigative Timeline

Year 1. Measure the IR spectra of nitrogen containing aromatics in argon as spectroscopic baselines for mixed molecular ice experiments. Expose to radiation to determine stability in inert Ar matrix. Year 2. Measure IR spectra of nitrogen containing aromatics in H_2O ice, at known concentrations for smaller more volatile ANHs for determination of Ns and Ks. Expose to radiation, measure rates of disappearance of starting materials. Compare IR spectra of ANHs in Ar matrix to astronomical observations of gas phase PAHs to set abundance limits. Year 3. Begin mass spectral analyses of ANH/H_2O irradiation experiments to determine products. Compare IR spectra of ANHs in H_2O to astronomical observations of ices to set abundance limits. Year 4. Begin chromatographic analyses choosing starting materials based on observations from previous years. Begin IR and mass spectral analyses of ANH irradiation experiments involving other ice components as indicated by astronomical observations. Year 5. Wrap up mass spectral and chromatographic analyses of ANHs irradiation experiments involving non-H_2O ice components. Isotopic (D_2O) labeling experiments if time allows. This research will be led by Dr. Max Bernstein, supervising a post-doc supported at the 45% level by this proposal.

11. Expanding the List of Target Stars for Next Generation SETI Searches

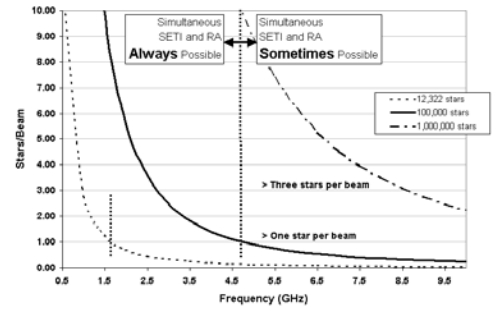
SETI attempts to take advantage of extraterrestrial technical intelligence in order to detect extraterrestrial life. The most commonly used characteristic to distinguish between astrophysics and technology (the SETI biosignature) is the product of the time duration (τ) and frequency bandwidth (B) of any detected signal. The classical uncertainty relation requires that the product $B\tau$ exceed about 1 (e.g. Elmore and Heald 1969). 21st century human technology often approaches this lower bound for reasons of economy (large signal to noise ratio at low cost), but natural emissions, even line-narrowed masers, have values of $B\tau >>1$. Since 1960, there have been over a hundred SETI searches reported in the literature. They have utilized a number of different strategies, and today are conducted at both optical and microwave frequencies, seeking narrowband, continuous signals as well as very short broadband pulses. While this may seem like a significant exploration, in fact it hardly begins to probe the 9-dimensional parameter space within which electromagnetic evidence of extraterrestrial technologies might be found (3 space, 1 time, 1 frequency, 2 polarizations, 1 modulation, and 1 signal strength). The most sensitive SETI searches must compete for time on observing facilities built and operated for other science, and therefore at any given moment these searches are usually off the air (Tarter 2001). This is about to change.

The SETI Institute has partnered with the UC Berkeley Radio Astronomy Laboratory to design and prototype the Allen Telescope Array (ATA) for both SETI and more traditional radio astronomy. When completed in 2005, this instrument (an array of over 350 6 m dishes) will be dedicated to a targeted SETI

search of nearby stars while simultaneously being used to do traditional astronomical surveys and research (see Ekers et al. 2002, Tarter et al 2002). The key to this dual-use strategy is a large list of target stars that represent credible abodes for technological civilizations—i.e. that are "habitable" from the point of view of complex life. The ATA will have a very large instantaneous primary field of view (PFOV) (because the array is constructed with small 6 m telescopes) and, if the target list is big enough, then on average several SETI target stars will be contained in the PFOV. The ATA will have a unique signal processing architecture permitting multiple SETI stellar targets to be observed while the PFOV is also being imaged for astronomical studies. The work being proposed in this section will concentrate on the scientific question of what constitutes 'Habstars' (those stars most likely to be suitable hosts for habitable planets), criteria for recognizing them, and in particular, the long-standing debate over whether M-stars should be considered among them. These results will be directly relevant to expanding existing target lists to 10^5 and eventually 10^6 stars that are good candidates for SETI observations from the ATA.

Turnbull and Tarter (in press, May 2003) have recently published a catalog of 17,129 'Habstars' based on the point source catalog from the Hipparcos mission. Selection of suitable stars was based on their spectral type, luminosity class, age, nature of any companions, metallicity, and flare activity. 12,322 of these stellar targets are visible from the northern California (Hat Creek) site of the ATA. In most instances, more is known about the physical attributes of the stars than about how these attributes actually influence the origin, evolution, and technical maturation of life in their vicinity. As illustrated in Fig. 11.1, a much larger list of candidate stars must be developed if SETI searches with the ATA are to be pushed to higher frequencies while still maintaining the ability to conduct SETI and traditional radio astronomy observations simultaneously. On average, for any direction on the sky, at frequencies below 1.5 GHz the current 'Habstar' list with 12,322 stars visible from the ATA (dotted curve) will yield at least one viable SETI target star within the PFOV. The 'quiet' terrestrially observable microwave window on the universe extends from ~1GHz to 10 GHz. Dual-use observing of the higher frequencies (at which the PFOV shrinks) will require a larger target list. Fig. 11.1 also illustrates the average number of stars within the PFOV as a function of frequency for two other cases; 100,000 visible stars (solid line), and one million visible stars (dash-dot line). It is apparent that a list of a million target stars provides at least two SETI targets within the PFOV at all frequencies up to 10 GHz. For a

Figure 1 - Stars per PFOV Beam --- with SETI target star list of 12,322 or 100,000 or 1,000,000 stars visible from Hat Creek



target list containing 100,000 stars, simultaneous SETI and radio astronomy observations will routinely be possible only below 4.5 GHz.

The FAME spacecraft (cancelled in 2001) would have provided astrometric distance measurements of ~40 million nearby stars, from which an expanded catalog of 'Habstars' could have been built. In the absence of these distance measurements, we will develop an expanded list of target stars in stages, by using the existing catalogs of point sources, and those that are now becoming available from ground-based observations. As a first step, the 'Habstar' results from the Hipparcos catalog will be augmented by stars selected from the All-Sky Compiled Catalogue of 2,501,968 stars (Karchenko 2001) that includes ground-based proper motion catalogs as well as data from the Hipparcos and the Tycho-1 and –2 catalogs (constructed from data collected by the Hipparcos star mapper during the Hipparcos extended mission). Poorer astrometric precision for this catalog (7 milliarcsec *vs*. 1 milliarcsec) and less accurate photometry for the non-Hipparcos stars will lead to greater uncertainty in distances and therefore in the intrinsic properties of the selected stars, but a list of ~10^5 targets should be achievable. To increase the list to ~10^6 stars will require combining entries in the recently released USNO B1.0 catalog of more than 10^9 objects (including proper motion measurements), with J,H,K

color data from the 2MASS survey, and the emerging color information from the Sloan Digital Sky Survey (u',g',r',i',z') in order to construct color-color relationships to determine stellar spectral types. The list will evolve over the lifetime of the ATA. Early on, it will inevitably be plagued by confusion between distant giants and nearby dwarfs, but the list will improve as distance measurements from future astrometric spacecraft missions (e.g. SIM in 2009 or GAIA in 2012) become available.

Of particular importance for the construction of the million target star catalog, is the selection (or not) of M stars since they comprise about 70% of the nearby stellar population (Henry et al. 1999). This is the SETI M-star dilemma. It is expected that the research conducted in a number of other sections in this proposal (e.g. Secs. 2, 3, 4, 7), as well as by other colleagues in the NASA Astrobiology

Institute, will inform the decision on which, if any, M stars should be included as ' Hab-stars'.

11.1 Do M Stars Have Habitable Planets?

Since the beginning of observational SETI programs, M stars have been excluded from most searches for three reasons—yet all three can be questioned. The habitable zone is so close to an M star that any planets would be locked into synchronous rotation (with potentially dire consequences for any atmosphere), the flaring activity of these stars during the first few Gyr of their lives would provide an untenable UV environment for the development of complex life on the planetary surface, and star spot activity would lead to unsuitable fluctuations in the total stellar luminosity. The "just right" requirements for complex life argued for by Rare-Earth proponents Ward and Brownlee (1998) reinforce this view, but other researchers refute it. Joshi et al. (1997) demonstrated that an atmosphere of 0.1 bars of CO_2 would be adequate to circulate heat to the planetary dark side, and prevent freeze-out of the atmosphere, while a CO_2 atmosphere as thick as 1.5 bars would permit temperatures compatible with the existence of liquid water at the sub-stellar point. Doyle and McKay (1991) argued that the flares from young M stars would increase atmospheric ozone production and provide protection for planetary surfaces, while Heath et al. (1999) calculated that atmospheric attenuation would prevent most UV flux shortward of 290 nm from reaching the surface and that damaging UV-B radiation would be comparable to solar fluxes on Earth. These authors also concluded that the luminosity variation from starspots would not impose undue stress.

Contradicting Heath et al. (1999), Scalo et al. (2002 preprint) claim that atmospheric reprocessing of ionizing X- and gamma-rays from M star flares transfers about 1% of the flare energy to the planetary surface, in the form of biologically damaging UV. However, instead of writing off planets around M stars as a result of these calculations, Scalo et al take the view that the strongly fluctuating UV environment due to stellar flares (on time scales of 100 hours for young M stars compared to 1000 years for the Sun), superimposed on a galactic-event background with a time scale of 10^ 6 years, may be ideal for forcing evolutionary turnover and innovation through mutation and sterilization (niche-emptying). A case can be made for the habitability of planets orbiting M-stars, but much work remains to be done to support it. In constructing the ' Habstar' catalog, Turnbull and Tarter (2003) opted to exclude any M star whose flare activity was sufficiently strong to be observed by Hipparcos, and retained ~ 600 M stars that met all the other culling criteria.

Deinococcus radiodurans (the gold-standard for radiation resistance among bacteria (Levin-Zaidman *et al* 2003) could possibly survive the radiation regime on a planet orbiting in the continuously habitable zone of an M star, but what does this really tell us about whether organisms might originate and evolve to intelligence there? We know that the high fluxes of ultraviolet light that radiated the surface of the early Earth (see Secs. 4 and 5 of this proposal) were obviously not inhibitory to the origin and early evolution of life. The effects of solar UV radiation on microbial life and evolution have been studied and reviewed extensively (e.g., Rothschild and Mancinelli, 2001). It has been well established that killing of cells by UV radiation is due primarily to its action on DNA, so that UV radiation near 260 nm is an effective lethal agent. The several defects in DNA caused by radiation include the formation of pyrimidine dimers, pyrimidine(6-4)pyrimidone photoadducts, DNA protein crosslinks and strand breaks.

More recently it has been reported that damage and plasmid inactivation increase with increasing photon energy. *D. radiodurans* possesses multiple copies of genes and is one of the most efficient organisms at repairing DNA damage, but these repair mechanisms are hardly universal and are thought to be incidental to its desiccation resistance (Mattimore and Battista 1996; Battista 1997). Microbes containing carotenoid pigments (e.g., Halobacteriaceae) and scytonemin (certain Cyanobacteria) are more resistant to solar UV radiation in the Earth's atmosphere than unpigmented species (Mancinelli and Shulls, 1978) because these pigments absorb UV radiation. Perhaps this is the key to evolutionary fitness on an M-star planet. The results on the sunscreen efficacy of iron compounds being studied in Sec. 4 of this proposal will help answer this. Research conducted in Sec. 5 of this proposal on UV resistance in bacteria living in Earth's highest altitude (and therefore highest UV) lakes should yield entirely new insights into this question, further informing this work. The organic molecules and aerosols that may survive in certain environments within the early atmospheres of M-star planets could also impact the UV environment on the surface, and the chemical modeling being proposed in Sec. 4 could help in evaluation of their habitability. The topic discussed in this final section will therefore serve to bring many of our team members together in a collaborative investigation that draws on much of their work. This work addresses Goals 1, 4, 5, and especially 7 of the Astrobiology Roadmap.

11.2 Work Plan: Bringing the Team Together

The work proposed here falls into two categories: (1) expanding the list of SETI target stars to ~10^6 using the most recent sky survey catalogs; and (2) once having approximately distinguished between the dwarf and the giant M stars on the basis of color-color relations (more easily accomplished for M's than for the F and G stars), decide which of the M dwarfs, if any, offer promising environs for the evolution of life—at first for life of any kind, and ultimately intelligent life. This proposal will partially fund Dr. Peter Backus, the SETI Observing Programs Manager, to accomplish the task of enlarging the target star list. Regrettably, nobody knows quite how to accomplish the second task. Therefore we propose a series of two focused workshops separated by 12 to 18 months to permit tangible results to appear from work done during the interval. The workshops will be held during the second and fourth years of this proposal at the SETI Institute, and be co-chaired by Dr. Tarter, the Director of the Center for SETI Research, and Dr. Mancinelli, a Co-I on this proposal (Sec. 6) with extensive research experience in environmentally induced stress in organisms. Dr. Cabrol, Co-I lead for Sec. 5, will participate, as will Drs. Rothschild (Secs. 2 and 4), Bishop (Sec. 4), Freund (Sec. 2), and Bakes (Sec. 3). Co-I Dr. Peter Backus, who will be preparing the target star catalogs and PI Chyba will round out the members of this proposal team whose expertise will be brought to bear on this problem. The remainder of participants will be invited from outside the SETI Institute, with an emphasis (but not exclusively so) on members of the Astrobiology Institute. In this way we will also reinforce ties between our astrobiology node and projects and those at other centers.

The workshops will be by invitation, include around two dozen scientists with specialties in particular microorganisms or atmospheric and planetary modeling, and will last 2.5 days each. Travel and per diem will be paid from this grant. The first workshop will be delayed until the second year of this proposal, in order to permit initial results from the Co-I's investigations to be included, and to allow advance preparation of the most complete description of the environment of putative M-star planets. Participants will be presented with the most recent "climate" calculations by Joshi et al. and others for a planet within the continuously habitable zone of an M star as well as the temporally varying biologically relevant UV surface fluxes calculated by Scalo and collaborators. The participants will be challenged to validate or refute these environmental conditions and to show whether the organism or radiation-damage repair mechanism in which they specialize would be viable under these conditions; work done in Secs. 2 and 4 of this proposal will be directly on point for these discussions. Results from the first workshop will feed into the second workshop held during the fourth year, at which participants will attempt to predict mutation rates and sensitivity to extinction. The goal of this latter exercise is to derive possible constraints on the frequency, duration, and magni-

tude of M star flaring events that can be used to answer the important question of whether M-star planets—which if they exist could be the most abundant planets in the galaxy, since 70% of stars are M-type—provide credible sites for life or even intelligent life.

These results will in turn have direct implications for determining observational strategy for the next generation SETI searches.

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The mission of the SETI Institute is to explore, understand and explain the origin, nature and prevalence of life in the universe.

SETI Institute Strategic Plan

Astrobiology is the study of the origins, evolution, distribution and future of life in the universe.

Astrobiology Roadmap

***Strengthening the Community*: The SETI Institute's 18 -Year Commitment to Astrobiology**

The SETI Institute is at the vanguard for understanding the nature of life, the search for life beyond Earth, and for communicating science and technology research to students, teachers and the public. The SETI Institute has become a premier example of an institution forging effective private/public partnerships. In 2002, the Committee on the Origins and Evolution of Life (COEL) of the Space Studies Board, National Research Council published *Life in the Universe: An Examination of United States and International Programs in Astrobiology* at the request of Congress. This report specifically cites the work of the SETI Institute, and its leadership in the search for life beyond Earth:

"Perhaps the most romantic venture in astrobiology is the search for extraterrestrial intelligence (SETI)….the current efforts are almost entirely privately funded. The SETI Institute in Mountain View, California, is the nexus of such efforts in the United States, and has accomplished in a spectacular way the founding of a science institute and the procurement of stable private funding to carry on the search. Because world-class scientists lead the SETI Institute, it is an effort carefully designed and worthy of notice by the scientific community and relevant federal agencies." (COEL Report, pp. 8-9.)

"The SETI Institute is in fact two different entities: a soft-money institution for research over a range of areas of astrobiology, largely with federal funding; and a donor-financed effort to develop technologies to search for intelligent life throughout a significant fraction of the Milky Way Galaxy.

The SETI institute has done an excellent job in developing programs in both arenas, which synergistically provide scientific breadth, vigor, and the resources for conducting a search that the federal government opted out of a decade ago. The SETI Institute's leadership—Frank Drake, Jill Tarter, and Christopher Chyba—are scientists who have personally invested their careers in the science of astrobiology. Students working at SETI from the Bay Area and elsewhere contribute their own intellectual energy and have produced high quality research projects that in some cases lead to Ph.D. dissertations. Overall the scientific quality of the program, and their output, is high….The leadership of the SETI Institute has forged a unique endeavor out of private and public funds, maintained a high standard of scientific research through its peer-reviewed research activities, and articulated clearly and authoritatively the rationale for approaches to a comprehensive search for extraterrestrial intelligence." (COEL, p. 72-73.)

Over the past 18 years, the SETI Institute has effectively administered $82.1 million from federal agencies (NASA, NSF, USGS, and others), and $54.4 million from private donations to support scientific research and educational projects. The Institute cultivates a quality research environment coupled with a commitment to maintain rigid cost controls in all administrative arenas. The SETI Institute is a 501(c)(3) non-profit scientific and educational research organization; our current negotiated overhead rate is 20% of direct labor and other direct costs excepting subcontracts and major equipment which are subject to lower rates. In practice, the average annual overhead rate for all projects combined at the Institute is 14.4% because of the low overhead on capital equipment. As a consequence of the Institute's low-cost environment, investigators are able to apply most of the awarded funds to research projects rather than infrastructure support. The SETI Institute provides a low-cost, highly effective institutional environment for the conduct of scientific research and education/outreach projects related to that research. In March, 1994, the SETI Institute was awarded a NASA Public Service Group Achievement Award "For meritorious service to NASA in carrying out research and education in areas of life in the universe and the search for extraterrestrial intelligence."

The Institute is governed Board of Directors, a leading group of individuals from science and private industry. The Board includes Nobel Laureate Dr. B. Blumberg and four members of the National Academy of Sciences: Drs. F. Drake, B. Blumberg, S. Faber, and W. Welch.

Board Member	Affiliation
Dr. Frank Drake	Astronomer & SETI Researcher
Dr. Greg Papadopoulos	Sr. Vice President and CTO Sun Microsystems
Mr. Thomas Pierson	CEO, SETI Institute
Mr. Al Bagley	General Manager and Engineering Manager for HP (Retired)
Ms. Linda Bernardi	Founder and CEO, ConnecTerra, Inc.
Dr. John Billingham	SETI Scientist & Chief of NASA Extraterrestrial Research (retired)
Dr. Joel Birnbaum	Dir. of HP Labs & Sr. Vice President for R. & D (retired)
Dr. Baruch Blumberg	Chase Fox Cancer Center &. NASA's Astrobiology Institute (1999-2002)
Dr. Sandra Faber	University Professor, University of California
Mr. Andrew Fraknoi	Chairman, Foothill College Astronomy Department
Mr. John Gertz	Founder, Zorro Productions
Dr. David Liddle	Partner U. S. Ventures; Co-founder & CEO of Interval Research
Mr. Steve Mourning	Executive Vice President, Kelmore Invertment Company
Dr. Lewis Platt	Chairman and CEO Hewlett Packard Company ('92-99)
Dr. Charles Townes	University Professor, University of California
Dr. William Welch	Watson and Marilyn Chair for SETI at UC Berkeley

Center for the Study of Life in the Universe: Dr. Christopher Chyba, holder of the endowed Carl Sagan Chair for the Study of Life in the Universe, leads the SETI Institute's Center for the Study of Life in the Universe (LITU). Under his leadership, leading researchers in the field of astrobiology conduct a wide variety of fundamental research projects funded by NASA, NSF, USGS, and private sources. Over sixty-four employees, more than half of them principal investigators on their own peer-reviewed research grants, are supported through the Center. The LITU science team explores a wide set of disciplines ranging from observing and modeling the precursors of life in the depths of outer space to studies of Earth, where we are attempting to learn more about how life began and how its many diverse forms have survived and evolved. Each LITU research project is related in some way to understanding the origin and evolution of life, and the extent to which life may be present beyond Earth. Appropriate to the sweeping scope of this research, the Center has many partners in our LITU work including NASA, NSF, and several major universities. In preparation for the science section of this proposal, LITU scientists at the Instutute defined a set of new coupled research proposals especially germane to the NAI. As an NAI Center, the SETI Institute NAI Co-Investigators would build on, and draw from, this much larger base of researchers at the Institute.

Strategic Planning Group:

Over the past two years, Chyba has co-chaired with Professor Ken Nealson, University of Southern California, a Strategic Planning Group (SPG) for the LITU component of the SETI Institute. The mandate of this group has been to identify novel and important research opportunities in the field of astrobiology for which the Institute could serve as a launching platform. The SPG consists of about fifteen prominent scientists across the field of astrobiology, including Barry Blumberg (NAI), Leslie Orgel (Salk Institute), Bill Schopf (UCLA), Rick Stevens (Argonne National Lab), Eric Mathur (Diversa Corporation), Lori Marino (Emory University), Dan McShea (Duke), Simon Conway Morris (Cambridge University), Pascale Ehrenfreund (Leiden), Jay Melosh (University of Arizona), Don Lowe (Stanford), and others. The final report of the SPG is still being written.

As a part of his astrobiology work, Chyba will continue his ongoing successful collaboration with Dr. Carol Cleland, a philospher of science at the University of

Colorado, Boulder and member of the U. Colorado NAI team. Cleland and Chyba have been examining a long-standing fundamental question of astrobiology, namely the definition of life. In their first published paper on this topic "Defining 'Life' "(Cleland and Chyba *Origns Life Evol.Biosph.* 32, 387-393, 2002) they draw on the philosophy of language to argue that defining 'life' currently poses a dilemma analogous to that faced by those hoping to define 'water' before the existence of molecular theory. They conclude that in the absence of an analogous theory of the nature of living systems, which may or may not prove possible, interminable controversy over the definition of life is inescapable. Chyba is well equipped to contribute to this collaboration not only because of his background in astrobiology, but also because he holds an M.Phil. in the history and philosophy of science from the University of Cambridge

Chyba is also contributing to the upcoming graduate textbook (edited by John Baross and Woody Sullivan) to be published by Cambridge University Press. Chyba and Co-I Phillips have submitted the textbook's chapter on Europa, and Cleland and Chyba have submitted the textbook's chapter on definitions of life. Chyba is to write the final epilogue chapter for the volume.

Center for SETI Research:

The SETI Institute leads the search for intelligent civilizations beyond our own solar system through its Center for SETI Research, led by Dr. Jill Tarter, holder of the endowed Bernard M. Oliver Chair for SETI. Over the past decade, the SETI Institute has raised over $50M dollars to privately fund ongoing SETI research, future planning, and the development of the Allen Telescope Array (ATA).

SETI Science and Technology Working Group (STWG) and *SETI 2020*:

The SETI Institute convened a distinguished panel of 39 scientists and technology leaders from around the world to create a roadmap for SETI research through 2020. Bringing together leading radio astronomy researchers and technologists from the Silicon Valley resulted in a technologically innovative and futuristic roadmap for SETI research and the next generation of radio telescopes. The major recommendations were to develop (1) a dedicated SETI radio telescope, (2) optical SETI research on existing optical telescopes, and (3) an omnidirectional SETI system. The report is published as *SETI 2020* (Ekers et al. 2002). All of these recommendations have been acted upon to the extent commensurate with the maturity of the underlying technologies.

Allen Telescope Array—The Next Generation Radio Telescope:

The Institute, in partnership with the Radio Astronomy Laboratory at the University of California Berkeley, is developing the 350-antenna Allen Telescope Array (ATA). It will be the world's premier radio telescope dedicated to SETI research while simultaneously conducting traditional astronomical studies, including studies of interstellar chemistry in regions of star and planet formation relevant to astrobiology. SETI searches seek a biomarker of extra-terrestrial life by sensing the presence of extraterrestrial technology, as endorsed within the Astrobiology Roadmap’s Goal 7. High sensitivity radio SETI searches will for the first time be conducted 24 hours per day, 7 days per week when the ATA is inaugurated in 2005. As of the date of this proposal, Paul Allen, co-founder of Microsoft, and Nathan Myhrvold, former CTO of Microsoft, have contributed more than $12 million to the design and prototyping of the ATA, and additional funds for the construction of the ATA are expected in the near future. The SETI Institute’s aggressive pursuit of private funding for this project will result in a superb instrument for astrobiology research and the partnership with UC Berkeley, a minority institution, will ensure that the ATA is used as an effective tool for training the next generation of researchers.

The ATA design utilizes hundreds of small dishes that can be manufactured inexpensively by the satellite dish industry. Signals from the individual antennas are combined using components developed for modern telecommunications and commercial high speed computing. This is a departure from the large-single-dish built from custom components that is the heritage of radio astronomy, and results in significant improvements in performance and reductions in cost. NASA is currently the beneficiary of the privately-funded ATA design concept since it is the model for the prototype array for the next generation of the deep-space network.

The ATA design is also informing the design of the US concept for the future Square Kilometer Array (SKA), an international project to build a radio telescope with approximately 100 times the collecting area of the ATA. Co-I Tarter has served as the Chair for the US SKA Consortium and is currently the Chairman of the International SKA Steering Committee. Thus, ongoing, privately-funded research at the SETI Institute will directly benefit the entire astronomical and astrobiological community through the coming decades. Tarter is also the former chair and a current member of the SETI Permanent Study Group of the International Academy of Astronautics.

The SETI Institute currently conducts dual-site SETI observations covering a frequency range of 1-3 GHz using the Arecibo Observatory and the Jodrell Bank Observatory as *Project Phoenix,* the world's most sensitive and comprehensive search for extraterrestrial intelligence. Since its beginning in 1995, *Project Phoenix* has surveyed almost 1,000 candidate stars utilizing Australia's Parkes and Mopra Observatories, the National Radio Astronomy Observatory in Green Bank, West Virginia coupled with the Woodbury observing facility in Georgia, and currently the large antennas in Puerto Rico and England. *Project Phoenix* doesn't scan the whole sky. Rather, it scrutinizes the vicinities of nearby, sun-like stars since such stars are most likely to host long-lived planets capable of supporting life. Center researchers naturally include stars that are known to have planets, all of them within 200 light years distance from our own planet. *Project Phoenix* is the privately funded successor to the ambitious NASA SETI program that was cancelled by a budget-conscious Congress in 1993. *Project Phoenix* will be superseded by the targeted SETI searches with the ATA, expanding the list of stars by two to three orders of magnitude, and expanding the frequency coverage to explore the entire 1- 10 GHz terrestrial microwave window. Scientific research proposed for funding elsewhere in this proposal will address the question of the habitability (for life and for advanced life) of planets around dwarf M-class stars, which comprise ~70% of solar neighborhood stars. That research will in turn be critical to helping the ATA choose its target stars for observations.

Optical SETI (OSETI):

Dr. Frank Drake leads an OSETI project with scientists from the SETI Institute, the University of California's Lick Observatory, UC Santa Cruz, and UC Berkeley. He searches for short, pulsed signals using Lick Observatory's 40-inch Nickel Telescope with a new pulse-detection system capable of finding laser beacons from civilizations many light-years distant. Unlike other OSETI searches that utilize a pair of fast photodiodes in coincidence, this new three-photodiode experiment is largely immune to false alarms that slow the reconnaissance of target stars. This is the most sensitive optical SETI search yet undertaken. Other OSETI research is conducted at UC Berkeley, Harvard, Princeton, and in Australia. Drake's OSETI research is privately funded by the Institute.

Omnidirectional SETI System (OSS):

Given sufficient, affordable high speed computing capacity, it will be possible to construct the equivalent of a radio fly's eye capable of observing all directions above the horizon at once, over a range of frequencies from 1-3 GHz. This is the ideal instrument for finding transient signals from either extraterrestrial technology or astrophysical processes. An array of 4096 small omnidirectional elements would have a reasonable amount of collecting area and require > 10^{16} ops to form all the beams on the sky and perform the signal processing for SETI. This much computing power is not affordable today, but will be in the future as a result of the exponential growth inherent in Moore's Law. Today preliminary work is being conducted by ElectroScience Lab at Ohio State University with funding from the Center for SETI Research to develop prototype technology for an OSS. An 8 element array (Argus Project) is now operational on the roof of the ESL building.

Funding New Science at the Institute:

The SETI Institute is a fertile breeding ground for new untried ideas in science through the private funding provided by the Institute's two endowed Chairs, C. Chyba and J. Tarter.

Center for Education and Public Outreach:

Through its Center for Education and Public Outreach (EPO) led by Co-I Edna

DeVore, the SETI Institute and its partners develop and conduct EPO programs supported by NASA, the NSF, and private donors. The EPO team has developed and is ready to publish an astrobiology curriculum entitled *Voyages Through Time* (VTT)*;* it's a standards-based course for a one-year high school integrated science class centered on the unifying theme of evolution. Scientists, teachers, curriculum writers, and media specialists created six modules that integrate astronomical, geological, and biological sciences. VTT was successfully field tested in more than 70 classrooms in 28 states by teachers during academic year, 2002-03. A Teacher Professional Development institute is proposed for EPO to enhance science teacher content knowledge and classroom implementation of VTT. The VTT project was led by the Institute's Dr. J Tarter and E. DeVore in close collaboration with co-investigators at NASA Ames Research Center (Y. Pendleton), California Academy of Sciences (Drs. M. Burke and S. Taylor), and San Francisco State University (Dr. K. O'Sullivan). San Francisco State University is a minority institution. Major funding was provided by the NSF IMD Grant # 9730693 ($2.3 million) with additional assistance from Hewlett Packard Company, Foundation for Microbiology, Educate America, and NASA's Astrobiology Institute and Fundamental Biology programs, other private support, and cost share. Over the 1998-2003 development period for VTT, project support totaled $ 3.3 million, a significant investment in astrobiology for students and teachers. The proposed EPO program brings this well-tested, standards-based astrobiology course to teachers and schools in our region, and offers the opportunity for other NAI sites to include their EPO staff in the training program. The Institute's manages EPO programs for NASA Flight Missions (see "Flight Missions" on page 101 below).

NOTE: The 5-page SETI Institute NAI-EPO Proposal appears at the end of this section.

Other "Strengthening" Elements:

The SETI Institute's NAI proposal team is a diverse group of scientists and educators, led by PI, Christopher Chyba. Each member of the team brings particular scientific expertise to the proposal, and all an engaged in activities that strengthen the overall field of astrobiology whether through participation on scientific panels, organizing scientific conferences, symposia and workshops, building new facilities relevant to astrobiology (the Allen Telescope Array), teaching courses and writing books, contributing to the professional development of pre-college teachers or simply bringing the adventure of science to students and the public. Several scientists are particularly interested in encouraging girls and young women to pursue careers related to astrobiology. This discussion describes "strengthening elements" and selected examples. The CVs in Appendix A include further documentation of the proposal team's contributions to the larger community.

1. Professional Community:

As noted in the COEL Report, the Institute's scientists are recognized leaders in the search for life beyond Earth. PI C. Chyba was awarded a MacArthur Award in 2001 for his work in both astrobiology and international security. In addition to his position at the Institute, Chyba is both Co-Director of the Stanford University Center for International Security and Cooperation (CISAC) and an associate professor (research) of geological and environmental sciences at Stanford. Co-I J. Tarter was inducted as a Fellow of the AAAS in 2003. Both are frequent plenary speakers for national and international meetings related to astrobiology. For example, at the 2002 *IAU Symposium 213 Bioastronomy Conference: Life Among the Stars*, C. Chyba, J. Tarter, K. Cullers, S. Shostak, F. Drake (all of SETI Institute), were 5 of the 10 plenary speakers. Simultaneously, the *Australian-American Fulbright Symposium 2002: Science Education in Partnership*, was held, and Co-I E. DeVore was US co-chair with K. Wilmoth (NAI), and Australian co-chairs C. Oliver and L. Vozzo of the Australian Centre for Astrobiology and University of Western Sydney respectively. C. Chyba was also an invited plenary speaker at the AAS Meeting (Jan. 2002), and the NAI Annual Meeting (Jan. 2003) addressing the question of life in the universe in both venues.

Notable Publications and Advocacy:

The PI and Co-Is for the proposed work are widely published in peer reviewed scientific

journals (See CVs.) We author books, and contribute chapters and articles to many reports and conference proceedings. We publish in the popular press, which continues to build support for the community through advocacy of the importance of astrobiology within the government funding cycles, as well as enhance general public literacy and interest in astrobiology.

Congressional Testimony: On July 12, 2001, Chyba testified before the Subcommittee on Space and Aeronautics, Committee on Science, of the House of Representatives at their hearing on Life in the Universe (serial no. 107-17, pp. 46-60). He continues to be consulted by staff of that committee on issues of astrobiology (including SETI) and planetary exploration missions. Chyba has personally briefed representatives Dana Rohrabacher (Subcommittee Chair, R, California), Lamar Smith (R, Texas), Zoe Lofgren (D, California), and Curt Weldon (R, Pennsylvania) on these issues.

"The Search for Extraterrestrial Life: A Core Mission for NASA" by C. Chyba in *Space Policy in the Twenty-First Century*, ed. W. Henry Lambright, Johns Hopkins University Press, 2002, pp. 198-231. Chyba argued in this paper that the search for extraterrestrial life provides a core theme for NASA's scientific research in the twenty-first century, and a centerpiece of its program for solar system exploration.

SETI 2020: A Roadmap for the Search for Extraterrestrial Intelligence, SETI Institute, 2002, Eds. R. Ekers, D. Cullers, J. Billingham, L. Scheffer. ***SETI 2020***, conceived by leading international scientists and technologists, it delineates the bright future of humanity's search for extraterrestrial intelligence. This distillation of four work-shops, held in the Silicon Valley from 1997 through 1999, sets forth and justifies expectations for the next twenty years in the search for extraterrestrial intelligence. A bold conceptual framework, with a liberal dose of practical engineering and financial planning, ***SETI 2020*** envisions a new path of growth.

"The Search for Extraterrestrial Intelligence (SETI)", *Annual Review of Astronomy and Astrophysics*, 2001, J. Tarter, 39:511-48. This paper places the search for evidence of extraterrestrial intelligence in the broader astronomical context of the search for extrasolar planets and biomarkers of primitive life elsewhere in the universe. A decision tree of possible search strategies is presented as well as a brief history of the search for extraterrestrial intelligence (SETI) project since 1960. The characteristics of 14 SETI projects currently operating on telescopes are discussed and compared using one of many possible figures of merit. Plans for SETI searches in the immediate and more distant future are outlined. Plans for success, the significance of null results, and some opinions on deliberate transmission of signals (as well as listening) are also included. SETI results to date are negative, but in reality, not much searching has yet been done.

"Is There Life Elsewhere in the Universe?" by J. Tarter and C. Chyba, *Scientific American*, Dec. 1999, p. 118-123. The article presents a straightforward discussion of the search for life beyond Earth in our own solar system at Mars and the moons of Jupiter. Then, the authors look forward in time to seeking evidence of life in other planetary systems by seeking chemical biomarkers and ultimately evidence of extraterrestrial technologies via SETI searches.

"Life's Far-Flung Raw Materials" by S. Sandford, L. Allamandola, and M. Bernstein (Co-I on this proposal), *Scientific American*, July 1999. This cover story suggests that the origins of life had major contributions from interplanetary and interstellar materials including the dust of comets and meteors. The complex molecules that exist in space could have peppered the primordial soup on early watery planets like Earth and Mars with the organic molecules we see in living systems today. The ubiquity of complex organic molecules across space, combined with the recent discoveries of planets around other stars, makes it more likely that the conditions conducive to life, if not life itself, have developed in other solar systems.

***"Introduction to Astrobiology"*,** textbook, for graduate students and senior level undergraduate, by Co-I E. Bakes, to be published Sept. 2005 by Johns Hopkins University Press. Astrobiology covers many aspects of physics, chemistry and biology and its subject matter ranges in diversity from the Big Bang through to the formation of galaxies, stars, planets, prebiotic chemistry concerning the emergence of RNA and DNA and the

emergence of life from these complex molecules respectively. This upper division, undergraduate text will include: the big bang and the formation of the first generation of stars; supernovae and subsequent next generation star formation, the interstellar medium--the site of the first prebiotic chemistry, the formation of planets--seeding the solar system for life, the evolution of a life bearing planet, the chemistry of prebiology, the origins and evolution of terrestrial life, the potential for extraterrestrial life, colonizing our solar system, and the technology of solar system and interstellar exploration.

New Encyclopedia Articles:

Mancinelli, R. L, and L. J. Rothschild: "Extremophiles: Who, What, Where & How". *McMillian Encyclopedia of Biology*, 2002.

Rothschild, L. J. "Astrobiology". *McGraw Hill Encyclopedia of Science & Technology* 2002

Tarter, J. C. "Search for Extraterrestrial Life". *Encyclopedia of Space Science and Technology*, Wiley & Sons, (in press 2003).

Proposed Workshop and Symposia:

The Institute's scientists are members and active participants in several professional organizations affiliated with astrobiology, and contribute plenary talks, invited talks, papers and presentations at a wide-variety of meetings, including those noted earlier. A review of the CVs documents the breadth of participation. For example, the Institute is directly involved in planning or having significant participation in the IAU General Assembly (July, 2003), for the International Astronautical Congress in Berlin, Germany (2003) the Division of Planetary Sciences (2003), Bioastronomy 2004: Habitable Worlds, in Iceland, AbSciCon (all meetings). The proposal team anticipates continued participation in the face-to-face conferences and symposia of the NAI community as well as in the virtual events hosted via NAI Central. This sustained scientific leadership strengthens the astrobiology community including the radio astronomical research efforts of the worldwide SETI research community

Cross-Disciplinary Scientific Workshop on M-Star Habitable Zones:

The work proposed by J. Tarter includes a series of two focused workshops in years two and four, separated by 12 to 18 months to permit tangible results to appear from work done during the interval. The purpose of these workshops is to bring together many of the Co-I's on this proposal and relevant experts from across the spectrum of astrobiology specialties to tackle again the question of whether any planets that might be in orbit around M stars offer a habitable environment. Inputs to these workshops will be the latest 'climate' models and calculated UV fluxes on the surface of a synchronously locked planet within the habitable zone, as well as a more complete understanding of the responses of terrestrial microorganisms to UV and desiccation stresses arising out of research in several sections of this proposal. The workshops will be held at the SETI Institute and co-chaired by Co-I's Dr. J. Tarter, and Dr. R. Mancinelli, who has extensive research experience in environmentally induced stress in organisms. The workshops will be by invitation, include less than two dozen scientists (whose travel and per diem costs will be paid) and will last 2.5 days each The participants will be challenged to show whether the organism or radiation-damage repair mechanism in which they specialize would be viable. Results from the first workshop will feed into the second workshop at which participants will attempt to predict mutation rates and sensitivity to extinction. The goal of this latter exercise is to derive possible constraints on the frequency, duration, and magnitude of M star flaring events that can be used to constrain the SETI target list. The workshops should be an ideal tool for bringing together the diverse set of skills and knowledge bases that are necessary to decide on whether or not to include M stars on the observing list.

Monthly SETI Institute—NAI Workshop Series

Under the auspices of this proposal, in addition to the focused workshops just described, the SETI Institute will sponsor a monthly workshop series at which the Co-Investigators of this NAI proposal will informally present their ongoing research in a constructive, problem-solving environment. These discussions will also be open, and advertised to researchers from NASA ARC, Stanford University, UC Santa Cruz and Berkeley as well as the regional California State Universities at San Francisco, Hayward, San Jose and Monterey Bay. (All of these CSU

campuses are minority institutions.) They will have both the effect of strengthening the Institute's team of researchers, and also of further strengthening that team's ties with researchers in the broader scientific community.

Examples of contributions to the professional community follow on the next page.

Investigator	Examples of Contributions to the Professional Community
C. Chyba	-NASA Planetary Protection Task Force -Chair, Science Definition Team for NASA's Proposed Europa Orbiter Mission, and have continued to investigate means of detecting Europa's subsurface ocean (Chyba et al. 1998, Kovach and Chyba 2001) and possible biosphere (Chyba 2000a,b; Chyba and Phillips 2001, 2002; Chyba and Hand 2001).
E. Bakes	-*Introduction to Astrobiology*, textbook for graduate students and Senior level undergraduates, Johns Hopkins Press, published 2005. -*The Astrochemical Evolution of the Interstellar Medium* Twinpress, 1997
A. Banin	-Reviewer for *Science, Icarus, JGR-Planets* and *Origins of Life and the Evolution of the Biosphere* -Senior NRC Resident Research Associate (2001-2) -Main Scientific Organizer: The Viking Commemorative Symposium, COSPAR'96; -Editor, Proceedings of Viking Commemorative Symposium published in *Advances in Space Research*, 1997.
M. Bernstein	-COSPAR: organized sessions at multiple meetings; editor of special issues of the Journal of Advances in Space Research (conference proceedings) -Member: Origins and Geochemistry Review panels -Member: NASA Specialized Center for Research and Training (NSCORT) Panel
J. Bishop	-NASA Peer Review Panels, External Reviewer for NASA Programs: PGG, EXO, MDAP, MFRP, SBR -Reviewer for *Science, Nature, Clays Clay Miner., J. Geophys. Res, Meteor, Planetary Science, Geophys. Res. Letters, Planet, Space Sci.,* and *others.*
F. Freund	-"Origins of Life and the Evolution of the Biosphere", "Astrobiology", "PNAS", and forthcoming "Astrophysical Journal". -Participant in the workshops like "Blue Dot I and II", Laboratory Astrophysics and others.
R. Mancinelli	-Member of editorial board of International Journal of Astrobiology. -Reviewer for Nature, European Planetary and Space Science Journal, Journal of Geophysicsl Research--Planets, Applied and Environmental Microbiology and Arctic and Alpine Research -Organized 2002 COSPAR meeting: Search for Signature of Life in the Solar System, Terrestrial Analogues and Simulation Experiments: Joint COSPAR/IAC Event
C. Phillips	-Co-author of chapter on the future of Europa Exploration, as part of the DPS decadal review ad-hoc panel on planetary science; -Co-author (with Chris Chyba) of chapter on Europa in upcoming Astrobiology textbook.
L. Rothschild	-Organized 1st and 2nd & 3rd Astrobiology Science Conferences, NASA ARC -Co-editor, *Astrobiology Journal* -Editor, "Evolution on Planet Earth: the Impact of the Physical Environment, Academic Press (publication in 2003) -Advisory Board, Denver Museum of Natural Science, New York Hall of Science -Co-organized Stanford Astrobiology course with Chyba
J. Tarter	-Kepler Science Working Group -Served on SOC for Bioastronomy Conferences, 1999, 2002, 2004: provided $10k conference support for each from endowed Chair.

2. Training

The SETI Institute is a nonprofit organization conducting scientific research and

education projects; it is not a degree-granting institution. Nevertheless, we are engaged in training future astrobiologists through work with regional and national university undergraduate and graduate students.

In addition to his position at the SETI Institute, PI Chyba is also a faculty member at Stanford University, which funds him at the 40% level. As an associate professor (research) in the Department of Geological and Environmental Sciences, he currently supervises three graduate student research projects, including that of Mr. Kevin Hand, support for whom is requested in this proposal. Dr. Chyba also teaches at both the undergraduate and graduate levels at Stanford. His courses—developed by him and now a regular part of the Department's curriculum—are the graduate course "The Origins of Life in the Solar System" and the undergraduate seminar "The Search for Life in the Solar System." Last year this undergraduate course had 65 applicants for 15 openings. The graduate seminar, taught the previous year, drew graduate students from six different university departments, creating exactly the kind of interdisciplinary mix astrobiology requires. Chyba's research group joins employees of the SETI Institute with Stanford graduate students in a transparent manner.

Working with Stanford colleague Professor Don Lowe, Chyba has been instrumental in the creation of an astrobiology and planetary sciences concentration within the Department of Geological and Environmental Sciences. This concentration has been approved by the faculty and is starting this academic year, built around a core of four (including Chyba's) new astrobiology and planetary sciences courses.

Chyba has also supervised summer undergraduate projects. This past summer he supervised a Swarthmore College undergraduate, Aaron Goldman, who joined Chyba's group at the SETI Institute and is coauthoring a paper with Chyba and Hand on the salts in Europa's ocean. Goldman has already lectured on this work in his department (the Department of Biology) at Swarthmore College.

Training Students:

At the Institute, we specialize on involving and training younger students, motivating them with the limitless possibilities of astrobiology, the search for life beyond Earth. Because we are very conscious of the potential multi-generational nature of much of our research, we take the job of training the next generation of scientists seriously. For that reason, we develop curriculum products around astrobiological themes for elementary, middle and high school students, and conduct an extensive education and outreach program (see Table 4). We mentor students over long periods of time, and assist them in entering the best graduate schools in order to earn the degrees that enable them to contribute to the field in the way that best suits their particular talents. Over the years, without any formal, funded undergraduate program or project at the Institute, we've been successful in bringing bright young people into the field. (See Table 1 for current students.) For example, Dr. Z. Webster, now Professor at CSU San Bernadino was mentored by J. Tarter through undergraduate work at UCSC, and PhD at UC Berkeley.

Name (Gender) (Investigator)	First Contact	Current Work	Scientific Focus
Amy Barr, (F) (J. Tarter)	6th grade	Graduate studies, U CO at Boulder	Liquid ocean on Europa
Sam Laroque, (M) (J. Tarter)	Undergraduate, UCSC	Graduate studies, U of Chicago	Cosmology & building instruments for CARMA
Josh Tasman, (M) (J. Tarter)	Computer science Brown U.	Graduate Studies	Psychology
Laura Langland-Shula, (F) (J. Tarter, E. DeVore)	High school; undergraduate UCLA	Graduate Studies, UC Santa Cruz	Astrophysics
Maggie Turnbull, (F)	Undergraduate, U. of	Graduate studies,	CATTS Fellow,

(J. Tarter)	Wisconsin	U of Arizona	Astrobiology
Nia Imara, (minority F) (J. Tarter, E. DeVore)	Undergraduate, Kenyon College	Graduate studies at UC Berkeley	Astrophysics Cosmology
John Keller, (M) (E. DeVore)	NASA ARC	Graduate studies U of Arizona	Astronomy, and Astronomy Education
Andrew Hock, (M) (N. Cabrol)	NASA ARC, Astrobiology Intern	Graduate Studies, UCLA	Geophysics
Vanessa Lancaster (F) (F. Freund; L. Rothschild)	Arizona State University	Graduate Studies, Arizona State Univ.	Detoxifying enzymes
Kevin Hand (M) (C. Chyba)	Stanford	Graduate Studies, Stanford University	Europa
Jamie Elsila (F) (M. Bernstein)	Undergraduate	Graduate Studies Stanford University	Chemistry
Ren Ping, (F) (A. Banin)	Hebrew University	Graduate Studies, Hebrew University	Weathering & soil formation on Mars
John Scoville (M) (F. Freund)	NASA Ames Research Center	Graduate Studies, Stanford University	Large scale computer modeling
Charles Apel (M) (M. Bernstein)	UCSC	Post-doc from UC Santa Cruz	Membrane biophysics
John Chaklader (M) (M. Bernstein)	Colgate University, Prof. Karne Harpp	Undergraduate research project	Parent body aqueous alteration
Aaron Goldman (M) (C.Chyba)	Swathmore	Undergraduate research project	Salt in oceans of Europa (paper in preparation)
Metages Sisay (B. Khare)	Santa Clara Univ.	Graduate Studies, MS	Physics
Patrick Wilhite (M) (B.Khare)	Santa Clara Univ.	Undergraduate	Physics
Hiroshi Imanaka (B. Khare)	Univ. of Tokyo, Japan	Graduate Studies	Physics
Brian Grigsby, (M) (N. Cabrol, E. DeVore)	Planetarium Director, Shasta Co. Schools	Graduate Studies MA CSU Chico, Sci.Ed.	Lincancabur expedition virtual field trip
Gretchen Walker (F) (E. DeVore)	High School Teacher	Graduate studies, U. of Maryland, MS	Astronomy

Table 1: Current Students and Post-Doctoral Students (F=female; M=Male)

SETI Institute's NAI team members are mentors for students in NASA's SHARP (summer high school minority interns), the Astrobiology Academy at NASA ARC, and at San Jose State University (SJSU), a minority institution for graduates and undergraduates.

3. Teaming with Minority Institutions:

As discussed in the Institute's proposed EPO program, the teacher professional development summer institute will be developed in collaboration with Dr. K. O'Sullivan, Professor of Science Education at San Francisco State University (SFSU), a minority institution. Continuing education credit for participants will be arranged either through SFSU, or San Jose State University, another regional minority institution.

The SETI Institute is also interested in working with the NASA Astrobiology Institute Minority Institution Involvement Program to support the Faculty Sabbatical program, pending our membership in the NAI. E. DeVore investigated this program at the NAI meeting at Arizona State University (2003), and will facilitate the Institute's participation. Our scientists work at the Institute's Mountain View offices, NASA ARC, in the field, and in partnership with the UC Berkeley Radio Astronomy Laboratory. We look forward to hosting faculty sabbaticals through this program.

As noted earlier, the Institute's NAI Monthly Workshops will be open to faculty from regional minority institutions.

4. Staff and IT:

At the SETI Institute, PI Dr. C. Chyba holds the endowed Carl Sagan Chair for the Study of Life in the Universe; and Co-I, Dr. J.

Tarter, holds the endowed Bernard M. Oliver Chair for SETI. Both are permanent positions, fully funded; their time in this proposal is a matching contribution to the Institute's NAI work. As described earlier, the Institute's mission is congruent with astrobiology, and the Institute's existence precedes the NASA Astrobiology Institute by many years. The entire scientific research program and the EPO program at the Institute (both those activities included in this proposal, and those that are separately funded) support the Goals and Objectives of the Astrobiology Roadmap, 2002.

The Institute's indirect costs provide the administrative support for our PI-led, NASA funded projects. This includes institutional management, procurement and contracts, business services, grant management, human resources, and information technology services. For Co-I's located at the Mountain View site, common IT services are provided from indirect costs; for Co-I's located at NASA ARC, IT services are provided via the ARC Division's subcontractors. The IT department at the Institute is staffed with 3.5 FTE's plus consultants. At the SETI Institute headquarters in Mountain View, a basic teleconference and videoconference system exists in the administrative conference room, a heavily used facility. At present, the Institute has one T-1 line for all internet based communications. In support of the Institute's NAI membership, it would be highly desirable to install higher speed communications, and a separate teleconference and videoconference system in the large conference room at the Center for the Study of Life in the Universe to insure ready access for the Institute's NAI team.

5. Information Technology:

Dr. E. Bakes is the Co-I responsible for "Titan, Photochemical Haze, and the Oxidation of Early Earth" (p. 17 and forward). In her work, she will develop code that models aerosols and photochemical evolution of large molecules. She will make a user-friendly version of the charging routines and the infrared spectral model available for analysis of Cassini/Huygens data. This is a concrete contribution to the larger community in the area of information technology tools.

6. Flight Missions:

Mars Exploration Rover: Dr. N. Cabrol is one of 28 scientists chosen by NASA for participation for the 2003 Mars Exploration Rover (MER) Mission. Since joining the SETI Institute, Cabrol has focused her research primarily on planetary geology and automated, human exploration of Mars. In the upcoming MER mission, Cabrol will be studying the traces left by water activity at the landing sites and wherever the rovers explore. Cabrol's investigation is divided into 4 primary tasks: (1) the geological and morphological identification of aqueous sedimentary landforms and deposits combining the analysis of PanCam and MI images at the MER sites with MOC and MOLA for insight into the local and regional geology and recommendations for preferred directions in which to send the rover; (2) the morphometrical and statistical analysis of PanCam and MI images to deduce properties of sediments, characteristics of emplacement, and nature of the transporting agents and depositional environments; (3) the generation of graphical and statistical material that will allow easy access and combination of the results of this investigation with those of other instrument teams and science investigators, and their archival in the Planetary Data System (PDS); (4) the active participation in the planning of science sequences and rover traverses, and the completion of tasks associated to the position in the Athena Science Team for which the principal investigator will be designated, and (5) the active participation to the EPO program of the Mars Exploration Program (JPL) and Center for Mars Exploration (ARC). It is critical that this project be performed *during* the Mars Landed Operations: (a) From the science perspective, it will ensure that appropriate images are acquired by both instruments to address issues that are key to this investigation and (b) From the rover operation perspective, it will assist the planning of science targets and traverse sequences by optimizing the understanding of the aqueous history of the MER sites, and assist the rover mobility by providing projected trafficability information as inferred from context images and experience acquired from terrestrial analogues. Finally, (c) Cabrol has acquired leading experience in the management of science teams and data, the generation of command

cycles, science sequences, and rover traverses during several field experiments.

Mars Reconnaissance Orbiter: Dr. J. Bishop is a current Co-I on the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) instrument for the 2005 Mars Reconnaissance Orbiter (MRO). CRISM is a visible/near-infrared imaging spectrometer that will produce global hyperspectral images of Mars at ~200m spot size, as well as increased spectral and spatial resolution images for selected regions. She is the lead for spectral identification tasks related to hydrated minerals and surface alteration. These are of particular interest to astrobiology on Mars in that remote identification of water and aqueous processes may be connected to the potential for habitability. Dr. Bishop participated in the Marsokhod rover tests at Silver Lake, CA, in 1999 as a remote scientist during rover field activities. This test was designed to assess techniques needed for rovers, such as the Mars Exploration Rovers that are launching in the coming months. She assisted in spectral analyses of visible/NIR and thermal IR data collected from the mission that resulted in three publications. Dr. Bishop was a member of the Pathfinder mineralogy Science Operations Working Group (SOWG) from 1997 to 1999. She performed spectral analyses of Martian rocks and soils based on lab spectra of analogues and assisted the team with analysis of the spectral, magnetic and chemical data.

Galileo Imaging Team: Dr. C. Phillips assisted the Galileo imaging team in planning observations of Europa and Io, including target selection, observation design, and technical delivery of observation information to JPL. She performed initial image processing and mosaic construction of those observations.

EPO Programs for NASA Flight Missions: The Institute manages EPO programs for NASA missions that conduct research complementary to our mission and that of astrobiology: (1) As a member of the Universities Space Research Association's consortium, and in partnership with the Astronomical Society of the Pacific, the Institute conducts the EPO program for NASA's *SOFIA*, the Stratospheric Observatory for Infrared Astronomy. *SOFIA* is the next-generation airborne observatory, featuring a 2.5 meter telescope mounted in a highly modified Boeing 747SP. *SOFIA* is home-based at NASA Ames Research Center, and begins observations in late 2004, flying research missions for 20 years. As an infrared astronomical observatory, SOFIA is especially suited to astrobiological research: the chemical composition of the interstellar medium, the ecosystems of galaxies, the nature of planetary atmospheres, and the formation of stars and planetary systems. For EPO, *SOFIA* offers the unique opportunity to train and integrate educators into the research environment on board the observatory. (2) The Institute is also a partner for the NASA Discovery Mission *Kepler* that will seek evidence for Earth-sized planets in orbit about sun-like stars. Observing approximately 100,000 stars simultaneously with extreme photometric precision, *Kepler* data will allow scientists to characterize the nature of other planetary systems, and determine the prevalence of terrestrial planets in the habitable zone. *Kepler* is on the central pathway toward space-based missions that seek evidence of life on other worlds. The 9-year *Kepler* EPO program will be conducted by experienced educators at the Lawrence Hall of Science at UC Berkeley, and the Institute. SETI Institute scientists are directly involved in *Kepler*; J. Tarter is a member of the *Kepler* Science Working Group; J. Jenkins is a *Kepler* Co-I and leads the data analysis team, and E. DeVore is a *Kepler* Co-I with responsibility for the EPO component

ESA Sponsored Missions: R. Mancinelli was the PI for the European Space Agency(ESA) sponsored BIOPAN3 "Survival of Osmophiles in Space" experiment. Since 1998, he has been the PI the ESA sponsored EXPOSE Experiment for the Space Station. 1994-1995, he was the Co-I for ESA sponsored BIOPAN 1-2 Survival Experiment. For the NASA ARC NAI CAN proposal for this same cycle, R. Mancinelli proposes space flight research. It is not disclosed to this proposal team.

7. Linkage to Other Agencies:

The PI and Co-Is receive current support and anticipate future support from other federal agencies, primarily the National Science Foundation (NSF), and a variety of private foundations. A representative sample of this linkage to other support appears below and in the Appendix B

Investigator	NASA Organization or Other Agency
C. Chyba	-Carl Sagan Chair for Life in the Universe, Endowed chair at SETI Institute -MacArthur Award, 2001 -Stanford University
N. Cabrol	-Director's Discretionary Fund: NASA ARC: Exploring Lincancabur (2002-03) $80K or 90K -Collaboration from the Universidad Catolicà del Norte which contributes 10% of a biologist work-year on this project equivalent to $10,000, and the logistical support from G. Chong (3 weeks full time) equivalent to about Sergeomin in Bolivia provides assistance in the field; dosimeters from other funding. Total contributed funding: $37,000 -National Geographic Society The project explores the Licancabur lake, including diving, to collect (1) critical astrobiological information about the limits of life in this unique extreme environment, (2) scientific clues about potential planetary analogs, and (3) elements to design science mission strategies for planetary exploration. $20,800
J. Tarter	-Bernard M. Oliver Chair for SETI Research, endowed by SETI Institute -NSF IMD Grant # 9730693: Voyages Through Time (completed) -Major private funding for SETI research from: David Packard and the Packard Foundation, William Hewlett, Gordon Moore, Paul Allen, Nathan Mhyrvold, and many other donors. -NSF requested white paper prepared indicating intention to apply for NSF annual funding to operate the Allen Telescope Array over 30 years..
R. Mancenilli	-NASA's Life Sciences, Planetary Protection, and NAI at Ames Research Center -Sole US PI on ROSE experiements; oversees the work on prokaryotes and testing all organisms in the Space Simulation Facility at the DLR in Germany; will co-coordinate flight experiments.
M. Bernstein	NASA's Origins, Planetary Geolog, Exobiology & NAI at Ames Research Center
F. Freund	NASA Goddard Space Flight Center Earth Sciences, and the Geophysical Laboratory at Carnegie Institution of Washington (Dr. G. cody & Dr. Y. Fei)
C. Phillips	Collaborated with USGS researchers; trained in using ISIS image processing software by USGS Flagstaff, and continue to assist them in the construction of a base map for Europa, as well as planetary nomenclature issues.
E. DeVore	NSF IMD Grant # 9730693: Voyages Through Time (completed) SOFIA EPO via USRA Prime Contract with NASA Kepler EPO, NASA ARC Grant #NAG 26066 NAI & Fundamental Biology, Grant # NAG 26051

Table 2: Examples of Linkage to Other Agencies

Other—Strengthening the participation of young people in Science: Dr. Janice Bishop volunteers as a Trustee for the Summer Science Program, Inc., an accelerated astronomy program for advanced high school students. This is a college-level astronomy program that teaches students from a variety of ethnic and social backgrounds about math, physics and astronomy. The curriculum has a strong hands-on component and each student spends many nights observing and developing plates, and many days calculating the orbit of their asteroid.

Dr. Cynthia Phillips authored *The Everything Astronomy Book*, an introduction to astronomy including a chapter on astrobiology, written for a general audience (Adams Media, 2002). She ran a website on Space & Astronomy for Kids from 1997-2001, at About.com providing weekly articles, annotated links, chat room and bulletin board, and answered questions from kids from around the world. She continues to provide occasional articles about Europa, and its potential for water and life for website Space.com as part of SETI Thursday.

Dr. J. Tarter is a high-profile figure for the media. As the role model for the Eleanor

Arroway character in Carl Sagan's *Contact*, she is often on camera or in press. She accedes to many media requests because she wishes to encourage the next generation of female scientists. One recent example of this commitment to young people is the book *Looking for Life in the Universe* by Ellen Jackson (2002). This book is a biography of Dr. Tarter written in a style that is intended to portray a career in science as rewarding and exciting. Tarter is also featured on *The Tech Club* a CD-ROM based collection of interviews with 54 women of science, engineering, mathematics and technology intended to provide positive female role models.

Dr. Nathalie Cabrol was awarded an IDEAS (Initiative to Develop Education through Astronomy and Space Sciences) one-year grant: to conduct a virtual field trip from Lincancabur for students, teachers and the public. Partnered with SETI Institute and the Shasta County Office of Education (rural schools in northeastern California), Cabrol's team and educator Brian Grigsby developed a web site, and interacted with students and the public during the 2001 research expedition. The project is the subject of Grigsby's MA in Science Education (2005); Cabrol anticipates integrating the activity into future research expeditions.

The SETI Institute's Proposal for NAI Education and Public Outreach (EPO):

Understanding the origins, evolution, distribution and future of life in the universe engages students, teachers and the public in the great adventure of 21st century science. Our Education and Public Outreach (EPO) program goal is to share the cutting-edge research and discoveries of astrobiology with educators and the public in order to inspire students to pursue careers related to astrobiology and to enhance science literacy.

The EPO program for the SETI Institute's "Planetary Biology, Evolution, and Intelligence" our NAI proposal focuses on these key audiences: (1) high school science teachers at schools with underserved students in urban, rural and minority communities; (2) science museum visitors--students, teachers, and families; and (3) the general public.

The SETI Institute's EPO programs have a long and successful history of engaging our scientists and educators with these key audiences in formal and informal education, and public outreach. Today, we have a broad and active EPO program. (See Table 4 below). The NAI-EPO activities we propose here will integrate into the Institute's ongoing programs to take advantage of natural synergies with existing projects and engage with the networks of people and institutions that have been developed by the Institute's EPO team over the past dozen years. This will substantially leverage NASA's investment in NAI-EPO. The Institute's proposed NAI-EPO program is comprised of five major activities:

- Conduct professional development for high school science teachers implementing *Voyages Through Time(*VTT*)*, and make VTT a resource available to the entire NAI
- Collaborate with the California Academy of Sciences for exhibits and outreach
- Facilitate education outreach, media opportunities, and public events that feature the Institute's NAI scientists
- Participate in the NAI EPO network and NASA OSS and Education Activities

The Institute's NAI EPO program will be developed and conducted in compliance with the OSS EPO guidelines described in *Partners in Education (1995)* and *Implementing the Office of Space Science Education/Public Outreach Strategy (1996)* as are the other NASA-funded EPO programs conducted by the SETI Institute.

The Institute's NAI EPO program will be directed by Co-I Edna DeVore (5%), Director of Education and Outreach at the Institute, with Pamela Harman (15%), Education Manager. Dr. Kathleen O'Sullivan, Professor of Science Education at San Francisco State University (SFSU) joins the team for her sabbatical in spring 2004 at no cost to the program. DeVore is an experienced educator and has more than a decade of experience developing and directing EPO programs for NASA missions and the SETI Institute. Like DeVore, Harman is an experienced classroom and NASA EPO educator, most recently presenting a teacher professional development workshop at the 2003 NAI Meeting at ASU.

Professional Development & *VTT*

*Voyages Through Time (*VTT*)* is an astrobiology curriculum for integrated high school science. It is a standards-based course centered on the unifying theme of evolution, that is delivered on CD-ROMs plus printed

readers. Over a period of five years at a cost of $3.3 million, the SETI Institute brought together scientists, teachers, curriculum writers, and media specialists to create six modules that integrate astronomical, geological, and biological sciences. They are:

- *Cosmic Evolution*: origin of the universe, life cycles of stars, & formation of planets
- *Planetary Evolution*: study of Earth, Venus and Mars, & what makes a planet habitable
- *Origin of Life*: origin hypotheses, common chemistry, life on Earth, multicellular life
- *Evolution of Life:* the great diversity & diversification of living things
- *Hominid Evolution*: processes & events in hominid evolution
- *Evolution of Technology:* technologies that meet human needs, evolution & impact of technology

Individually, the modules are appropriate as supplementary materials for Earth Science, Biology, Life Science, Astronomy, and Space Science courses. Together, the six modules comprise one year of science for grades 9 or 10. The VTT curriculum is based upon the *National Science Education Standards* and *The Benchmarks for Science Literacy*. The goals and objectives of these two key documents are specifically referenced and stated throughout the curriculum materials at both the module and lesson levels.

VTT's themes, goals and objectives align closely with the Fundamental Questions and the Goals and Objectives of NASA's astrobiology program (The Astrobiology Roadmap, 2002) and introduce students to astrobiology early in their education. VTT's goal is to engage students in understanding that evolution is the result of cumulative changes over time; that it occurs in all realms of the natural world; and that various processes underlie evolving systems and organisms as reflected in the differing time scales and rates of change. The intent is that students understand connections and relationships across these realms of change, and learn that science is a process of advancing our understanding of the natural world, rather than a set of final answers. The major overarching questions posed in each module are: What is changing? What is the rate of change? What are the mechanisms of change?

The VTT project was led by J. Tarter (PI) and E. DeVore (SETI Institute); M. Burke and S. Taylor at California Academy of Sciences (CAS), K. O'Sullivan at SFSU (a minority institution), and Y. Pendleton at NASA ARC. The NSF IMD Grant #9730693 provided major funding with additional assistance from the Foundation for Microbiology, Hewlett Packard Company, Educate America, and NASA's Astrobiology Institute and Fundamental Biology programs. The SETI Institute (a non-profit organization) is publishing VTT in partnership with *Learning in Motion*, a woman-owned small business that develops and publishes software products for K-16 education. VTT will be available commercially in summer, 2003.

The proposed VTT Teacher Professional Development (VTT-PD) will feature a 5-day, summer institute for 20 teachers plus NAI EPO participants each year. (Total is 100 teachers plus NAI participants over 5 years.) Other NAI EPO teams will be invited to participate in the VTT-PD with funding from their home institution. One VTT-PD institute will be held each summer, calendar year 2004 -2008. As recommended by the *National Science Education Standards*, the VTT-PD teachers will participate in science content training led by scientists from the Institute's NAI team, NASA ARC, and other collaborating organizations such as CAS, hands-on training with curriculum materials, planning time for classroom use, and on-going, school-year online-mentoring via the VTT-PD web site at the SETI Institute. The VTT Online Community web site will be separately developed by the Institute and *Learning in Motion* to support VTT implementation and represents cost-share on this proposal.

Each VTT-PD teacher will receive a set of the 6 VTT modules, and a stipend to be used for additional materials or continuing education college credit. We anticipate holding the VTT-PD institutes at various regional sites in the San Francisco Bay Area and greater Northern California. Potential venues are regional high school districts, NASA ARC in Santa Clara County, CAS in San Francisco, Chabot Space and Science Center in Alameda County, CSU Monterey Bay in Monterey County, The Tech Museum in Santa Clara County, and Shasta County Office of Education which serves rural northeastern

counties. These sites represent an extension of the collaborations that developed the VTT curriculum as well as a network of other organizations working with the Institute's current EPO projects. Continuing education credit for participating teachers will be offered via SFSU or San Jose State University (SJSU), both minority institutions well-known for training science teachers, and universities with existing relationships with the Institute's EPO programs. Dr. K. O'Sullivan, Professor of Science Education (SFSU) will spend one semester on sabbatical at the Institute spring 2004 to collaborate on planning the VTT-PD institute, and additional dissemination strategies and proposals for VTT. Dr. O'Sullivan's participation is evidence of ongoing collaboration of the VTT development team, and represents institutional commitment of both SFSU and the Institute. Teachers will be recruited for VTT-PD specifically from school districts dominated by minority and under-served students with the goal of assisting these teachers and schools to effectively implement a tested, research-based, standards derived curriculum for freshman or sophomore science. As California is a minority-majority state, and the schools of California reflect great ethnic and cultural diversity, we expect to achieve this goal. The VTT-PD time-line appears in Table 3.

The VTT-PD institute program will be evaluated by the participants and over duration of the 5-year program and will be tracked via regular communication with the teachers and schools to understand the implementation of the curriculum in the classrooms. Evaluation tools will be developed with the assistance of Dr. O'Sullivan during her sabbatical. O'Sullivan's participation is cost shared.

VTT-PD Events	**Summer 03**	**School Yr. 03-04**	**Summer 04**	**School Yr. 04-05**	**Summer 05**	**School Yr. 05-06**	**Summer 06**	**School Yr. 06-07**	**Summer 07**	**School Yr. 07-08**	**Summer 08**
NAI proposal awarded											
VTT Published											
Build *VTT* Online Community											
Recruit teachers & schools											
Plan workshop											
Conduct workshop											
Evaluate & Report to NAI											

Table 3: VTT-PD Timeline

Collaboration with CAS

The California Academy of Sciences (CAS) is the oldest scientific institution in the West and houses one of the largest natural history museums in the world, attracting about 1 million visitors each year. The CAS Education Division serves over 400,000 participants, many of them from urban, disadvantaged backgrounds. While the main museum in Golden Gate Park is undergoing major renovation beginning January 2004, a temporary facility with exhibit and aquarium facilities will be open in downtown San Francisco. Over the 4 years of construction, the Institute's EPO team will collaborate with CAS by providing scientific and educational expertise, and by co-sponsoring and participating in CAS EPO activities. In addition to collaboration for VTT-PD, the Institute's scientists will act as scientific and educational advisors for CAS in planning and developing exhibits for the new facility on the theme of "Earth and its Place in the Universe." Because CAS scientists are primarily involved in systematic and organismal biology, the Institute's expertise will complement existing resources and enrich the physical science and astronomical components of the new exhibitry. The Institute will work closely with Drs. M. Burke and C. Tang (a CAS astrobiologist and educator) to coordinate efforts. This will be collaborative work that grows from the long-term relationship between the Institute and CAS which has been both collegial and formal

as partners in the development of curriculum materials, presentation of courses and workshops, and lectures at CAS. Beyond advising on future exhibits and programs, the Institute will be a co-sponsor for select CAS education outreach programs. The opportunity for continued and ongoing collaboration with CAS's education group effectively leverages NASA's EPO funding through NAI. The Institute will participate in and provide financial support (see Table 5) for the CAS education program as follows:

(1) ScienceNow, museum exhibit: The Institute NAI EPO program will contribute scientific information, images, and expertise for the development of two ScienceNow panels each year for public display. The panels will also be featured and archived on the CAS web site.

(2) New Exhibit Development for "Earth in its Place in the Universe": The Institute's EPO team will identify and support NAI team members as advisors and reviewers for development of exhibits and classroom materials providing scientific and educational expertise. Included is support for new exhibit prototyping and "road testing" of materials for both exhibits and curriculum.

(3) Public Education: Institute scientists and educators will be speakers and presenters for educator (teachers, docents, staff and interns) workshops, the BioForum series, and other public lectures such as Member's Lectures, the Planetarium's Dean Lecture series, the Leakey Series, book signings, and other Special Forums. Support for these events is provided in the Institute's proposed EPO budget. A letter of support from CAS affirming the collaboration appears in Appendix C.

Education Outreach, Media Opportunities and Public Events: As detailed in Table 4, the Institute has an active and far-reaching EPO program supported by the EPO team and the Institute's Public Information Office that often works in close coordination with NASA's Public Information Office with media contacts for our PIs and Co-Is. The Institute's NAI team on this proposal has been involved in our Public Outreach Program activities, and we plan to facilitate additional opportunities for them over the next 5 years. Further, the Institute's EPO team conducts an active program of workshops and short courses at regional and national science teacher meetings; NAI-EPO workshops and short courses will be proposed and conducted as a part of our overall outreach for educators..

NAI EPO Network Participation

The Institute expects to participate fully with other NAI members in the NAI EPO virtual network to share successful EPO programs and projects. For example, other current NAI members have already expressed interest in conducting VTT workshops or institutes at their sites. The Institute's planning and strategies will be shared openly; Institute staff could be available to assist at other NAI sites as appropriate. The SETI Institute expects to provide activity reporting in a timely and accurate way to NAI Central. Because of the experience as the NASA EPO lead on other projects, the Institute's EPO team understands the requirements for such reporting. The Institute anticipates participation in the OSS Origins Forum, and other NASA programmatic activities related to EPO in concert with the Institute's other NASA EPO program requirements.

Curriculum Projects for Formal Education
• *Voyages Through Time, (VTT)* a standards-based, astrobiology curriculum for high school integrated science centered on the unifying theme of evolution • *Life in the Universe Series (LITU Series)*a supplementary series of six science teaching guides for grades 3-9 on the theme of the search for life in the universe
Outreach & Professional Development for Science Educators
• *Australian-American Fulbright Symposium 2002: Science Education in Partnership*, E. DeVore US co-chair with K. Wilmoth, NAI, and C. Oliver and L. Vozzo of the Australian Centre for Astrobiology and University of Western Sydney; held simultaneously with the *IAU Symposium 213 Bioastronomy Conference: Life Among the Stars*, July 2002 • *Educator Day for Division of Planetary Sciences*, Institute EPO staff are the lead organizers for the DPS day-long teacher workshop, Sept. 2003 • *Short Courses for Science Teachers*, professional development courses for *SOFIA, LITU Series, VTT*, and others at major regional and national science teacher meetings, 10 over past 2 years

• *Workshops for Science Teachers*, 1-2 hour workshops regional and national science teacher and astronomy meetings, 130 over the past 6 years • *Invited Talks*, SETI and astronomy including SOFIA and Kepler, teaching astrobiology and evolution, and teacher professional development at American Association of Physics Teachers, American Geological Institute, and several different State Science Teacher Associations
Online, Science Museum & Planetarium Projects for Informal Education:
• *Other World's, Other Beings*: interactive science museum exhibition and 2 planetarium programs developed by Pacific Science Center; Institute advised on all exhibits; the Institute's SETI scientists were featured in interactive kiosks; and the Institute's EPO team produced the two planetarium programs (1997-present: on exhibition) • *Cosmic Origins:* interactive science museum exhibition under development by the Space Science Institute involves SETI, NAI, *SOFIA*, *Kepler*, *VTT* and the *LITU series* (NSF & NASA funded) • *SETI*, an animated, video planetarium program developed with the National Space Center, UK • *NASA's QUEST* and *Women of NASA* feature several Institute projects and scientists • *Virtual Field Trip to Lincancabur*, an IDEAS project led by Co-I N. Cabrol that supported a virtual field trip for students and the public during her exploration of the world's highest lake, Fall 2002, in cooperation with Shasta County Schools, a rural underserved community
NASA Mission Education & Public Outreach Programs
• *SOFIA*, NASA's Stratospheric Observatory for Infrared Astronomy; EPO program that trains and integrates K-14 educators into the research environment onboard *SOFIA* (1996-current) • *Kepler*, NASA's Discovery Mission that seeks evidence of Earth-like planets in habitable orbits around Sun-like stars (2002-2011) • *ABE*, Astrobiology Explorer, a pending mission with a consortium of EPO groups; the Institute's participation will support dissemination of *Voyages Through Time*.(pending) • *Origins Forum*, Institute staff participate fully in OSS's Origins Forum (ongoing)
Public Outreach Program
• *Public Lectures:* Institute scientists have presented more than 100 public talks at science museums, civic organizations, colleges and universities throughout the nation and world during the past year • *Television Documentaries*: Institute scientists are often featured in documentary programs, e.g., Voyager Missions, SETI, solar system exploration, comets and meteors--all related to astrobiology • *Are We Alone?* A weekly one-hour talk radio show that emphasizes the search for life in the universe • *SETI: The Search For Life* on *Space.com*: a weekly column by Institute scientists and educators on since November 2000, more than 100 articles published on astrobiology-related topics • *SETI Institute News*: the quarterly publication of the SETI Institute • *Silicon Valley Astronomy Lecture Series*: co-sponsor with Astronomical Society of the Pacific, NASA Ames Research Center, and Foothill Community College, the host institution • *SETI Institute Web Site:* established 1994; the Institute's popular web site provides information on the scientific search for life in the universe to over 100,000 unique visitors each month. • *Astronomy & SETI Classes for the Public*: taught for California Academy of Sciences and Elderhostel • *CONTACT*: provided advice and role models for this feature film starring Jody Foster
Recent Textbooks and Popular Books:
• *Life in the Universe*, J. Bennett, S. Shostak, & B. Jarkowsky, college introductory astrobiology 2003 • *The Everything Astronomy Book*, C. Phillips (Co-I on proposal) and S. Priwer, introductory astronomy for the general public, 2002 • *Cosmic Company*, S. Shostak & A. Barnett, companion to *SETI* planetarium program, 2003 • *SETI 2020: A Roadmap for the Search for Extraterrestrial Intelligence*, Institute's report of the Science and Technology Working Group that outlines the future of SETI research, 2001

Table 4: SETI Institute's Current Education and Outreach Programs

Facilities and Equipment

Section 2.

Co-I Freund has the necessary lab space at NASA Ames including access to collaborator Dr. Rothschild's labs. These two labs are located at NASA Ames Research Center and include shared facilities with SETI PI Rocco Mancinelli. Jointly the labs have all of the basic equipment for studies of microbial physiology, DNA damage and ecology including a Zeiss Axioscope microscope with phase optics, fluorescence, DIC optics and both film and digital cameras with OpenLab software for image analysis. In addition, their labs have assorted equipment for biochemical and molecular analyses (e.g., speed vac centrifuge, thermal cycler, horizontal and vertical gel electrophoresis equipment, UV and white light boxes and cameras). Centrifuges, autoclaves, combustion oven and drying ovens are all available on the same floor. An EPA-certified analytical lab is located one floor below Rothschild's lab. This lab uses EC-HPLC to analyze DNA that Rothschild's lab prepares for analysis of DNA damage.

Section 3.

Co-I Bakes currently has all the facilities and computational resources necessary at NASA Ames Research Center to investigate quantum photochemical models of Titan's haze.

Section 4.

Dr. Bishop has laboratory space at NASA-Ames Research Center (building 239, SSX Branch) for synthesis of the iron oxide/oxyhydroxide minerals as described in section 4. Collaborator Dr. Rothschild has two labs at NASA Ames Research Center and shared facilities with SETI PI Rocco Mancinelli. Jointly the labs have all of the basic equipment for studies of microbial physiology, DNA damage and ecology including a Zeiss Axioscope microscope with phase optics, fluorescence, DIC optics and both film and digital cameras with OpenLab software for image analysis. Additionally, they have a model Z1 Coulter Counter, a UV/Visible plate reading spectrophotometer and fluorometer (Molecular Devices), glassware, balance, pH meter, microprobes for pH, O_2 and CO_2 measurement, a Li-Cor model LI-185B quantum/ radiometer/ photometer with an spherical sensor, a broad band UVA/UVB meter (Solar Light Company), an underwater temperature sensor, several microfuge centrifuges, a radioisotope hood, anaerobic hood, gas manifolds, incubators, freezers, chromatography refrigerators, and assorted supplies. In addition, their labs have assorted equipment for biochemical and molecular analyses (e.g., speed vac centrifuge, thermal cycler, horizontal and vertical gel electrophoresis equipment, UV and white light boxes and cameras). Centrifuges, autoclaves, combustion oven and drying ovens are all available on the same floor. An EPA-certified analytical lab is located one floor below Rothschild's lab. This lab uses EC-HPLC to analyze DNA that Rothschild's lab prepares for analysis of DNA damage.

One piece of specialized equipment is described under "Iron, the Oxygen Transition, and Photosynthesis" (Bishop and Rothschild), a constant-temperature water bath on the roof. This facility was custom-built with a long, six-inch deep plexiglass container, recirculating water and a heater and cooler. Thus, organisms can be grown exposed to natural solar radiation but grown at a set temperature.

Section 5.

Co-Is Cabrol and Grin have access at to all the equipment and facilities necessary for the completion of this investigation at NASA Ames Research Center, UCLA, and Universidad Catolicà del Norte (UCN), Chile. This includes: I) General: Access to UNIX machines, Macintosh computers, color, black & white printers, map printers, and software (e.g., Statistica 4., Mathematica, Excel, Deltagraph, Kaleidagraph, Adobe Photoshop 5.1, GraphiConverter, NIH Image 1.6.1, Word, and Powerpoint) for processing of both images and data allowing mapping, modeling, and 3-D rendering of data; Video conference room of Ames as well as conference phone lines for telecons. II) for "Geophysics": computer time and CPU for analysis of logger and

on site data; III) "Chemistry": laboratory (care of UCLA Dept. of Civil and Environmental Engineering) for Ion Chromatography, use of UCLA Earth and Space Sciences Inductively Coupled Mass Spectrometer; IV) For biology, use of NASA Ames Research Center lab (see Lynn's Rothschild facility description), including cold chambers for preservation of samples; Use of UCN biology laboratory; V) GPS units, RangeFinders and laser target, field flags, soil, water thermometers, soil temperature profiler (heat flux probe) + short term logger (small PC or similar), hobo environmental dataloggers (for water temp, air temp, relative humidity), ELDONET UV dosimeters, UV acrylite plates, sample containers as well as lab equipment that may be necessary at the time of analysis (flasks, additional reagents, pippettors, etc.), water sampling kits, DGGE equipments, microscopes, culture facililties.

Section 6.

Co-I Amos Banin will perform advanced soil and water research and analyses laboratory at the Department of Soil and Water Science, Hebrew University, Rehovot. Laboratories are well-equipped for advanced analyses of soils and water, using ion selective electrodes, various potentiometric methods, UV-VIS spectroscopy, pyrolytic DOC analysis and microwave analytical digestion system (CME) for controlled-conditions (temperature and pressure) digestion of organic and mineral materials. Mineralogical analyses employing XRD and DTA and TEM and SEM instrumentation. Available at the Interdepartmental Equipment Unit of the Faculty of Agricultural, Food and Environmental Quality Sciences in Rehovot are: Two advanced ICP-AES instruments (Spectro) for multi-elemental analysis in high-electrolyte matrices. A state-of-the-art ICP-MS instrument (Elan 6000, Perkin-Elmer) has in general 2 - 3 orders-of-magnitude better detection limits than the ICP-AES for trace metals in low-electrolyte matrices, enabling analyses at the ng/l (ppt) range. Pyrolytic-GC elemental analysis system (Fissons EA 1108) for analyses of C, N, H, S, O, in solids, liquids and gases has a measuring range of concentrations from 0.01% to 100%.

Co-I Rocco Mancinelli has lab space at NASA Ames Research Center and shared facilities with collaborator, Dr. Lynn Rothchild. These are described in section 4. Additionally, this team has constructed on the roof of the laboratory building an artificial intertidal facility for growing microbial mats in the open air using artificial sea water.

Section 7.

Dr. Summers has access to laboratories at the NASA Ames Research Center. His laboratory is equipped with chemicals, glassware, analytical balances sonic bath, strip chart recorders, pH meters, ovens, centrifuges, thermostatic baths, fumehoods, etc. The laboratory has a nitrogen line for purging samples and glassware. A vacuum pump is available for conversion to a schlenk line. The laboratory contains a 450 watt medium pressure mercury arc lamp, a CHEMTRIX Type 45 pH controller with Sage Inst. 341B syringe pump, and a glove box. The usual computers (PC and Macintosh) are present. Also available are a Hewlett Packard Series II Gas Chromatographs with a 5971 Series Mass sensitive Detector, a Nicolet Nexus 670 FT-IR, a Cary 3 UV-visible spectrophotometer, a Dionex Ion Chromatograph, a HP 1084 HPLC, A Technics Lab One reverse osmosis water purification system, a Hitachi 4000 FESEM with Noran Voyager EDS, and a Modified Phillips X-ray Diffraction. (All instruments are attached to suitable computers.) Co-I, Dr. Khare has much of the necessary specialized glassware already made up and has some additional gas handling equipment. Dr. Khare also has access to facilities in the Center for Nanotechnology to conduct the proposed research. Available are: Lyman-alpha source; reactor set-up; FTIR, Raman, UV-Vis-NR, NMR, SEM, TEM, AFM and STM and other characterization facilities; ion beam sputtering; general chemistry lab facilities; plasma generator.

Section 8.

At the Center for the Study of Life in the Universe at the SETI Institute (which Dr. Chyba directs), Drs. Phillips and Chyba have access to the full computational resources and imaging necessary to complete their research under section 8 of this proposal.

Section 9.

At the Center for the Study of Life in the Universe at the SETI Institute and at Stanford University where Mr. Hand is a graduate student, Drs. Hand and Chyba have access to the full computational resources necessary to complete their research under section 9 of this proposal.

Section 10.

Dr. Bernstein has all of the equipment needed to carry out the tasks described in this proposal, currently available at NASA-Ames Research Center. This includes multiple high vacuum ice simulation chambers with closed cycle helium refrigerators, IR spectrometers, various sources of UV radiation, two electron sources, and analytical techniques. More information about the scientists, the Astrochemistry Lab, ongoing projects, recent publications and pictures of the facilities can be found on line at the Astrochemistry Lab web site at http://www.astrochem.org

A. Sample Preparation

In order to reproduce the unusual conditions experienced by an ice on the surface of an icy satellite we employ a high vacuum - closed cycle helium cryostat apparatus. With this equipment we can (under a dynamic vacuum at ~10-8 Torr) hold a substrate anywhere from 12 to over 300 K. It is on this substrate that the ice analog is formed as a thin film of ice by vapor deposition. Volatile samples (such as water vapor) are introduced into the stainless steel vacuum system as gases that are previously mixed in glass bulbs using a glass manifold at room temperature. Non volatiles, such as PAHs are vapor deposited from an evacuated Pyrex tube, heated if needed. Our cryostat is mounted on a double O-ring seal so once the sample is deposited we can rotate the window to face the source of UV radiation, or electrons. We have many UV sources including mercury, deuterium and xenon lamps but typically use a microwave-powered, hydrogen flow, discharge lamp for the production of UV radiation (C.2). We have a Kimball Physics EGG electron gun which allows us to irradiate our sample with electrons tuned to any energy between ~100 eV and 10 keV, and a 20 keV electron gun extracted from a CRT. After irradiation (and spectroscopy) at low temperature we warm the sample monitoring the evolution of the ice using IR spectroscopy. Once the volatiles have sublimed away, the residual, non-volatile, organic film that remains is washed from the substrate with water and analyzed by attaching a chiral fluorescent tag to any primary amines and separation by HPLC, or by attaching a trimethylsilyl (TMS) functional group to reactive -OH and NH2 moieties followed by analysis by GC-MS.

B. Sample Analysis:

IR Spectroscopy: Our experimental set-up allows us to take infrared (IR) spectra at low temperature while an experiment in progress so we use IR spectroscopy on the low temperature ices to check the starting materials before the reaction has begun and to monitor the progress of the reaction. Also, we use our IR spectrometer to monitor volatile molecules that sublime from the ice as it is warmed under vacuum, and take spectra of the non-volatile organics that remain. We have a Nicolet 740 IR spectrometer with a spectral coverage from 12,000 to 600 cm-1 (0.83-17 mm) and a Bio Rad excalibur with a spectral range of 15,000 to 75 cm-1 (0.67-130 mm) both which have a resolution of 0.5 cm-1.

GC-MS: Identifications are based not only on the retention time but also the mass spectrum on the unknown, and this allows us to perform isotopic labeling experiments and more securely identify unknown compounds. We use an HP 5971 series II GC-MS and bis[trimethylsilyl] acetamide to derivatize the residual organic materials from our simulations.

HPLC: We use an Hewlett Packard 1100 series HPLC consisting of a ternary pump with both a diode array UV detector and a four channel fluorescence detector. This HPLC, used in conjunction with a variety of columns (reverse phase, anion, cation, etc.), is an ideal configuration for the separation and detection of a wide range of polar compounds. While many compounds can be detected via their UV absorption, fluorescence generally increases sensitivity by orders of magnitude, so we intend to use fluorescence labeling to increase our sensitivity.

Section 11.

The work undertaken by Drs. Backus and Tarter to generate an enlarged list of 'Habstars' for the SETI targeted search with the Allen Telescope Array, will involve extensive manipulation of large data catalogs. All of these will be acquired prior to the commencement date of this proposal by exchanging adequate disk storage medium with the USNO or directly from the CDS portal in Strasbourg. Construction of the new target list is expected to be feasible with the existing computational resources of the SETI Institute. If additional capacity is required, it will be acquired as a charitable donation (the SETI Institute has enjoyed consistent success with this approach), or provided from funds available in Dr. Tarter's endowed chair, and constitute additional matching funds to this proposal.

University of Arizona
Tucson, AZ

An Astronomical Search for the Essential Ingredients for Life: Placing our Habitable System in Context

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The Astronomical Search for the Essential Ingredients for Life: Placing Our Habitable System in Context

Executive Summary

We plan to carryout three investigations concerning the astronomical constraints on the possible origins of life in the Universe.

Our first module will focus on astrochemical investigations concerning the building blocks of life. Under the leadership of L. Ziurys, we plan to: (i) undertake laboratory studies to determine signatures for pre-biotic compounds that might exist in the interstellar medium, with special emphasis on ribose; (ii) initiate an observational search for these gas-phase molecules that are important for life using millimeter and sub-millimeter radio telescopes to which we have access; and (iii) conduct theoretical studies into the stability of complex organic molecules in the gas phase ISM and the evolution of their abundances in circumstellar disks in order to interpret the broader implications of our findings for the possibility of life elsewhere in the Universe. This module will address Goal #3 in the NAI revised roadmap.

Our second module will focus on the formation and evolution of habitable worlds under the leadership of S. Strom and J. Najita (NOAO). We plan to: (i) undertake an observational program to learn when, when, where, and how frequently planets form around young stars to provide the possibility for constraints on habitable planet formation; (ii) conduct an observational characterization of circumstellar environments that give rise to life and initiate a theoretical modeling program to determine the frequency of giant impacts as traced through circumstellar dust disk evolution, connecting the evolution of dusty disks around sunlike stars to the history of our solar system; and (iii) investigate the time evolution of the UV/x-ray flux of young solar-type stars from the protstellar phase through the epoch of terrestrial planet formation to mature planetary systems. The investigation will also include studies of stars that have evolved further than the Sun, so as to predict the future variability of the Sun. This module addresses Goal #1 of the NAI Revised Roadmap, as well as aspects of #4 and #6.

Our third module, under the leadership of R. Angel and P. Hinz and is aimed at the characterization of planetary systems. We plan to: (i) initiate an observational program aimed at the direct detection and characterization of astrobiologically relevant extra-solar giant planets (EGPs) to determine their frequency around solar-type stars and characterize their composition through spectroscopy; (ii) initiate a theoretical study of giant planet atmospheres extending current modeling efforts down to 0.1 $M_{Jupiter}$ as a first step in understanding the detectability of biosignatures in planet atmospheres with liquid water present; and (iii) extend observational work on the observed earthshine spectrum to near-infrared wavelengths to close a gap in our understanding as well as initiate a monitoring program. This module addresses goals #1 and #7 in the NAI Revised Roadmap.

Our special emphasis on the revised roadmap Goal #1 arises because of current lack of understanding of how the Solar System arose, and how it fits with the many other planetary systems which are being discovered, and which have substantial differences from the Solar System. Our goal is to set a better, more appropriate starting point for consideration of the origin of life, and to start to explore the question of whether Earth is or is not rare.

Our plans to strengthen the astrobiology community revolve around the creation of the Laplace Center for interdisciplinary astrobiology studies and the Astrobiology Winter School to be held annually here at the University of Arizona. The Laplace Center will be an interdisciplinary program (IDP), a standard system for interdisciplinary research and teaching at UA. It will serve as a focus for our interdisciplinary research efforts and strengthen the growing ties between the Departments of Astronomy, Planetary Sciences, NOAO, Geosciences, Chemistry and Biochemistry. Future plans call for including also Microbiology

and Ecology and Evolutionary Biology as well as the Tree Ring Laboratory, Optical Sciences, and parts of the Medical College.

The Center will host 2-3 extended visitors per year and organize meetings to increase scientific interactions across the boundaries that exist within the College of Science. The Winter School will train up to twenty students per year over the period of performance of this proposal. We plan to host visiting faculty for up to two months per year from partner NAI node institutions as well as 10 graduate students for a four-month curriculum in the origins of life. In collaboration with colleagues throughout the College of Science we will offer two interdisciplinary courses for graduate credit that focus on the boundaries between the disciplines and take advantage of the unique observational and laboratory facilities on the University of Arizona campus.

Our efforts to "Strengthen the Astrobiology Community" will include production of first rate scholars in areas important to the future success of the field. In addition to extant interdisciplinary graduate programs at the University of Arizona such as those in Planetary Science (Astrobiology Minor), Chemistry (Astrochemistry emphasis), and Optical Sciences (various joint degree programs) we will introduce an Astrobiology minor as part of the IDP for the departments within the College of Science.

A particularly innovative part of this proposed project is for a significant and integrated education and public outreach component led by Tim Slater. Because a comprehensive program to improve the public's understanding of the this interdisciplinary science requires targeting schools, we will work directly with secondary school teachers on improving their understanding of the myriad of underlying concepts surrounding the search for other worlds. Initially, we plan to conduct systematic studies of the understanding and beliefs about the scientific search for other worlds held by K-14 students, teachers, and the general public. In addition to contributing to the scholarly literature base of science education, a detailed understanding of the existing notions and attitudes people have about this interdisciplinary science are crucial to designing the most effective education and public outreach programs. This will be accomplished through a systematic series of surveys, interviews, and carefully monitored instructional interventions. The results will be disseminated through journal articles and presentations at professional education conferences.

Finally, we also plan to help other graduate students and university faculty supported by this program to become informed about the reasoning difficulties K-12 students, teachers, and the general public have with understanding the search for other worlds. If research scientists have an appreciation for the specific parts of this science that people find difficult, they will be better able to communicate the exciting results and enhance the public's attitudes toward supporting this endeavor. This will be accomplished by conducting frequent workshops and contributing scholarly papers at professional science conferences as well as regularly contributing to the program's seminar series. In support of these efforts, the team will undertake the creation and dissemination of an astrobiology public speakers toolkit and a dynamic FAQ (frequently asked questions).

Summary of Personnel, Commitments, and Costs

Section deleted

An Astronomical Search for the Essential Ingredients for Life: Placing Our Habitable System in Context

I. Introduction

I.1 Rationale for the Proposal.

The ultimate goal of astrobiology is to understand the origin of life and its prevalence in the universe. There would be no purpose for astrobiological studies if it were known that life is unique to the Earth or even to the Solar System. If it is not, astrobiology studies must include investigation of a wide range of phenomena that have traditionally been viewed as principally astronomical in nature. These include the composition and dynamics of material involved in the process of star and planet formation from its initial state in interstellar clouds to its final state in planetary systems. It is also critical to understand the nature of planetary systems, the stability of the planetary environment and the influence of this environment on the evolution of life forms.

Progress in each of these areas depends on the developing the right overall balance of astronomy, geosciences, chemistry and biology input in NASA's Astrobiology Institute (NAI) as contributed by each of its individual members. It is also important to develop a common language and understanding between the various sub-disciplines, especially among the young scientists entering the field. As noted by the National Academy Committee on Extra-Terrestrial Life (COEL), the NAI program would benefit from strengthening its astronomical aspects. In submitting this proposal, we seek to contribute to the pioneering efforts at the NAI, building on the strengths and interests of the proposing organizations, the University of Arizona (UA) and the National Optical Astronomy Observatories (NOAO), both based in Tucson, Arizona. Our goal is to bring our existing strengths together to focus on the needed astronomically oriented aspects of astrobiology and to ensure that these are well meshed with other parts of the NAI program. We believe that the search for the origin of life, and the related question of the frequency of the occurrence of life in our galaxy and the universe are potentially the most interesting and challenging topics in all of 21st century science.

I.2 The Astrobiology Challenge

The current goals of NASA's Astrobiology program and hence for the NAI have been set out in the 2002 Astrobiology Roadmap. They are, in no particular order of priority:

1. Understand the nature and distribution of habitable environments in the Universe.
2. Explore for past and present habitable environments, prebiotic chemistry and signs of life elsewhere in our Solar System.
3. Understand how life emerges from cosmic and planetary precursors
4. Understand how past life on Earth interacted with its changing planetary and Solar System environment.
5. Understand the evolutionary mechanisms and the environmental limits of life.
6. Understand the principles that will shape the future of life both on Earth and beyond.
7. Determine how to recognize signatures of life on other worlds and on early Earth.

This is indeed a challenging list of objectives and explains why the NAI is a virtual institute by necessity. It is recognized within and outside of NASA that no single academic or research institution has or can have the breadth of research disciplines to meet all the Astrobiology

Roadmap goals. In its short history thus far, it has been helpful for the NAI to get started with specialized organizations, but the progress of astrobiology requires broader range of science capabilities than is currently in the NAI mix. Thus the COEL, in its review of NASA's astrobiology program, notes:

> *" The "astro" in astrobiology: Astronomy remains the key fundamental discipline that has yet to fully impact the discipline of astrobiology."*

and

> *" The research interactions between these two areas [Astronomical Origins and Astrobiology] seems much weaker at the moment than they could be, and certainly much weaker than between Astrobiology and the evolutionary biology or geobiological communities."*

The goal of the present collaboration is to contribute a strong astronomical element to the NAI program and to develop connections with chemistry and biochemistry. We believe that, in this way, we can help strengthen the overall NAI program in exactly the way envisaged by COEL.

I.3 The Proposed Collaborators

This proposal represents a collaboration between the UA and NOAO. The UA is a major public university in the southwestern United States with a dual mission in research and education in fields relevant to the NAI such as astronomy, planetary sciences, chemistry, geosciences, and the biological sciences. The NOAO is a major national institution for research in astronomy located on the UA campus. Both institutions can offer access to significant research facilities, with observational capability over the wavelength range from 3mm to 0.3 microns. The UA will, through Steward Observatory, be able to support the work proposed here with access to optical/infrared telescopes such as the Large Binocular Telescope (11.8m effective aperture), the MMT (6.5m), many smaller telescopes in the Tucson area, and access to the twin Magellan 6.5m telescopes in the southern hemisphere. It will also provide access to mm-wave (12m) and submm-wave (10m) telescopes. NOAO operates observatory facilities both in Tucson and in Chile. Through the UA, the collaborators can address another COEL concern, namely the needed access to major astronomical facilities.

> *" Much larger optical telescopes, of mirror aperture 6 meters or larger and equipped with advanced adaptive optics capabilities, are required to examine the broader class of extra-solar planets that are not in the unusual orbital orientation necessary to make studies of transit possible. Such facilities, supported outside of NASA with other federal, private or state funds, represent a substantial monetary contribution to the field of astrobiology, on a level of hundreds of millions of dollars in infrastructure."*

In addition the UA has major programs in astrochemistry, planetary sciences and optical sciences that will contribute to a Tucson node of the NAI. The science of astrobiology and the activities of an Astrobiology Institute will thrive in a university setting where cross-disciplinary interactions can occur readily.

I.4 Proposed Location

We also believe that establishing an Astrobiology node in Tucson will also bring enormous benefits to the national astrobiology program. In addition to the collaborating institutions, there are many other organizations that own or share astronomical facilities based in Tucson. These include the National Radio Astronomy Observatories, the Harvard Smithsonian Center for Astrophysics, international organizations such as the German (Max-Planck-Society) and Italian (Istituto Nacional di Astrofisica) partnership in the Large Binocular Telescope, as well as individual private and public universities. Thus Tucson is a crossroads for the international astronomical community. An astrobiology center in Tucson would have a wide reaching impact on astronomical studies as well as providing a forum for interdisciplinary research across the fields of the physical and biological sciences throughout the University of Arizona. It would also complement the more geology-oriented efforts being made at the Arizona State University under the NAI program. The Center will be housed in the Astronomy Department/Steward Observatory building on campus, in close proximity to the Planetary Sciences Department/Lunar and Planetary Lab and the NOAO headquarters building.

I.5 Proposal Overview

Our proposal consists of three research modules, which range from biochemistry to astrophysics but focused on the astronomical origin of and likely harbors for life. These three modules deal respectively with questions in astrochemistry, planet formation and evolution, and characterization of extra-solar planets. They are summarized in section I.8. Section 6 describes an education and outreach plan designed to strengthen astrobiology in the community and to develop the next (perhaps the first) generation of true astrobiologists.

If selected, we plan to create a center, the **L**ife **A**nd **PL**anets **A**strobiology **Ce**nter—the **LAPLACE**—within the College of Science at the UA to support and promote interdisciplinary studies towards astrobiology. The center is named to recognize the work of 18th century mathematician, Pierre Simon de Laplace, on the origin and the stability of our solar system, in *Systeme du Monde* and *Mechanique Celeste*. He also developed ideas in probability theory that will be essential in discriminating between different concepts about the origin and development of life.

The purpose of the Laplace Center is to address the problems facing the development of the astrobiology community, namely the range of disciplines and hence language required to make progress in the field and the current unwillingness of many scientists to venture across disciplinary boundaries. It is our conviction that, in time, astronomy will become increasingly concerned with astrobiology and vice-versa. The Laplace Center will accelerate that process, providing a focus for astrobiology in Tucson that transcends departmental and institutional boundaries.

A novel and major item in our proposal is the development of a Winter Astrobiology School at the UA, where we would for one complete semester each year train 20 graduate students (10 from outside the UA) in astrobiology. We would also bring in visiting faculty to complement our local expertise, especially in areas such as molecular biology of organic molecules, the physical chemistry of life, and artificial and model life, where we do not have enough internal

breadth. Over the five years of this proposal we would train 100 students, and make significant strides to develop a broad astrobiology program at the UA and to provide well-trained scientists for astrobiology endeavors at other institutions.

As noted above, the Astrobiology Roadmap and the COEL report have suggested that the Astrobiology Institute should be more concerned with the astronomical environment that shapes planetary systems. We believe that this is indeed essential to astrobiology. In the present proposal, we draw heavily upon our current strengths in astronomical research programs that relate directly (often unknowingly) to astrobiology, and we propose to broaden the scope of these to include many other astrobiology-oriented activities in the College of Science at the University. Further, we expect this program to encourage dedication of new faculty positions to areas where we have major gaps. This is consistent with the UA goals to place increased emphasis on development in life sciences.

I.6 Access to Telescope Facilities

The problem of incorporating more astronomy into the astrobiology activity has received much attention. It might naively be assumed that the need for astrobiology is widely accepted among astronomers, and all that is needed is to let astronomy into the astrobiology tent. Nothing could be further than the truth. It is clear that many in both astronomy and planetary sciences see broad based astrobiological studies as in competition with their more traditional work. The problem is greatest in the allocation of telescope time, which is essential to make progress on many of the Roadmap goals.

An example of this problem may be seen in the recent studies of earthshine to discover the nature of Earth's visible spectrum and help plan for Terrestrial Planet Finder like studies. There have been three requests to NASA facilities to study earthshine, two to HST and one to IRTF. All three proposals were rejected by telescope time allocation committees. In the same era, there were three requests for observing time at ground telescopes outside NASA control, by members of the groups that control those facilities, Steward Observatory and Apache Point Observatory. Those three proposals were accepted. The first study (Woolf et al 2001) showed that in predicting the spectrum of Earthshine we had failed to predict both the Rayleigh scattering of the atmosphere or the "red vegetation edge" due to reflection from land plants but not aqueous photosynthetic organisms. Turner (2003) showed that the identification of the red edge was correct by showing the difference between observing when the illuminated earth was substantially vegetated and when it was desert. Finally, Turnbull *et al.* (in preparation) show that the Rayleigh scattering study is likely affected by cloud cover, and gave a measured strength of the 0.94 micron water band, the strongest band in the region of the spectrum available to CCD detectors.

The above example is a demonstration that the astronomical facilities that will be made available to this work both at Steward Observatory and NOAO are urgently needed. The Laplace Center will provide the appropriate vehicle for this to occur.

I.7 Impact on NASA Missions

The COEL Committee also noted a need for greater astrobiology input to the planning of space missions. Thus:

> *" An important operational goal of astrobiology is to inform NASA missions with respect to the techniques and targets for the search for life elsewhere, and the search for clues to the steps leading to the origin of life on Earth"*

The UA group has already made significant contributions to astrobiologically relevant space missions. For example, the initial suggestion for detection of terrestrial planets in the infrared, and the use of ozone as a biomarker was made by Angel, Cheng and Woolf (1986); the work was funded by a NASA grant for studies of advanced optics in space. The development of the concept for a Terrestrial Planet Finder interferometer was supported as part of the Ex-NPS study. The development of a concept for a Life Finder device was developed with funds from the NASA Institute for Advanced concepts. The first earthshine studies (see above) were made using funds from JPLs industrial study of different possible TPF missions. The one consistent theme through all of these is that the science part has been tacked on to what were supposedly engineering studies, rather than being supported in their own right. Direct support for the science must be provided if the science is to flourish. This is why linking our work to the Astrobiology Institute is necessary. It will be even more important in the future as, for example, detailed planning of the TPF program will require major input from astronomical studies of the nature of exo-planetary systems. The Laplace Center can bring greater coherence to development of concepts for both astrobiology-related space sciences, which has been supported largely in an incoherent fashion. And the Tucson group can continue to make substantial contributions to this aspect of the NAI program.

I.8 The Proposed Research Program

As noted above our program is strongly oriented to the NAI need for greater connection with astronomy and builds on our strength in that area. As background we note that when TPF was conceived almost a decade ago, it was assumed that essentially all planetary systems had architectures like the solar system. Now, 104 more planets and 12 multiple planet systems later (http://www.exoplanets.org), we find that our solar system is unlike the other systems, and plans based on the idea of the solar system as the "typical" planetary system are being questioned. This impacts directly on fundamental questions of Astrobiology such as “Does life exist elsewhere in the universe?” and "How does life begin and evolve?"

In its broadest sense, astrobiology is not just a question of how life arose in a known environment such as Earth’s. The chemical, physical and astronomical environment of early Earth is not well known. The origin of life on Earth is deeply linked to the question, "How typical is the Solar System?” If we try to put the Solar System into the context of the current information, including the recently discovered exo-planetary systems, it is possible to arrive at two wildly different conclusions. One is that earth-like planets are very common, and could be found by observing the environments of stars no more than 10 light years away. The second is that earth-like planets are so rare that only one or two are likely to exist in our entire galaxy. Obviously there is a flaw in at least one of these analyses! The answer is crucial to planning missions such as TPF.

It is generally agreed that the planetary systems being found by radial velocity studies are dynamically saturated, with little or no possibility of harboring rocky planets. Dynamical stability studies show that the relatively large eccentricities of the Jovian planets of these systems make retention of terrestrial planets in habitable zones nearly impossible over long time scales.

The conclusion that Earth-like planets are nonetheless common considers the radial velocity systems to be anomalous due to selection and rests on the observational result that the key ingredients of Earth's biosphere, rock materials and water are common in the galaxy. Laplace had hypothesized in the late 18th century that the planets of our solar system had formed from a disk of gas and dust orbiting the young Sun. The great strides in infrared astronomy in the last three decades have confirmed the basic picture of Laplace's model. Dusty disks are common around young stars in formation. The interpretation here is that *all* such disks form planets. The statistical argument is then made that the radial velocity systems cannot represent more than 20-30% of all stars and that these are the ones that did not retain earth-like planets. All others did form and retain earth-like planets, and so we should find them around most stars, even those in the immediate neighborhood of the sun.

The contrary conclusion obtains if one assumes that the planetary systems that are being observed with the radial velocity method are representative of all the planetary systems that form. We then calculate the fraction of those systems that would be like the solar system. Outside the regime where tidal forces circularize orbits, known extra-solar planets range widely in eccentricity, with their eccentricities uniformly distributed between 0 and 1. The solar system is very different. The eccentricities of the 8 main planets, Mercury through Neptune are all less than 0.5, and average 0.06. The probability that these arise from a uniform distribution from 0 to 1 is 6.6×10^{-10}. Then, because only < 25-30% of stars have planetary systems, the probability of a solar type star having a Solar System like ours is 1.6×10^{-10}. Since there are only $\sim10^{10}$ solar type stars in our galaxy, there will be at most one or two systems.

Clearly we urgently need to discover the frequency of earth-like planets, both for the scientific interest in the issue, but also to plan future space missions such as Terrestrial Planet Finder. Either earth-like planets are essential for its goals, or the goals must change to discover the character of planetary systems. The concept of the mission is currently focused on the spectral regions where terrestrial planets will appear best, and so is not optimized for finding cool giant planets. We hope for the Kepler mission to discover the frequency of earth-sized planets in earth-like thermal environments by searching for the planets occulting their star. But those results would come very late to totally change the concept for a TPF mission.

Even if earth-like planets are common, there is still remains a crucial question, whether earth sized, earth temperature objects can be expected to be just rock spheres, or whether they may be expected to have volatiles, and so be habitable. Recent dynamical models suggest that the acquisition of volatiles by earths may be controlled by the masses and orbits of giant planets in the planetary system (Chambers 2003). If so will there be totally different environments where life might develop? On the other hand, even if Earths are rare, life could be abundant. One possible place for life to develop would be on the satellites of giant planets inside a star's habitable zone. The problem then becomes one of detecting life, and the problem of observing

satellites of a warm Jovian planet orbiting a star. These are beyond the range of current planet finder concepts. Thus it is important to learn as much now as we possibly can about how planetary systems form and develop.

Our research plan thus has a substantial effort focused on determining the character of extra-solar planetary systems. If systems like the solar system are not uncommon, there will be an abrupt increase in the frequency of giant planets at distances 5-10 times greater than the distances where an earth would form. We would expect to see signs of this in both the formation and development of planetary systems.

There are three facets of this problem where it can be explored observationally. First we can try to find out in young planetary systems where the planets are forming. This requires both low and high-resolution spectrophotometry of pre-planetary circumstellar disks. Spatial variations in the density of matter would indicate the characteristics of forming planets. Second we can study "second generation dust" in mature planetary systems. This dust is a product of collisions amongst minor planets (asteroids and Kuiper belt objects) and caused by the gravitational perturbations of massive planets. The spectral distribution of radiation from such a dusty debris disk will tell its temperature, and so its spatial distribution in the planetary system. Giant planets will sweep out dust-free zones. Thus observations of dust will tell where giant planets are present. The third method is to search for the planets themselves. We have been developing techniques for looking at such planets from the ground using adaptive optics and the novel approach of nulling interferometry. Large telescopes with adaptive optics are essential for this work and provided under this proposal. The Large Binocular Telescope is an ideal instrument and should be available for research for the majority of the 5-year period of this research.

The above paragraphs hopefully provide a useful background to the need for astronomical observations in the pursuit of astrobiology. Our proposed research program follows the likely time sequence of planetary evolution beginning with the most diffuse state of pre-planetary material and concluding with studies of planets themselves. Here we provide a brief summary of each of our three modules; details are to be found in sections 1, 2 and 3 respectively.

I.8.1 Module 1

In the first module we plan studies of prospective pre-planetary material in dense interstellar clouds and in circumstellar envelopes and disks, Our focus is on the search for complex organic molecules both to understand their prevalence, their formation processes and their ultimate impact on the materials available after planet formation. One key program involves the search for the presence of ribose in space. Ribose is a relatively simple organic molecule essential to the formation of ATP (adeninosine triphosphate); ATP is the molecule used universally for energy storage and transport in all known biological processes. It is also a key ingredient in the monophosphate molecules that link together to form RNA and DNA The question of biological significance is why ribose is favored over other sugars the formation of which are energetically favored at higher densities (thermodynamically). On the other hand ribose formation is favored in conditions of kinetic control (low densities). The possibility that original ribose was formed by gas reactions in space rather than by aqueous reactions may solve a chicken-and-egg issue in the formation of RNA and DNA. We see in this an exciting possibility that nature may have jump-started life thus in the cosmos. This work will involve extensive laboratory efforts and follow up

observational work using the facilities of the UA Radio Observatories. We also plan to carry out a program of theoretical studies, backed by observational follow up on the formation of organic molecules in dense cores of interstellar clouds and circumstellar (especially carbon star) envelopes and in the interior of protoplanetary disks. This module addresses *Astrobiology goal 3.1,* sources of organic molecules and catalysts.

I.8.2 Module 2

In the second module, we plan several astrophysical studies related to the search for habitable planetary systems. We will study circumstellar dust disks as indicators of the presence and characteristics of giant planets, of the populations of minor bodies, and of the asteroidal and cometary bombardment history of terrestrial planets. The latter processes are dependent on the masses and orbits of planets in the system, and are significant for their effects both on the transport of volatiles across the system, and on habitability and the development of life. This study will address *Astrobiology goal 1.2* [direct and indirect observations of extrasolar habitable planets], *goal 1.1* [how solid planets acquire liquid water and other volatiles], and *Astrobiology goal 4.3* [effects of extraterrestrial events upon the biosphere].

We will also study the time-variability of sunlike stars. The high sensitivity of the earth's climate to the solar radiation received, and where it is received is well known. Solar changes have demonstrated severe effects on Earth's climate in the past, e.g. the “little ice age” that accompanied the Maunder sunspot minimum. Here we will examine how the current variability of the sun is likely to change if it follows the historical pattern of otherwise similar stars. We wish to quantify this to consider the effect of solar variation in early epochs, such as those in which photosynthesis developed, and in future epochs, to understand the effects of variability superposed on the continual brightening of the Sun. This study will also address *Astrobiology goal 4.3* as above, as well as *goal 6* [to understand the principles which will shape the future of life both on Earth and beyond].

I.8.3 Module 3

This module consists of searching for and studying planets with a view to establishing criteria for habitability. While the number of known exo-planetary systems (determined from radial velocity measures) now exceeds 100, there has to date been no direct detection of the radiation from a single extra-solar planet. This is clearly a problem in technology development, and we have included such a component in our program. We believe our experience and our record of innovation will lead to success in this area, and allow us to start characterizing planetary systems. Using the large telescopes available to the Laplace Center, we plan to develop and implement techniques for direct detection and (crude) spectroscopic analysis of planets around nearby stars.

A second component of this module is to advance our understanding, through modeling of the atmospheres of giant planets. This work will be an essential tool in interpreting the data expected from both ground-based and space observations during the next decade.

Also in this module is a component directed towards the study of radiation from the earth by further observations of earthshine on the moon. We have two goals for this work. The first is to make observations of earthshine in the spectral region between 0.8 and 2.3 microns. This is the

remaining unexplored region of the whole earth spectrum. Just as we discovered unexpected results in the visible spectrum, we are hopeful here. There are open questions on how the red vegetation edge feature changes with wavelength, and possible rock identification features that may show when desert regions dominate the field of view. We will also investigate how the visible/UV part of the spectrum changes with time, and attempt to interpret the spectrum, so as to be prepared for a Planet Finder mission.

Module 3 contributes to *Astrobiology goals 1.1 and 1.2*.

I.9 Management Plan The goal of our management plan (see section 4) is to ensure proper coordination of the Astrobiology node while maximizing the opportunities for the individual investigators to develop their ideas. The key to success will be communications. With the proper level of information flow and communication, coordination of the diverse science and educational elements will be straightforward.

Overall responsibility for the program will be the responsibility of the PI and Laplace Center Director, Neville Woolf. He will be assisted by Program Manager Tom McMahon and by Research module leaders, Lucy Ziurys, Steve Strom and Roger Angel. Michael Meyer will coordinate the Winter Institute and Tim Slater will lead the education and public outreach components.

The team plans regular internal meetings to assess progress and to fine-tune the research effort. It also plans close coordination with the NAI and with other NAI nodes.

I.10 Education and Public Outreach

Our EPO proposal (section 6) describes how we plan to strengthen astrobiology as an academic discipline and to provide opportunities for students to develop their interests in the field despite the barriers often presented by existing departments. It also contains an educational and public outreach component (EPO).

We have described above our plans for the Laplace Center. This will be the focus of an Interdisciplinary Program (IDP) at the University of Arizona. It will be run by a Director (initially Woolf) and will provide coordination of the entire Astrobiology node in Tucson. Members of the IDP will be drawn mainly from the current departments of Astronomy, Chemistry (and Biochemistry) and Planetary Sciences and from NOAO; it will be open to faculty members from other departments. The UA has a successful record in multi-departmental, multi-college IDPs, including those in Applied Mathematics and in Neurosciences. IDP programs of study will be developed so that students can earn academic qualifications within the IDP including minors in any of the departmental specializations of vice-versa. The initial focus will be on departments within the College of Science and the Winter Institute.

The Laplace Center will also coordinate interchange of faculty with other members of the NAI to develop understanding of issues in all areas of the roadmap. Some of this interchange will focus on the Winter Institute, which has been described briefly above and will also be coordinated by the Laplace Center. Students will not only participate in formal class work and seminars on astrobiology, but will be actively involved in both the astronomical, instrumental and laboratory activities described in I.8.

On the education side we will ensure that astrobiology components are available in the UA General Education programs in Natural Sciences - that is science for non-specialists. Astrobiology is a good topic for capturing interest as a general science education topic for undergraduate students in non-science fields. Much of the student enrollment Natural Sciences is in the Astronomy and Planetary Sciences departments, which together provide instruction for over 1,500 students per semester. The Laplace Center will ensure that suitable astrobiology materials are available for these and similar courses in Geosciences, the Tree Ring Lab, Ecology and Evolutionary Biology.

In our EPO program, we have an integrated education and public outreach component. EPO Lead T.F. Slater's primary area of scholarship is the teaching and learning of astronomy and geosciences. He is a member of the College of Science high school science teacher preparation program, the director of the UA Science and Mathematics Education Center and an associate professor of astronomy at UA. His astronomy education research group focuses on identifying students' misconceptions in astronomy and designing effective instructional materials to improve student understanding, both in formal courses and for museums and informal science centers. This unique research group has earned national recognition in the area of astrobiology education by conducting seminal research that systematically studies student beliefs and reasoning difficulties regarding the search for life in the universe. This work serves as the foundation for a new laboratory activities manual for undergraduate astrobiology courses for non-science majors. Dr. Slater is also working with Discovery Park, a science education center located in Safford, Arizona at the foot of Mt. Graham. His goal is to use the center and the proximity of major astronomical facilities on Mt. Graham to extend the outreach program to parts of rural Arizona and to the San Carlos Indian reservation. The Laplace Center will provide material and support to these efforts.

In addition Dr. Don McCarthy has, for many years, run a very successful Astronomy Camp for high school and university students as well as for teachers using the astronomical facilities on Mt. Lemmon and incorporating components of geosciences and tree ring studies as well as astronomy. If selected, the Laplace Center will seek to expand this activity and to ensure that suitable materials on astrobiology are available for each of the above program elements.

1. Module 1: The Building Blocks of Life: Astrochemistry of Simple Sugars and Other Prebiotic Molecules

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1.1 Introduction

The key life processes of metabolism and reproduction have a related chemistry. The breakdown of intake proteins into amino acids is achieved by hydrolysis, and the reconstruction of desired proteins requires a condensation reaction in which energy is required to put more water into the aqueous solution. This energy comes from the conversion of ATP (adenosine triphosphate) into ADP (adenosine diphosphate). The reproductive helices of RNA and DNA are constructed from the monophosphates of adenosine (AMP), guanosine (GMP), etc. Common to both of these biological processes is Nature's exclusive use of pentose sugars, namely ribose and deoxyribose, in the structures of all nucleotides. The origin of ATP, ADP and AMP from prebiotic material is obscure since reproduction of them requires both helix formation and condensation reactions. In space, the presence of simple sugars and important organic molecules is made possible because ion-molecule and other low-barrier reactions are achieved in a space medium. In this module we explore the possibility that ribose forms in space, is collected into comets and primitive meteorites and falls to earth, into an appropriate version of Darwin's warm pond, acting to jump-start life processes. Theoretical modeling of pathways leading to interstellar ribose is also discussed, as well as calculations of abundances of complex molecules both in molecular clouds and protostellar disks. In addition, we express the possibility of formation of organized nanostructures, which may function as prebiotic membrane systems. This study therefore belongs to objective 3.1 of the astrobiology roadmap.

1.2 The Investigation of Interstellar Molecules: Beginnings of Astrochemistry

The field of astrochemistry really began in 1963 when the lambda-doubling transitions of the OH radical were detected at 18 cm towards dense gas in Casseopia A, a supernova remnant (Weinreb et al 1963). This species was the first interstellar molecule observed at radio wavelengths. Up to that time, it was generally thought in astronomy circles that the gas between the stars, the so-called "interstellar medium," was, at its densest, only 10-15 particles per cm^3. Given this low

Table 1.1. Known Interstellar Molecules

2		3		4	5	6	7	8	9	10
H_2	CH^+	H_2O	C_3	NH_3	SiH_4	CH_3OH	CH_3CHO	CH_3CO_2H	CH_3CH_2OH	CH_3COCH_3
OH	CN	H_2S	MgNC	H_3O^+	CH_4	NH_2CHO	CH_3NH_2	HCO_2CH_3	$(CH_3)_2O$	$CH_3(C{\equiv}C)_2CN$
SO	CO	SO_2	NaCN	H_2CO	CHOOH	CH_3CN	CH_3CCH	CH_3C_2CN	CH_3CH_2CN	$(CH_2OH)_2$
SO^+	CS	NNH^+	CH_2	H_2CS	$HC{\equiv}CCN$	CH_3NC	CH_2CHCN	C_7H	$H(C{\equiv}C)_3CN$	
SiO	C_2	HNO	MgCN	HNCO	CH_2NH	CH_3SH	$H(C{\equiv}C)_2CN$	H_2C_6	$H(C{\equiv}C)_2CH_3$	
SiS	SiC	SiH_2	HOC^+	HNCS	NH_2CN	C_5H	C_6H	CH_2OHCHO	C_8H	**11**
NO	CP	NH_2	HCN	**CCCN**	H_2CCO	HC_2CHO	c-CH_2OCH_2	HC_6H		$H(C{\equiv}C)_4CN$
NS	CO^+	H_3^+	HNC	HCO_2^+	C_4H	$CH_2{=}CH_2$	$H_2CC(OH)H$			
HCl	HF	NNO	AlNC	**CCCH**	c-C_3H_2	H_2C_4				**12**
NaCl	SH	HCO	SiCN	c-C_3H	CH_2CN	HC_3NH+				C_6H_6
KCl	HD	HCO^+	SiNC	**CCCO**	C_5	C_5N				
AlCl	FeO?	OCS	H_2D^+	C_3S	SiC_4	C_5S?	15 ions			**13**
AlF		**CCH**	NH_2	**HCCH**	H_2C_3		6 rings			$H(C{\equiv}C)_5CN$
PN		HCS^+	KCN?	$HCNH^+$	**HCCNC**		~100 Carbon Molecules			
SiN		c-SiCC	SiH_2?	**HCCN**	**HNCCC**		19 Refractories			Total = 134
NH		**CCO**		H_2CN	H_2COH^+					
CH		**CCS**		c-SiC_3						
				CH_2D^+?						

density, and the presence of a strong ultraviolet radiation field from background stars, it seemed unlikely that anything more than a few diatomic molecules could exist in interstellar gas. This assumption was supported by observations at the time, primarily 21 cm and continuum radio astronomy, which showed that large amounts of hydrogen atoms ("HI", in astronomy terms), and ionized hydrogen (H^+ or HII) existed throughout the galaxy. Observations at optical wavelengths did result in the detection of a few diatomic species, such as CH^+, CN, and CH (e.g. Adams 1948), which were observed in diffuse gas in absorption against background stars. The dusty regions of "dark clouds" that often obscured measurements of optical astronomers were usually ignored, that is, until the discovery of OH. Several years later, NH_3, H_2O, and H_2CO were also detected at radio wavelengths (1.2 cm and 6 cm) by Cheung *et al.* (1968) and Snyder *et al.* (1969), added to the inventory of interstellar compounds. The largest breakthrough in astrochemistry occurred in 1970, when Wilson *et al.* (1970) observed emission from carbon monoxide from the Orion Nebula at 3 mm, using the NRAO 36 ft. radio telescope (now the 12 m). This unique discovery and years of subsequent observations have shown that the CO molecule is abundant and ubiquitous throughout the Milky Way Galaxy. Moreover, emission from this species is very intense and readily detected. In addition, these measurements showed that molecular emission was easily observed at short radio (i.e. millimeter) wavelengths. In fact, following 1970, there was very rapid progress in the identification of new interstellar compounds, primarily at millimeter wavelengths. The list continues to grow. Currently, there are over 120 securely identified unique interstellar molecules as shown in Table 1.1.

Contrary to earlier notions, the interstellar medium is sufficiently dense to support a complex chemistry. This chemistry occurs throughout the Galaxy, and even in external galaxies. This fact is illustrated by recent "all-sky" surveys of certain species such as carbon monoxide, as shown in Figure 1.1. This figure shows a plot of CO, $J = 1 \rightarrow 0$ spectral line emission (peak intensity) versus position across the galaxy, overlaid across the optical picture of the Milky Way.

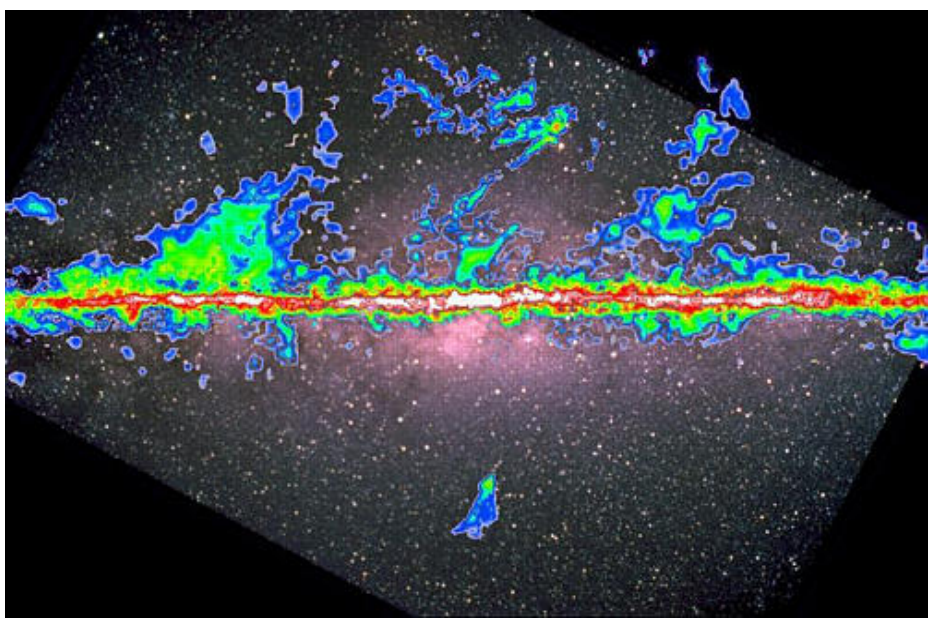


Figure 1.1. The "Molecular Milky Way:" a plot of CO, $J = 1 \rightarrow 0$ spectral line emission at 115 GHz (3 mm) as a function of position, illustrating the predominance of molecular gas. In the background is the optical picture of the Galaxy.

Relative intensity is shown as a color scale, white indicating the strongest emission. The figure illustrates the molecular gas is present in most regions where stars exist, but also in areas far from the galactic plane.

Astronomy conducted at millimeter (0.3-3 mm) wavelengths has therefore been a primary tool of interstellar chemistry, not only for the discovery of new molecular species, but also in examining their abundances and distributions in the Galaxy. Millimeter astronomy has distinct advantages for studying such molecules because the spatial resolution at these wavelengths matches the sizes of molecular emission on the sky. Also, quantum mechanics favors millimeter wavelengths. Because most dense gas in the interstellar medium is cold, with temperatures T_k~10 – 100 K, in general only the rotational energy levels of a molecule are populated, as opposed to vibrational and electronic. Hence, interstellar species are identified on the basis of their "pure rotational spectrum," which occur primarily at millimeter wavelengths.

Emission lines are produced by collisions, primarily with H_2, which excite higher rotational levels, followed by spontaneous decay. Collisional excitation in colder gas therefore means that linewidths of interstellar emission features are very narrow, typically 1 part in 10^6. Hence, the spectra obtained from interstellar gas are "high resolution," such that not only are individual rotational transitions well defined, but fine and hyperfine structure as well. Hence, interstellar molecules can be securely identified on the basis of their "fingerprint" patterns. Illustrations of such patterns are show in Figures 1.2. Given sufficient measurements, followed by a knowledgeable spectral analysis, a given chemical compound can be identified in interstellar gas beyond any doubt.

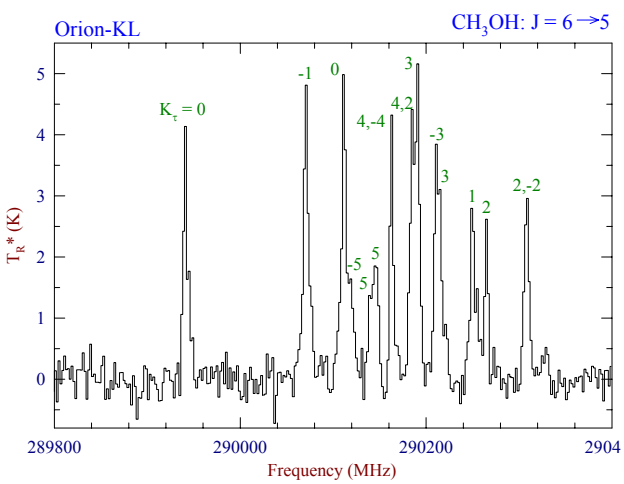


Figure 1.2a. Spectra of the J = 6→5 transition of methanol (CH_3OH) observed toward the Orion molecular cloud near 290 GHz (1 mm). Individual features arise from "K- structure," the pattern, which unique to methanol, is based on its molecular weight and geometry.

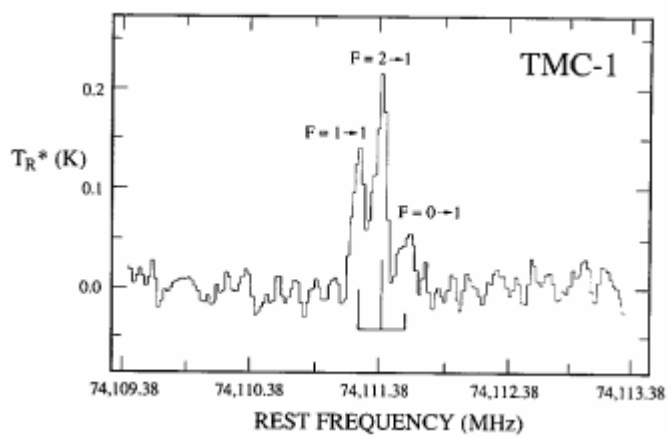


Figure 1.2b. Spectrum of the J = 1→0 rotational transition of $HCNH^+$, observed at 74 GHz towards the Taurus molecular cloud. The three separate features arise from nuclear quadruple splittings due to the nitrogen (^{14}N) nuclear spin. This unique "hyperfine" structure serves as a fingerprint for $HCNH^+$.

A key ingredient to the study of interstellar molecules has been laboratory spectroscopy. The spectral signature of a new species must be known before it can be identified in interstellar gas.

This fact has become particularly clear as millimeter astronomy achieved higher sensitivities through advanced detector development. It is very common that, in addition to known spectral features, any sensitive observation at millimeter or sub-millimeter wavelengths will contain so called "U-lines," i.e., unidentified lines. These are emission features arising from yet to be discovered interstellar compounds. Currently, hundreds of U-lines are known to arise from the Orion molecular cloud alone; a small subset is shown in Figure 1.3. Amazingly, not one of these spectral lines can be securely identified, although one may arise from ethanol and two others from methyl formate. The common appearance of U-lines in interstellar spectra means that chance coincidences are not unusual. Consequently, the pure rotational spectrum of any proposed new molecule must be very accurately measured in the laboratory (1 part in 10^7-10^8) over a large wavelength region. Direct measurements in the lab are preferable, but extrapolated predictions, in particular to higher frequencies (i.e. higher rotational levels) can be reliable as well, provided sufficient lower frequency measurements are made such that all the necessary spectroscopic constants are well characterized. Current laboratory techniques in the rotational regime, such as direct millimeter-wave absorption spectroscopy (e.g. Hirota 1985, Ziurys *et al.* 1994) and Fourier transform microwave methods (Balle & Flygare 1981) are extremely powerful and have both the sensitivity and resolution to tackle most astrophysical problems.

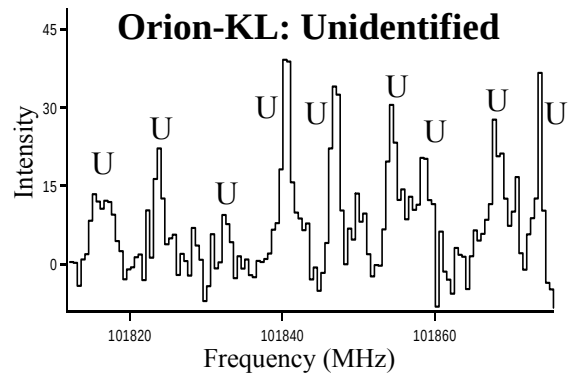


Figure 1.3. Unidentified emission lines observed towards the Orion molecular cloud near 101 GHz. Such features remain a puzzle for astronomer and spectroscopist alike.

1.3 Organic Chemistry in the Gas-Phase: Towards Greater Chemical Complexity

The current compendium of interstellar compounds clearly demonstrates that the chemistry in space is primarily organic. Not only do 75% of the known species contain carbon, but also all the basic organic functional groups are present. For example, the carbonyl group is represented by common species like formaldehyde ($H_2C{=}O$) and acetone ($(CH_3)_2C{=}O$). There are various amides (NH_2CH_3, NH_2CN), acids ($HCOOH$, CH_3COOH), and many compounds with the CN moiety (HCN, HC_3N, HC_5N, CH_3CN, EtCN, etc.) Basic aldehydes, ketones, esters, and ethers are additionally present. Some representative organic compounds are shown in Table 1.2.

The basic mechanisms thought to form organic interstellar molecules are two-body ion-molecule reactions (e.g. Herbst and Klemperer 1976), of the basic form:

$$A^+ + B \rightarrow C^+ + D \qquad (1)$$

These processes were initially postulated because they usually have no activation barriers and proceed at the collisional (or Langevin) rate ($k \sim 10^{-9}$ cm^3 s^{-1}). Hence, they can readily proceed at the low temperature ($T_K \sim$ 10-100 K), low density ($n \sim 10^3$-10^6 particles/cm^3) environment of interstellar clouds, where 3-body collisions do not occur and reactions with normal neutral-neutral type barriers would never occur. Chemical modeling has shown (Lee, Bettens & Herbst 1996) that ion-molecule reactions can produce the more complex species within the postulated lifetime of a molecular cloud (10^6 – 10^7 years), including dimethyl ether, methyl formate and

ethanol. For example, methyl formate can be created via the reaction (Millar, Herbst and Charnley 1991):

$$H_2CO + CH_3OH_2^+ \rightarrow H_2COOCH_3^+ + H_2. \qquad (2a)$$

Neutral methyl formate is then produced by:

$$H_2COOCH_3^+ + e^- \rightarrow HCOOCH_3 + H. \qquad (2b)$$

This process is quite viable because both H_2CO and CH_3OH have large interstellar abundances (e.g. Minier & Booth 2002). The ion $CH_3OH_2^+$ could be created by the process:

$$CH_3OH + H_3^+ \rightarrow CH_3OH_2^+ + H_2. \qquad (3)$$

H_3^+ is the dominant ion in an astrophysical plasma, and it is readily created from H_2 and cosmic rays from the sequence:

$$H_2 + \text{c.r.} \rightarrow H_2^+ + e^- \qquad (4)$$

$$H_2^+ + H_2 \rightarrow H_3^+ + H. \qquad (5)$$

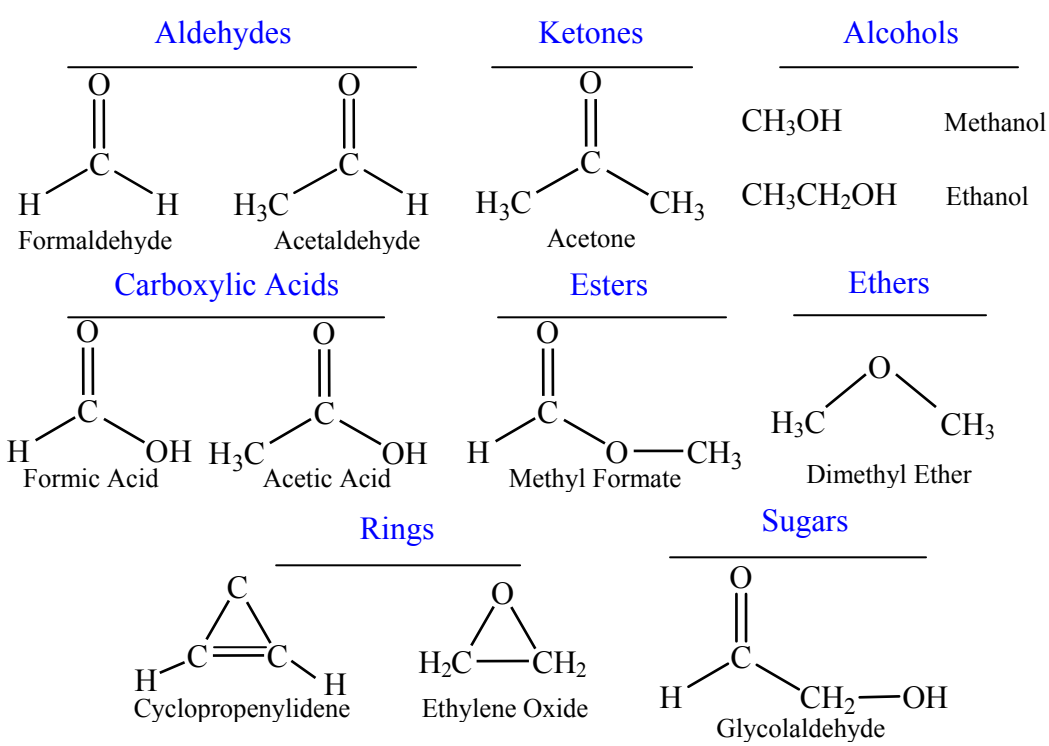
Because H_2 is the most abundant interstellar molecule and cosmic rays are common, large amounts of H_3^+ must exist in dense gas. There is sufficient H_3^+ for many types of proton transfer processes. Some common reactions are:

$$CO + H_3^+ \rightarrow HCO^+ + H_2 \qquad (6)$$

$$H_2CO + H_3^+ \rightarrow H_2COH^+ + H_2 \qquad (7)$$

Given the relatively large abundances of HCO^+ and H_2COH^+ in interstellar clouds (e.g. Ohishi *et al.* 1996), these reactions must occur.

Table 1.2: Interstellar Organic Molecules



It is clear that as millimeter-wave telescope systems become more sensitive, more complex organic species are being found in the gas phase in interstellar space. Acetone and acetic acid, the next step in complexity from formaldehyde and formic acid, for example, have just recently been identified (Ramijan *et al.* 2002; Snyder *et al.* 2002). Such compounds, of course, are very simple in comparison to proteins or even sugars. However, given the observation of large numbers of unidentified features in interstellar spectra, and the fact that millimeter telescopes can do ever increasingly sensitive measurements, it would seem to be self-evident that the complexity of detected interstellar compounds will increase. The fundamental question is, what are the limits for gas-phase interstellar chemistry? Can it produce molecules of sufficient complexity to be of biological significance? In other words, are the building blocks of life present already in interstellar gas clouds?

1.4 Interstellar Ribose: A Likely New Species?

Although there are many biologically important molecules, perhaps one of the most fundamental is DNA and its single-strand counterpart, RNA. Together, DNA and RNA are responsible for storage, transport, and expression of genetic information. DNA, or deoxyribonucleic acid, is a double-stranded polymer consisting of repeating nucleotide units. Each nucleotide contains a phosphoric acid group, the pentose β-D-2-deoxyribose, and a purine or pyrimidine base. An example of such a nucleotide is shown in Figure 1.4.

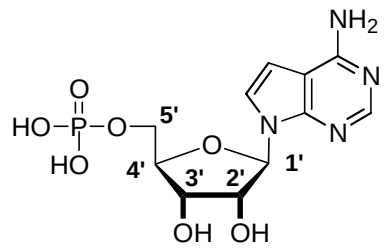


Figure 1.4:. Adenosine 5-monophosphate, otherwise known as AMP, one of the nucleotides that forms RNA.

The two strands that form DNA, the so-called "double helix," attach to each other via hydrogen bonds between the nucleotide bases. Such bonds are possible only between certain base pairs, such as cytosine and guanine, or adenine and thymine. RNA, or ribonucleic acid, is often single stranded, with the pentose in this case being β-D-ribose, with the base uracil replacing thymine. The individual nucleotides bond together via the pentose in both RNA and DNA; the linkages are made between the 3' and 5' carbons of the pentose using a phosphate (P-O-C) bond.

Ribose and deoxyribose are thus critical to the structures of RNA and DNA. They are both 5-membered rings consisting of four carbons and one oxygen atom. The fifth carbon is attached to

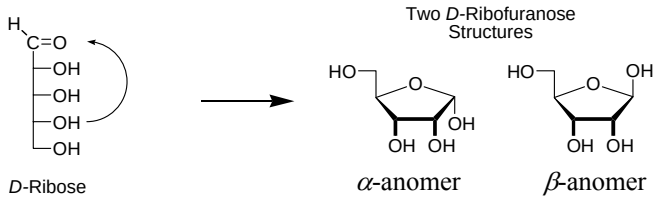


Figure 1.5: Cyclization of *D*-ribose leads to the two anomeric ribofuranosides, thus preventing further chain extension *via* the formose reaction.

the ring at the 4' carbon. Structures for these compounds are given in Figure 1.5.

To appreciate the structures of these pentoses, it is useful to consider that of monosaccharides in general. The simplest monosaccharides are trioses, which contain three carbon atoms. Examples of trioses are L- and D- glyceraldehyde. These species are chains as opposed to rings, and they have one chiral center and hence, two enantiomers, D and L, which are mirror images of each other.

Four carbon sugars (tetroses) also have chain geometries with two chiral centers and four stereoisomers. By the time pentoses form, i.e. five carbon sugars, the structures are large enough such that the most stable geometry is cyclic, generating four chiral centers and 16 stereoisomers. Cyclization of these compounds prevents further chain extension. The α- and β- designations refer to the orientation of the C-1 OH group relative to the other functional groups on the ring. All naturally occurring sugars on Earth are D-sugars; similarly all protogenic amino acids exist as a single antipode (L-isomers, e.g. Engel & Macko 1997).

Ribose and deoxyribose are certainly not large biological molecules, indicative of life. On the other hand, they are essential for life as we know it. Detection of these molecules in the interstellar gas would certainly revolutionize our thinking about where and how life began. Ribose could in fact be synthesized in space from ion-molecule reactions, as is postulated for other smaller organic compounds. Sugars in the terrestrial laboratory are readily synthesized via the formose reaction, which is the successive base-catalyzed addition of formaldehyde, H_2CO, (Butlerow 1861). By analogy, sugars could be formed in interstellar space via successive additions of protonated formaldehyde, H_3CO^+, a common interstellar molecule. Because of the high vacuum conditions, an acid-catalyzed process is feasible in space. The starting materials could be glycolaldehyde ($CHO\text{-}CH_2OH$), or *cis*-1,2-dihydroxyethylene (HO)HC=CH(OH), which are enol-keto tautomers with the keto form more stable by 15.4 kcal mol^{-1} (Su *et al.* 1999). There is some evidence that glycolaldehyde may be present in interstellar gas from the work of Hollis, Lovas and Jewell (2000). (The laboratory spectrum of this species is known, unlike the latter molecule.) Either species could react with H_3CO^+ via a radiative association ion-molecule process, which should occur relatively rapidly in the interstellar environment, resulting in the protonated form of the desired sugar:

$$CHO\text{-}CH_2OH + H_3CO^+ \rightarrow CHOCHOHCH_2OH_2^+ + h\nu. \qquad (8)$$

This reaction can occur quickly because the large protonated complex can be stabilized through vibrational channels (Petrie 1996). The neutral molecule can be formed in a number of ways; for example, by recombination with an electron, proton transfer with H_2 or CO, etc.

$$CHOCHOHCH_2OH_2^+ + e^- \rightarrow CHOCHOHCH_2OH + H. \qquad (9)$$

The sugar glyceraldehydes can thus be synthesized, either the D or L forms. The addition of H_3CO^+ to glycolaldehyde could also result in other products, such as dihydroxyacetone and several keto-sugars. Glyceraldehyde is a triose; formation of tetrose sugars would occur by the addition of H_3CO^+ to the trioses. Addition to D-glyceraldehyde would create D-erythrose and D-threose. From D-erythrose, the next addition would result in D-ribose and D-arabinose. This

synthetic scheme is presented in Figure 1.6. Deoxyribose could be produced from D-erythrose, as well in a fashion analogous to that depicted in reactions (8) and (9).

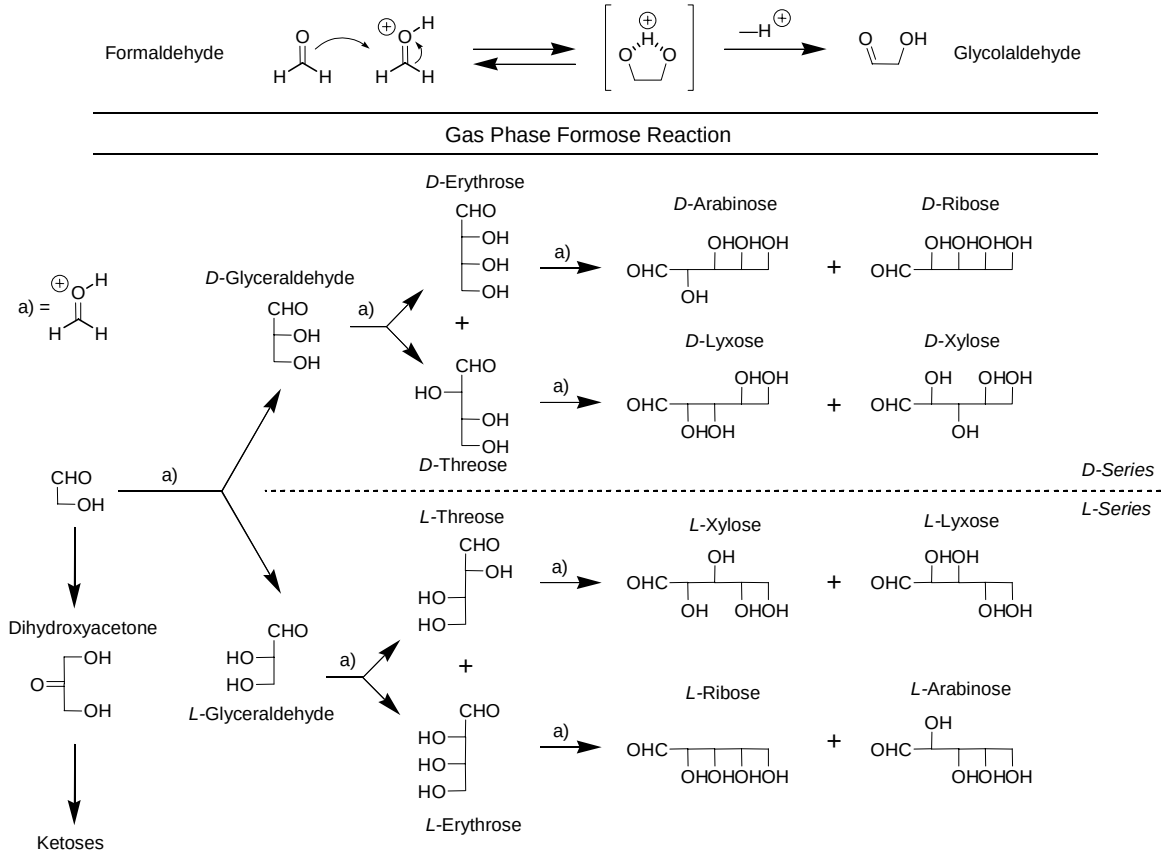


Figure 1. 6. Production of D-ribose from glycolaldehyde via ion-molecule addition of H_3CO

There are other possible schemes leading to the creation of sugars besides the one proposed here. For example, certain neutral-neutral reactions might be involved in the scheme, or other ion-molecule reactions. The point of this exercise is to show that ion-molecule chemistry in principle could produce sugars as large as ribose. Several organic ring species are already known to exist in interstellar gas (C_3H_2, C_2H_4O). These are three-membered rings, and therefore, should suffer more strain than larger structures. It is suggested that the process could be inhibited at the 5-carbon (ribose) stage because cyclization would remove the aldehyde functionality necessary for the formose reaction to proceed to a 6-carbon sugar.

If ion-molecule type reactions produced ribose and deoxyribose, this formation process would be kinetically rather than thermodynamically controlled. Studies by Eschenmöser and collaborators (e.g. Wilds *et al.* 2002) suggest that sugars other than ribose are just as likely to form RNA-like structures. In fact, the capability of Watson-Crick base-pairing is widespread among potentially natural nucleic acid alternatives that are close in structure to RNA (Eschenmöser 1999). Why then D-ribose, and not another isomeric pentose? This remains an open question. It could well be that the structure of RNA resulted from synthetic contingency, not from combinatorial generation and functional bias. Ion-molecule chemistry might selectively generate ribose as the primary pentose, providing exactly this contingency. Experiments have indeed shown that ribose is preferentially formed in kinetically controlled aldolization reactions (Müller *et al.* 1990).

Kinetically controlled interstellar chemistry could be guided by the same molecular forces, i.e. dipole-dipole interactions and hydrogen bonding).

If reaction rates were known for the ion-molecule processes leading to ribose, then this problem could be readily attacked. However, these rates are not known, and it would take a long-term experimental endeavor to actually measure them at interstellar temperatures. On the other hand, identifying ribose and its simpler sugar precursors in interstellar gas would be highly suggestive that gas-phase chemistry preferentially provided the starting materials for RNA, especially if other pentose alternatives were not found, or found in lesser abundance.

The purpose of this section of the proposal is to carry out systematic searches for ribose and its sugar precursors in interstellar gas using the highly selective techniques of high-resolution spectroscopy and radio/mm astronomy. These combined methods are so exact that with careful, thorough studies, the existence of these compounds in interstellar space can be established beyond any shadow of a doubt, as in the case of other interstellar molecules. To carry out this program, however, extensive laboratory spectroscopy must be completed first. Of the precursors proposed for ribose in Figure 1.6, only the pure rotational spectrum of glycolaldehyde has been measured with sufficient accuracy with a large enough data set for meaningful interstellar measurements. Some of the species of interest include hydroxyacetone, dihydroxyacetone, D-glyceraldehyde, D-erythrose, D-threose, D-xylose, D-ribose, and D-lyxose. A new absorption spectrometer will be built to study these molecules in a wavelength range comparable with their most intense rotational transitions (~10-100 GHz). Once the necessary laboratory studies have been completed, a search for each molecule will be conducted in interstellar gas using the University of Arizona's two radio telescopes, the Kitt Peak 12 m, and the Sub-mm telescope (SMT). These facilities are operated by the Arizona Radio Observatory (ARO); the director is a co-PI on this proposal. Large amounts of telescope time will have to be devoted to searching for such molecules. The ARO is committed to providing this time, if this proposal is funded. Because the proposed molecules are large species, by interstellar standards, they will have hundreds of favorable rotational transitions across the millimeter spectrum. This plethora of lines will be measured and analyzed as a complete data set to avoid fortuitous coincidences with other species, and to secure an accurate identification. Our computational group will carry out theoretical investigations in parallel to establish which interstellar processes could actually lead to sugars and other complex species.

1.5 The Astrochemistry Team

The astrochemistry team consists of three expert high resolution molecular spectroscopists (Ziurys, Apponi & Herbst, who is very experienced in the analysis of organic molecules with complicated spectra), two theorists (Adamowicz & Herbst), who will explore the interstellar pathways for formation of the sugars and examine abundances of large organic molecules in dense clouds and protostellar disks, one synthetic organic chemist (Polt), who will provide the necessary precursors for the lab work and bioorganic interpretations of interstellar mechanisms, and one biochemist (Brown), who will interpret the implications of these results for RNA and DNA structure. In addition, three members of the team are highly experienced radio observers who have discovered numerous other interstellar molecules (Ziurys, Apponi & Halfen).

In the next sections, the laboratory spectroscopy, radioastronomical observations, theoretical calculations, and work with condensed membrane systems will be discussed in more detail.

1.6 Laboratory Spectroscopy of Proposed Interstellar Sugars

1.6.1 Experimental Apparatus

The spectrometer to be designed is a classic absorption experiment consisting of a radiation source, an absorption cell with a supersonic jet nozzle for molecular cooling, and a sensitive microwave detection system. This instrument will be designed to operate over a frequency range of 10 – 110 GHz where the signals from the species of interest are expected to be the strongest. For example, at an operating temperature of 50 K (a result of jet cooling), the spectrum of glyceraldehyde exhibits its peak intensity near 89 GHz (see Figure 1.8). Although the spectrometer as a whole is not commercially available, most of the individual parts can be purchased from commercial vendors, and these parts will be discussed in the following four sections.

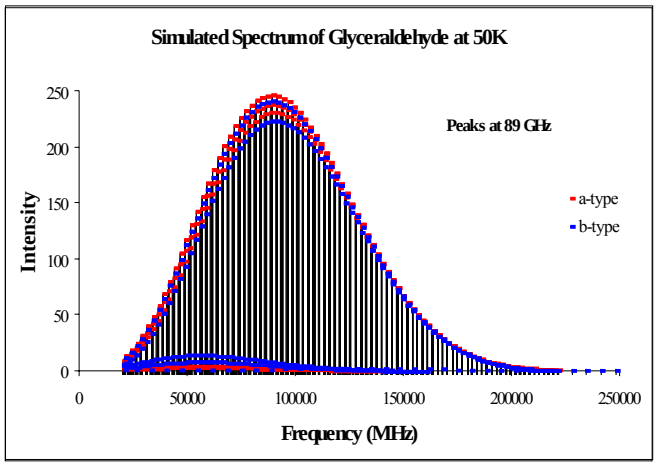


Figure 1.7. Calculated intensities for the individual rotational transitions of glyceraldehydes at 50 K.

Radiation Source:

The radiation source for this experiment will be a low phase noise, high power Agilent E8247C series signal generator operating in the frequency range of 250 kHz – 40 GHz. Phase-locked Gunn oscillators will be used to generate frequencies higher than 75 GHz, which we already have in our laboratory.

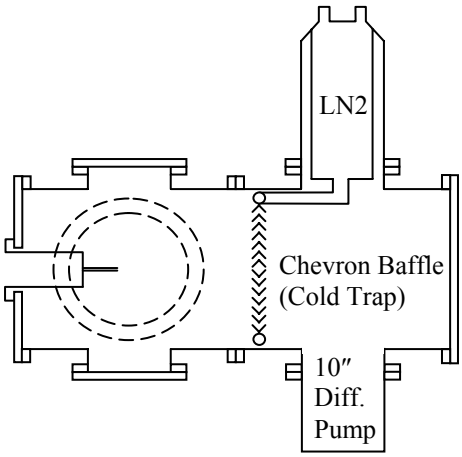


Figure 1.8. Elevation sectional view of vacuum chamber used for free-jet microwave spectroscopy

Absorption Cell and Optical Components:

A diagram of the gas cell is shown in Figure 1.9. The cell will be approximately 0.30 m × 0.60 m, and will be evacuated by a Varian VHS-10 (5300 liter/s) oil diffusion pump equipped with a Leybold D65B (53 cfm) roughing pump, creating a vacuum of better than 10^{-7} torr. The supersonic beam expands perpendicular to the radiation, and the molecules are terminated on a liquid nitrogen cooled Chevron baffle to help reduce contamination in the pump oil. The radiation will be launched from tapered feedhorns and focused to a waist of about 2 cm at the center of the cell where absorption by the molecules will occur. The radiation is then allowed to pass out of the cell and is refocused onto the detector (see Figure 1.9).

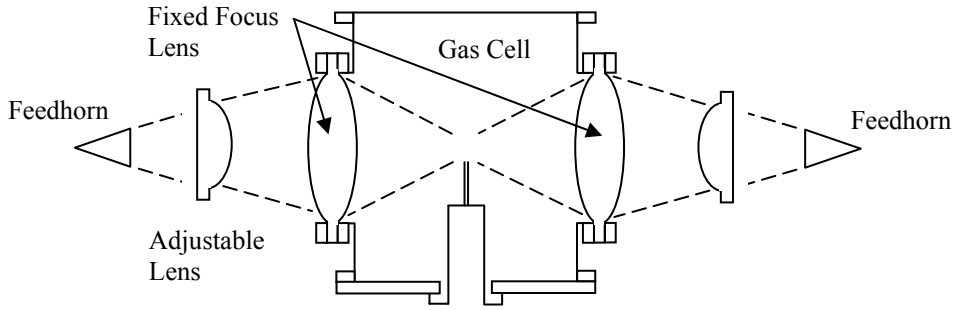


Figure 1.9. Top sectional view of gas cell. Dashed lines show the propagation of the microwave radiation.

Molecular Beam Apparatus:

The supersonic jet will be produced from a continuous flow or pulsed nozzle as shown in Figure 1.10.

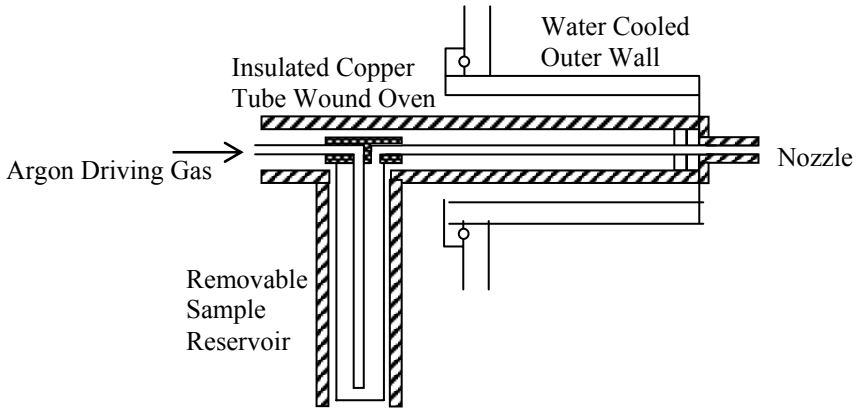


Figure 1.10. Water cooled insert to main chamber showing sample inlet and nozzle layout.

Detection System:

A standard phase sensitive detection scheme will be employed for this experiment incorporating a liquid He cooled (4.2K) InSb hot-electron bolometer (Cochise Instruments). The radiation source will be frequency modulated at a 20 – 50 kHz rate and demodulated by a lock-in amplifier. Data acquisition will be under computer control via GPIB interface.

1.6.2 Proposed Molecules

High-resolution spectra will be obtained from this system on the large molecules proposed (see Table 1.3). Sensitive searches over large frequency ranges are necessary to positively identify these species. The advantage of this system over many other microwave and millimeter wave systems is that automated data taking is possible without user intervention. Samples in the oven are expected to last for many hours, possibly overnight, without being exhausted. High-resolution spectra will be recorded using this technique with linewidths <100 kilohertz, and measured frequencies will be accurate to a few kilohertz—necessary for assignment of the interstellar spectrum. Unlike vibrational or electronic spectroscopy, rotational spectroscopy offers an unambiguous assignment of the molecular species where each measured line is unique to a given species. Each line measured, however, is like a piece in a puzzle that the spectroscopist must first assemble in the laboratory with limited contamination before attempting to assign the complicated interstellar spectrum.

Initial work will be done on the smaller molecules, 1,2-dihydroxyethylene, and hydroxyacetone; then the spectra of the larger sugars, dihydroxyacetone, D-glyceraldehyde, D-erythrose, and D-ribose, will be measured. Analysis of these data for the sugar molecules proposed will yield

Table 1.3: Proposed Laboratory Species

Molecule	Ground State	Estimated Constants (GHz) A	B	C	Dipole Moment	Commercially Available Form
1,2-Dihydroxyethylene	^1A	26.56	2.16	1.99	a, b-types	Must Synthesize
Hydroxyacetone	^1A	10.34 [a]	3.82 [a]	2.86 [a]	a, b-types	Solid Dimer
Dihydroxyacetone	$^1A_1$	4.73	1.86	1.33	b-type	Solid Dimer
D-Glyceraldehyde	^1A	~3.0	~1.8	~1.2	a, b, c-types	80% in Solution
D-Erythrose	^1A	~2.0	~1.2	~0.8	a, b, c-types	Pure Syrup
D-Threose	^1A	~1.8	~1.4	~0.8	a, b, c-types	60% Syrup
D-Xylose	^1A	~1.0	~0.8	~0.4	a, b, c-types	Pure Solid
D-Arabinose	^1A	~1.0	~0.8	~0.4	a, b, c-types	Pure Solid
D-Lyxose	^1A	~0.9	~0.9	~0.4	a, b, c-types	Pure Solid
D-Ribose	^1A	~1.1	~0.7	~0.4	a, b, c-types	Pure Solid

[a] Experimentally determined by Kattija-Ari, & Harmony (1980)

spectroscopic constants for these species. The rotational constants, A, B, and C, as well as centrifugal distortion constants will be determined from their spectra. Significant K-ladder structure will be observed, which will establish the spectroscopic constants to high accuracy, which is necessary for subsequent astronomical searches.

1.7 Astronomical Observations:

To detect the proposed compounds in space, high sensitivity searches will be conducted; primarily at the Kitt Peak 12m telescope. This instrument is, in fact, currently being used to confirm the presence of glycolaldehyde in interstellar gas in the Galactic center in a source called "Sgr B2(N)." Some confirming spectral lines are shown in Figure 1.12. The measurements will be primarily conducted in the 3 mm and 2 mm bands (65-180 GHz). The objects to be studied include a variety of Galactic dense molecular clouds, such as Sgr B2 (Galactic center), the Orion molecular cloud, W51, W49, NGC7538, DR21(OH) and G34.3. These sources are known to contain star forming regions and hence potential new solar systems. Typical sensitivities to be reached will be ~10 mK, but deeper signal-averaging can be conducted if necessary. It should be noted that signal strength is not a limiting issue. Measurements at the

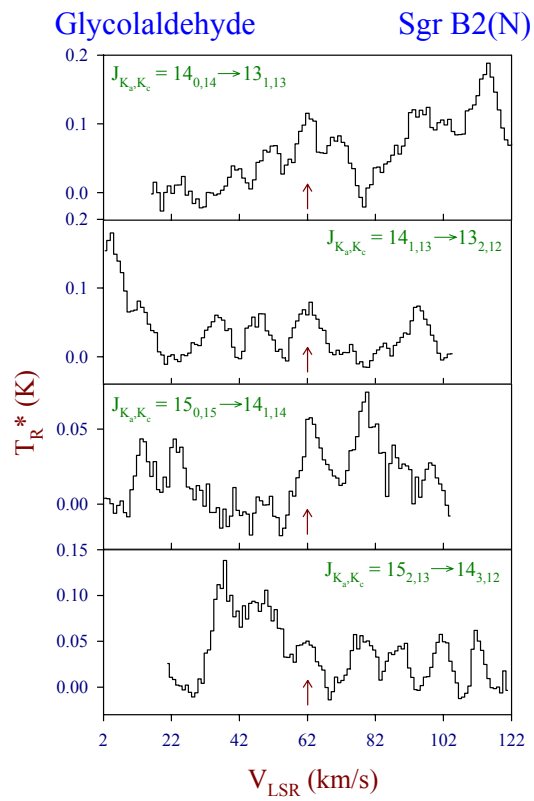


Figure 1.12. Some observed rotational transitions of Glycolaldehyde measured towards Sgr B2(N), the Galactic center molecular cloud, using the ARO 12 m telescope.

KP12m telescope have been conducted for as long as 130 hours of signal-averaging, to achieve levels of T_{rms} ~0.0005 K. More damaging will be contamination from other spectral features. However, given sufficient frequency coverage, enough rotational transitions can be studied to be certain about a given molecular identification.

1.8 Computational Modeling of Elemental Reactions Leading to Interstellar Ribose

The material falling from the interstellar space on Earth is a rich source of organic molecules. Recent assessments indicate that very significant quantities of organic material from meteoritic and cometary sources have been deposited on the primitive Earth (approximately 20g/cm^2; e.g. Anders 1989) Seventeen amino acids, in every way identical to those made in the course of the famous Miller experiment, have been identified in the Murchison meteorite, which fell on Australia in 1969 (Chyba *et al.* 1990). It is estimated that more than 100 tons of meteoritic material fall on our planet each year; at the time of formation of the Earth, the bombardment was 10,000 times more intense. Thus, meteorites have brought an enormous quantity of organic molecules to Earth (Maurette *et al.* 1987).

The origin of chirality of living molecules (e.g. the exclusive involvement of D-ribose) is at present not satisfactorily explained. Engel and Macko (1997) have demonstrated that the Murchison meteorite contains an enantiomeric excess of certain L-amino acids that are today almost exclusively present in living systems. This result suggests that an extraterrestrial source for the excess enantiomers in the Solar System may predate the origin of life on Earth (Cronin & Pizarello 1997). This excess may have resulted from the alteration of initially abiotic racemic mixtures by a process such as preferential decomposition from exposure to circularly polarized light (e.g. Bonner, Micura & Eschenmöser 1997).

Several difficulties were encountered in the synthesis of all pieces of an RNA molecule under primitive conditions (Joyce 1989). When one tries to produce ribose from formaldehyde and glycolaldehyde in the presence of sodium hydroxide and calcium acetate, by so-called "formose reaction", a very complex mixture is obtained, in which ribose is only a very minor component. Moreover, ribose is unstable on a geological time scale (Larralde, Robertson & Miller 1995). However, pentose and hexose phosphates can be obtained in good yields from glycolaldehyde phosphate when the reaction is run in the presence of formaldehyde (Müller *et al.* 1990). The outstanding problem at present is related to the synthesis of ribose with the exclusion of other aldopentoses (Zubay & Mui 2001).

The computational modeling will be one of the core activities in this project and will complement the experimental effort. The combination of astrophysical observations, laboratory and theoretical studies is needed to understand the complexity of the physico-chemical phenomena that occur in space. These are essential for testing and improving the existing astrophysical models and for the identification of molecules by laboratory studies and by observation.

Although much is known about the chemistry of dense interstellar clouds, much also remains uncertain, as a result of the variety of heterogeneous processes which may be important, including the effects of radiation fields and cosmic rays. Such conditions may vary significantly among and within different environments. Thus, the additional constraints on models supplied by

the identification of new molecular species and the determination of their abundance remains very important. Astrobiological studies that address the origin, evolution and distribution of life in the Galaxy require a fundamental understanding of the chemical processes and cycles that lead to organic chemistry in molecular clouds. Molecular clouds provide the initial organic inventory available to protostellar disks, and they can be the source of volatile organic material for the development of organic complexity of biogenic material. Energetic processes, such as irradiation and thermal processing may further increase the complexity of organics, as evidenced by laboratory studies. In this context the development of theoretical models of molecular synthesis and chemical pathways that can occur in the interstellar media in combination with laboratory data provide a basis to understand how molecular chemistry develops in interstellar material at different evolutionary stages.

The objective of this work will be to develop and implement multilevel modeling and simulation capabilities for understanding the elemental chemical reactions that may lead to formation of sugars (ribose) in the interstellar medium under various conditions. In particular, we will study the elemental processes involved in the formose reaction including its ion-molecule analog. The issues that will be considered in relation to the production of ribose in the formose reaction are: (i) the product selectivity under various conditions that can occur in the interstellar clouds, and (ii) the efficiency of the benzoin type condensation and the aldol condensations.

The Adamowicz research group has experience in developing methods in the area of molecular modeling, molecular quantum mechanics and molecular dynamics simulations. They have also used these methods to study chemical and physical properties of atomic and molecular systems relevant to interstellar chemistry (e.g. Lee & Adamowicz 2001). These include a series of studies concerning gas-phase amino acids performed with the use of molecular quantum mechanical methods and IR matrix-isolation spectroscopy (e.g. Stepanian *et al.* 1999, 2001). The effort in this project will focus on multilevel simulations of bi-molecular chemical reactions that can occur under various conditions (temperature, pressure, density, irradiation exposure, etc.) in space and leading towards synthesis of sugars.

In the simulation work performed with the use of quantum modeling and quantum mechanics interfaced with molecular dynamics, we will develop static and dynamic models enabling simulation of reaction cycles involving elementary molecular precursors and leading to synthesis of sugars. The simulation methodology will be implemented on a massive parallel processing system (MPPS) using novel computational architectures. The models will serve as a roadmap towards developing a fundamental scientific understanding of the investigated molecular systems and their interactions occurring under conditions that may involve high collisional velocities and intense irradiation.

1.9 Tracing Abundances of Organic Molecules from Clouds to Protoplanetary Disks

Complex molecules are produced in a variety of environments, including the inner envelopes (and possibly the outer envelopes) of carbon-rich AGB stars as well as the cores of dense interstellar clouds. As of the present, very little research has gone into the subject of how these molecules are formed, except for two specific classes of species. The production of PAH's (polycyclic aromatic hydrocarbons), ranging in size from species with perhaps 30 carbon atoms to much larger objects, has been considered in warm inner regions surrounding AGB stars. The

basic idea is that under appropriate conditions, the large amount of acetylene present can be partially converted into simple aromatic species such as benzene and naphthalene. These molecules can then grow into much larger PAH's via a high-temperature radical-based synthesis akin to the production of soot via combustion of petroleum in terrestrial laboratories and automobiles (Frenklach & Feigelson 1989). The production of fullerenes (C_{60}, etc.) has been studied in the more rarefied and oxygen-rich atmospheres of interstellar clouds, where a detailed scheme involving low-temperature ion-molecule reactions has been proposed (Bettens & Herbst 1995, 1996, 1997) based on the laboratory work of Bowers and co-workers (von Helden *et al.* 1993), in which fullerene production proceeds through the initial production of straight-chain carbon clusters, followed by large single-ring and double-ring systems. The production of large clusters of carbon atoms, of unspecified structure, has been considered in supernova remnants by Clayton *et al.* (1999) who extended the methods of Bettens & Herbst (1995, 1996, 1997) to form actual carbonaceous grains.

1.9.1 Molecular Cloud Chemistry:

The gas-phase formation of PAH's under interstellar conditions is not likely unless the PAH's are hydrogen-poor because it is difficult for gas-phase processes to hydrogenate large molecules at low temperature (Bettens & Herbst 1995). This constraint is important for the production of other classes of organic molecules. Most of the larger ones found in typical dense interstellar clouds are exceedingly unsaturated, with species such as C_nH radicals and the polyynes (HC_nN) important. Indeed, what relatively hydrogen-rich species exist unambiguously in space are found in local regions of dense interstellar clouds near sites of high-mass star formation. These sources, known as hot cores, are typically much warmer than the ambient dense interstellar medium (100-300 K rather than 10-30 K). Hydrogenation is thought to occur not in the gas, but on the surfaces of dust particles in a prior and cooler stage, where it is brought about by fast-diffusing hydrogen atoms. The observations of hydrogen-rich, or "saturated," molecules in the gas phase in hot cores is thought to signify that molecules on the mantles of dust particles have been released into the gas owing to the rise in temperature. The largest molecules detected in such hot cores are simple ethers (dimethyl ether, and possibly ethyl methyl ether and diethyl ether - see Charnley *et al.* 2001), alcohols (methanol and ethanol), acetone, and methyl formate. Intensive searches in the past for glycine have only achieved ambiguous results. Larger molecules than these, including biogenic species, are certainly present in hot cores, since the abundances of these smaller organic species are often quite high. But, progress awaits the measurement and analysis of the spectra of larger species in the laboratory.

To understand the abundance and distribution of complex molecules (defined roughly as organic species with greater than 10-15 atoms) throughout the interstellar medium, we propose to enlarge our current chemical model networks to include both the formation and destruction of a wide variety of such species. Although a few studies of the actual rate of destruction of complex molecules via both chemical reactions and photons have been published, on balance little is known about complex molecular lifetimes in assorted regions of interstellar space. In the absence of detailed information on the rates of formation and depletion, we will use a variety of theoretical methods, principally statistical theories of reaction rates, as detailed by Bettens & Herbst (1995) in their fullerene analysis. Such methods have indicated that large molecules become quite stable against photodissociation and can even exist for long periods in relatively unshielded diffuse interstellar clouds. We currently have three networks that already have some

complex species in them: (i) a model for the gas-phase chemistry that occurs in the carbon-rich, low-density outer envelopes of AGB stars and protoplanetary nebulae (Millar *et al.* 2000; Woods *et al.* 2003), which currently contains a small number of species through 25 carbon atoms in size, (ii) the model for fullerene production in the interstellar medium (Bettens & Herbst 1997; Ruffle *et al.* 1999), which also contains the chemistry of other types of very unsaturated hydrocarbons, and (iii) a model for hot-core chemistry that contains both gas-phase and surface chemistry (Caselli *et al.* 1993). These model networks will be extended to include larger varieties of complex species, especially molecules, such as the simple amino acids and heterocyclic rings, important in the context of pre-biotic synthesis. A suggested interstellar synthesis of glycine and other amino acids has recently been published (Blagojevic et al. 2003).

It is likely that the more saturated alcohols, esters, and ethers will be produced efficiently in star formation regions, where dust chemistry is important (Charnley *et al.* 2001). Until quite recently, models of the chemistry occurring on dust particles have suffered from a basic problem: typical rate equations are not adequate to explain what happens when only a small number of reactive species are present on a given grain at any time. A new method, known as the direct master equation approach (Green *et al.* 2001, Biham *et al.* 2001) has now been adapted by us (Stantcheva *et al.* 2002; Stantcheva & Herbst 2003) and should be able to solve the surface chemistry problem exactly if the correct surface reactions are delineated and their rates understood. The actual chemistry of star formation regions such as hot cores involves a close interplay between gas and dust. Molecules such as methanol, produced mainly on grains, can desorb into the gas and be the precursors for more complex species formed via gas-phase reactions. We are currently collaborating with a group of Norwegian and Canadian chemists to measure some of the gas-phase reactions that have been suggested.

1.9.2 Complex molecules in protoplanetary disks:

The protoplanetary disk stage of stellar evolution, in which a young, low mass star known as a T Tauri star, is at the center of a dense, optically thick disk of gas and dust, is of extreme interest because these disks are the precursors to planetary systems. Observations of gas-phase molecules by radio astronomers show that the fractional abundances of heavy species such as CO and HCN are significantly lower than in dense interstellar clouds (Dutrey *et al.* 1997). The obvious interpretation, that many of these molecules have been accreted onto dust particles in disks, appears to be the right one. In fact, current models of the chemistry occurring in protoplanetary disks (Aikawa *et al.* 2002; Willacy & Langer 2000) show that at distances greater than ~50 AU from the star, all molecules heavier than H_2 in the midplane of the disks should condense out in far less time than the typical million-year-lifetime of a T Tauri star/disk system. But disks are not flat despite their name; they are known to flare out with increasing distance from the star, so that there is a coordinate for height (Z) as well as a radial coordinate. At very large heights, gas-phase molecules tend not to be present in current models, mainly because the molecules are photodissociated by relatively unimpeded UV radiation and X-rays, both from the central star and from the interstellar medium. Sizeable abundances of gas-phase molecules exist, however, mainly in regions of intermediate height, where the temperature is warm enough to evaporate the molecules from grain surfaces after they accrete onto them, and the radiation field is not intense.

The current state of chemical models of protoplanetary disks is as follows. Models that follow the chemistry of disks with both a vertical and a radial structure are static in nature; that is, the physical conditions, though strongly heterogeneous, are assumed to be constant (Willacy & Langer 2002; Aikawa *et al.* 2002). In these models, sedimentation and coagulation are not included although they are clearly of great importance for planetary formation. Models that include some dynamics do not include the vertical coordinate; i.e., they are one-dimensional models of the midplane. In these models, chemistry occurs during the inside-out collapse from a dense interstellar core and continues to occur as parcels of gas move inwards towards the central star under steady accretion due to angular momentum loss via viscosity (Aikawa & Herbst 1999). The material becomes almost totally solid-state in nature except for molecular hydrogen but can evaporate from the dust particles once the distance to the star becomes sufficiently short. No model that we are aware of contains complex molecules, although it is vital to follow their fate in a protoplanetary disk.

We propose to remove the current shortfalls of one-dimensional and two-dimensional chemical models. First, we will include complex molecules in them. The complex molecules will have initial abundances produced in our dense cloud models. But, since grain chemistry will be active, and since temperatures will rise near the T Tauri star, it is likely that we will form more complex molecules in processes similar to those occurring in hot cores. We will focus initially on one-dimensional models because the accretion of large molecules onto dust particles is clearly important for astrobiology. In addition to both gas-phase and surface chemistry, processes to be considered include coagulation of small dust particles into larger objects, accretion shocks, and inward radial motions. To determine photodestruction rates of complex molecules, we will utilize statistical theories (Bettens & Herbst 1995). Once one-dimensional models have been successfully upgraded, we will turn to our two-dimensional models. One specific question of interest is the height of the zone in which molecules are depleted from the gaseous state. This height would certainly be related to the planarity or lack thereof of the planetary system that is produced.

1.10 Role of Membranes in the Evolution of Cellular Life

Clearly the presence of the molecules of life is a necessary prerequisite; yet it is also necessary for them to be confined to surfaces or contained within a suitable compartment within which the formation of macromolecules may be possible. In either case, the effective concentration is increased and can promote further reactions, which would be improbable in the gas phase. The formation of membranes involves amphiphilic compounds, which in aqueous solution are known as surfactants (detergents) and biological lipids.

Biological membranes mediate many of the distinctive functions of life on earth, and their formation is prerequisite to any conceivable form of cellular life. It is known that membranes comprise a lipid bilayer, which provides a permeability barrier to the passage of ions and polar molecules, together with proteins which mediate various biological functions, including active transport, photoreception, light energy transduction, and the generation of electrical signals. At the present time two generic schemes for the origin of cellular life on earth are under discussion. The first supposes initially that small molecules were formed within a primordial aqueous environment, leading eventually to their polymerization and subsequent incorporation within a membrane-encapsulated volume to yield the earliest cells. In the second scheme, spontaneous

self-assembly of amphiphiles into bilayer vesicles preceded polymerization of small molecules, with further membrane growth occurring by a photosynthetic process. Moreover, a further consequence of this compartmentalization is that gradients of chemical potentials of various solute species present in the prebiotic aqueous environment would be possible, e.g. as a consequence of photosynthetically produced gradients of H^+ or other ions. Thus a source of free energy for polymerization systems involving small molecules would exist, e.g. involving nucleotides or activated amino acids.

The latter mechanism is plausible since common amphiphiles can be formed from relatively simple precursors such as long chain alcohols and inorganic phosphate (Chachaty *et al.* 1988; Caniparoli *et al.* 1988). Such organic amphiphiles are found in carbonaceous meteorites (Deamer 1985; Deamer & Pashley 1989). The proposed studies will focus upon the investigation of dialkyl phosphates as plausible models for prebiotic amphiphiles. These compounds exhibit sufficient richness of polymorphic phase behavior that they are able, depending on the environment, to form a variety of self assembled structures.

The specific objectives of the proposed work will encompass the following:

1) Determination of phases diagrams for aqueous dispersions of a homologous series of dialkyl phosphates, in which the number of chain carbons n = 6, 10, 14, 18;

2) Characterization of influences of salt and pH on temperature-dependent polymorphism of aqueous dispersions of dialkyl phosphates;

3) Characterization of isotropic micelles and lamellar and reversed hexagonal phases of dialkyl phosphates; investigations of conditions necessary for spontaneous vesicle formation;

4) Comparative studies of average properties of dialkyl phosphates in different organized assemblies by deuterium (^2H) NMR lineshape analysis (Brown 1996); investigation of dynamical properties at the molecular level by nuclear spin relaxation measurements (Martinez *et al.* 2002).

Very briefly, modern state-of-the-art nuclear magnetic resonance (NMR) methods will be applied to investigate the average thermodynamic properties and phase behavior of organized assemblies of dialkyl phosphates in water (Brown & Chan 1996). These and related amphiphilic compounds have been hypothesized to be implicated in early protobiological assemblies leading to encapsulated membrane systems, self replication by photosynthetic growth, polymerization of small molecules, and eventually life on earth as we know it. The polymorphic phase behavior of a series of dialkyl phosphates will be investigated as a function of variables to include temperature, salt, and pH. These studies will provide knowledge of the requirements for formation of highly curved surfaces, leading to the formation of closed membranous structures such as vesicles. We will test the hypothesis that the curvature free energy of the membranous assemblies is a crucial variable leading to the formation of closed membrane vesicles. In addition the microscopic configurational and dynamical behavior of the lipid systems will be investigated (Nevzorov, Trouard & Brown 1998). The latter will establish a connection between microscopic properties and bulk or average properties of the systems, which may be important both with regard to the polymorphism of the assemblies, as well as the interaction of the amphiphiles with other lipid constituents such as polyisoprenoids, which may represent chromophores, found in the prebiotic milieu.

2.0 Module 2: Formation and Evolution of Habitable Worlds

Team: L. Close, M. Giampapa, A. Glassgold, P. Hinz, D. Kring, J. Lunine, R. Malhotra, M. Meyer, J. Najita (Deputy), and S. Strom (Lead).

2.1 Introduction

Gas giant planets are the most readily observable diagnostic signatures of emerging and mature planetary systems. They play a decisive role in determining whether earth-like planets can form and survive in habitable zones. They sculpt the remnant debris disk of planetesimals, they control, both the influx of volatiles delivered to terrestrial planets in the inner solar system as well as the rate of giant impacts over geologic time. Our proposed program provides measurements which directly constrain when giant planets form, and where they are located in mature planetary systems—observations central to understanding the likelihood that life-bearing planets can form, survive, develop oceans and atmospheres, and sustain biological trends for long periods absent cataclysmic events.

We make use of ground- and space-based spectroscopic observations mapping the gas content of disks surrounding stars of differing ages to determine how long gas sufficient to form giant planets is present. The existence and location of giant planets can then be inferred from spectroscopic signatures of "tidal gaps".

In addition, we make use of ground- and space-based infrared and submillimeter emission arising from small dust grains in circumstellar disks with ages from 3 Myr to 3 Gyr—from when planets are forming to when planetary systems are mature. Spectral energy distributions and low-resolution spectra probe the total dust mass as well as its radial distribution and composition. In combination with theoretical models, these observations provide the basis for inferring the location of giant planets, planetesimal collision rates in exo-terrestrial planet zones and the rate at which volatiles are transported from the outer to inner solar system.

The proposed efforts thus speaks directly to road map goal 1, and provides data which serves as context for the design and science strategy of a TPF mission, goal 1.2.

The third part of the module provides essential constraints on the development of atmospheres and climatic variations through observations of variability and activity among sun-like stars, spanning ages from the earliest phases of accretion activity to characterization of the range of variations for sun-like stars throughout their lives.

In the pre-main sequence phase (1-30 Myr), large variations in the x-ray and UV flux could play a significant role in ionizing the circumstellar disk and driving chemistry important for determining abundances of organic molecules. Indeed the results from this study will be important for the work described in Module 1, as well as the modeling of observational results on gas-rich disks in this module.

Over longer timescales, we will characterize the mean and dispersion in solar flux by observing sun-like stars from 30 Myr to the age of the Sun. The sensitivity of earth's climate to modest

variations in insolation is well known. The Maunder sunspot minimum is associated with a severe cooling of the Northern Hemisphere, and associated famine. Here we are both interested in how solar cycle variability has changed with time, and how it can be expected to change in the future. The decline in stellar activity with age is well known, and here we focus on a quantified analysis not only of general trends, but of excursions about the average. The Sun is apparently much less variable than typical stars of its age, and far less variable than younger stars. Is the Sun's relative stability responsible for providing a hospitable evolutionary environment on earth? If so, how frequently do solar-type stars exhibit such benign activity excursions?

These measurements provide crucial connection between studies of complex organic chemistry in circumstellar disks (Module 1) to investigations of climate variability such as those undertaken by the NAI focus group ``Mission to Early Earth'' addressing NAI Roadmap Goals 4.1 and 6.1.

2.2 Task A: Gas Rich Disks

2.2.1 Overview

Understanding the formation of giant planets is critical to understanding the origin of the Earth and the potential for habitable extra-solar planets. On the one hand, Wetherill has suggested that the development of life on the Earth may be a consequence of the existence of Jupiter, because Jupiter probably cleared the inner solar system of planetesimals that would otherwise have impacted the Earth at a damagingly high rate. On the other hand, at earlier times, giant planets may have worked *against* the survival of earth-like planets. For example, the discovery of extra-solar giant planets at small orbital radii (~0.1 AU; e.g., Marcy and Butler 1998) suggests that giant planets migrate readily inward from their formation distances at larger radii (~5 AU; e.g., Boss 1995). Giant planets that form early, e.g., during the disk accretion phase in which orbital migration is likely to be rapid, are likely to migrate in close to their stars, sweeping along any earth-like planets that have already formed at smaller radii. Solar systems in which giant planets form late, at the end of the disk accretion phase, have the best chance of preserving earth-like planets at AU distances (e.g., Trilling *et al.* 1998). These considerations raise fundamental questions regarding the origin of planetary systems: where and when do giant planets form?

Where do Giant Planets Form? Traditionally, theories of giant planet formation have focused on the growth of planets under the protoplanetary disk conditions expected at ~5 AU. However, the large spread in the orbital radii of the known extra-solar planets may indicate that giant planets actually form over a range of disk radii and physical conditions. Indeed, it is probably inaccurate to assume that planets formed where they are now observed to be because dynamical effects, such as orbital migration due to tidal interactions between the disk and protoplanet and dynamical scattering between planets following disk dissipation (e.g., Lin and Ida 1997), are likely to significantly alter planetary systems. In order to determine the formation distances of planets, we need to carry out a census of young (1 – 10 Myr) planetary systems. Ideally, we would hope to measure the masses and orbital radii of planetary companions, for comparison with the properties of older (several Gyr old) systems, in order to begin to chart out the evolution of planetary systems.

When do Giant Planets Form? What is the likelihood that disks that have ceased accreting (at ages t > 1 Myr) still have the gas reservoirs needed to form giant planets? Moreover, is the

actual gas dissipation timescale consistent with present theories of giant planet formation? Current theory requires that the gas in disks survive for ~10 Myr in order for planetary cores to accrete a massive gaseous envelope (e.g., Podolak *et al.* 1993). However, existing studies of the gas in outer disks (>50 AU; e.g., Zuckerman *et al.* 1995), indicate a giant planet formation timescale <10 Myr. Additional evidence for the rapid dissipation of disks comes from studies of the IR excesses of young stars, which show that inner disks (r <1 AU) become optically thin in concert with the cessation of stellar accretion, i.e., on timescales <10 Myr (e.g., Strom *et al.* 1989). These results favor the *early formation* of giant planets which is less advantageous for the survival of Earth-like planets. Moreover, these results, combined with the now frequent detection of extra-solar planets via radial velocity techniques, suggest that giant planet formation theory may require serious revision. Unfortunately, the observational motivation for a theoretical overhaul is far from definitive because there have been no direct measurements of the gas content of disks within ~5 AU. We propose to test this scenario by measuring the gas dissipation timescale in disks at radii < 5 AU. If we find that abundant reservoirs of gas persist beyond the stellar accretion phase, we will establish the possibility of an extended period of giant planet formation and the likely preservation of earth-like planets. The proposed study will also place a fundamental constraint on theories of giant planet formation. Finally, the spectroscopic diagnostics that we propose to develop will lay the groundwork for future opportunities: the indirect detection of forming protoplanets and the measurement of planet formation distances (see Section 2.3).

2.2.2 Proposed Program

Observational: We will search for molecular emission from disks at <5 AU surrounding nearby (< 150 pc) sun-like stars in the age range 1-10 Myr. Since the angular scales involved are much beyond our current capability to resolve spatially (1 AU at the nearest star forming regions at 150pc is <10 milli-arcseconds), we will use high-resolution spectroscopy to determine where in the disk the detected gas resides. The detected emission strengths will be converted into gas content as a function of disk radius using the results of the theoretical studies described below. Diagnostics in the thermal infrared (4-30μm) are ideal for this study since the Planck function for disk material at < 5 AU peaks in the mid-infrared. At the warm temperatures (100 – 2000 K) and high densities of disks at these radii, molecules are expected to be abundant in the gas phase, and sufficiently excited to produce a rich ro-vibrational and rotational spectrum. We are part of a team that will utilize modest resolution (R=600) spectra to be obtained with SIRTF through the Legacy Science Program (Meyer *et al.*) to constrain the lifetime of gas-rich disks capable of forming gas giant planets. Abundant molecules such as CO, OH, H_2O, and H_2 have transitions in the mid-infrared, and can be used to trace the structure and dynamics of disks. Work to date on high-resolution IR spectroscopy of disks has shown that the CO overtone (2.3μm), CO fundamental (4.6 μm), and H_2O (~2μm) ro-vibrational lines can be used to probe the kinematics and physical structure of disks at radii < 2 AU (Fig. 2.1; see also Najita *et al.* 1996, 2000, 2003), i.e., in what is today the terrestrial planet region of the solar system. By extending these studies to other molecular species and wavelengths (e.g., OH, H_2 and longer wavelength H_2O lines), it should be possible to extend these studies to the larger distances traditionally considered in giant planet formation theories (r > 5 AU). We plan a two-pronged approach which includes: 1) complete pilot survey using high resolution near-IR spectroscopy to survey nearby young stars; and 2) utilize ground-based mid-IR echelle spectrometers to follow-up objects detected in the SIRTF Legacy Science Program of Meyer *et al.* (described below).

Theoretical: The observations will be complemented by a detailed theoretical study of the thermal and chemical structure of gaseous inner disk atmospheres. Disk atmosphere calculations to date refer primarily to the temperature structure in the dust component of the disk (e.g., D'Alessio *et al.*1998; Chiang and Goldreich 1997). However, the gas and dust components are poorly coupled thermally in the upper disk atmosphere. Consequently, the temperature structure of the gaseous atmosphere requires its own calculation. For the proposed program, we will build a detailed study of the thermal and chemical structure of disk atmospheres that includes all of the diagnostics to be studied observationally.

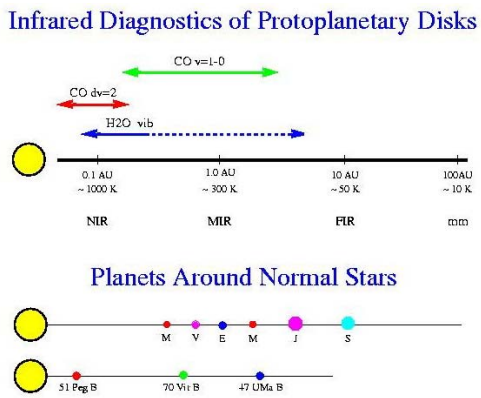


Figure 2.1 ***Infrared spectral line diagnostics of disk:*** The CO overtone (Δv=2, 2.3μm), CO fundamental (v=1-0, 4.6μm), and the ro-vibrational water lines (K -band), probe the structure of disks.

2.2.3 Future Opportunities

The spectroscopic diagnostics that we have proposed to develop will also lay the ground-work for future opportunities: the indirect detection of forming protoplanets and the measurement of planet formation distances. We will develop this potential technique to the extent possible during the proposed funding period.

Indirect Detection of Forming Protoplanets: It may be difficult to detect young planets directly during the epoch of their formation (i.e., when a substantial circumstellar disk is still present): the much larger emitting area of the disk compared to the planet may make it difficult to detect the planet in the glare of the disk. Thus, we may have to rely on a more indirect, dynamical signature of the presence of planetary companions. Dynamical theory predicts that as a giant planet forms, tidal interactions between the planet and disk clear a region in the disk, a ``gap", within which the planet orbits (Lin and Papaloizou 1993; Takeuchi *et al.* 1996). Since the width of the gap depends on planetary mass, measurements of the location and width of gaps in protoplanetary disks can ultimately provide us with a method of inferring both the masses and orbital radii of planets at their epoch of formation. Given the small angular scales subtended by inner disks (and the even smaller angular width of gaps created by giant planets) e.g., Jupiter-mass companions will produce ~ 0.3 AU wide gaps at an orbital distance of 1 AU; Takeuchi *et al.* 1996). The most promising approach by which it would be possible to search for forming giant planets in the next ~ 5 years is through high resolution spectroscopy. With this technique, we would search for line emission from the gap which will appear bright against the absent continuum emission from the gap. High spectral resolution will be used in lieu of high angular resolution to identify the range of disk radii over which the gap extends. The resulting spectral energy distribution (SED) will be indistinguishable from that of a disk without a gap. Even with the high photometric accuracy and continuous wavelength coverage of SIRTF (or JWST) observations of disks in the mid-infrared, such small gaps will be impossible to detect given the ambiguities involved in interpreting SEDs. Our simulations indicate that 8-10m telescopes have the sensitivity to detect spectroscopically gaps created by Jupiter-mass companions in the nearest star-forming regions.

2.3 Task B: Evolution of Planetary Systems

2.3.1 Overview of Debris disks

The growth and survival of earth-like planets depends critically on the architecture of the parent planetary system: Jovian mass planets in or near the terrestrial zone will doom the formation of earth-like planets (Jones, Sleep, & Chambers 2001), but may have life bearing satellites of their own. The likelihood that planets in the terrestrial zone will have oceans and life-friendly atmospheres may well depend on this architecture as well, since transport of volatiles from the outer solar system is believed linked intimately with the relative location of the terrestrial planets, giant planets and outlying cometary material. Finally, whether life on an earth-like planet has sufficient time to evolve—even on apparently hospitable worlds—depends on the number and time between cataclysmic collisions of the sort that resulted in the extinction of the dinosaurs. The collision frequency in turn is linked to the architecture of the parent planetary system: 'favorably' located giant planets can quickly remove planetesimals from the terrestrial zone, while earths in systems with distant Jupiters or none at all may suffer high, potentially life-stultifying collision rates.

The Space Infrared Telescope Facility (SIRTF) is a cryogenically cooled mid- to far-infrared space telescope with unprecedented sensitivity to be launched by NASA this spring. The University of Arizona and NOAO are heavily involved with this mission, contributing one of the scientific instruments and leading two of the major data collection ("Legacy") projects. This expertise and the data will be made available to the NAI as part of the Arizona membership. With the advent of the SIRTF satellite, we can for the first time carry out observations diagnostic of (i) planetary architectures, and (ii) the rate of collisions among large bodies in terrestrial zones for a large number of sun-like stars. These span a wide range of ages—from 1 Myr to 5 Gyr, equivalent to pre-Hadean to the present. These observations, combined with dynamical modeling of simulated exo-solar systems can provide the basis for placing our solar system in context. We can assess the likelihood that habitable planets exist and the potential for life's surviving and evolving on those planets.

We plan to use precise infrared spectral energy distributions from SIRTF to infer the surface density, temperature and approximate mineralogy distributions of small grains surrounding sunlike stars. Ground-based imaging both of light scattered earthward by the dust, and of thermal emission from dust grains provide incontrovertible evidence that this dust is arrayed in a disk of dimension several hundred astronomical units.

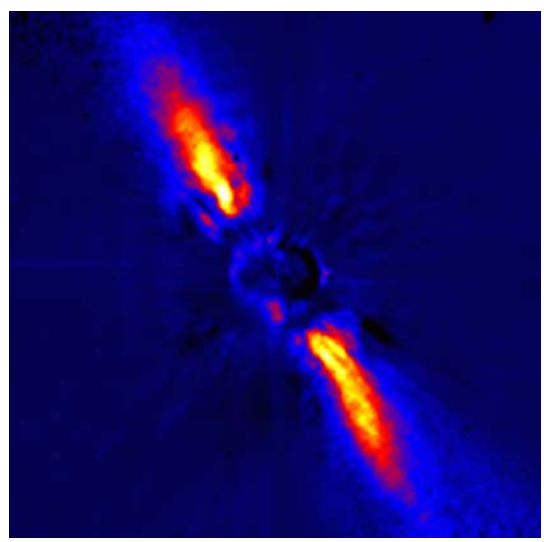


Figure 2.2: Adaptive optics image of light scattered earthward by the dust disk surrounding the nearby star, β Pictoris (ESO NTT). The light of the central star has been occulted by a coronagraphic disk.

The lifetime of dust grains with sizes of order 1 micron (typical of those responsible for producing both thermal emission and scattered light) is well less than 1 Myr—the time required for Poynting-Robertson drag to cause such grains to spiral inward toward the surface of the parent star. The dust disks are thus "second generation" material—dust released

from larger parent bodies such as asteroids or comets through fragmenting collisions or sublimation. Further, the presence of large, planetary masses is *required* in order to stir the smaller planetesimals; planetesimals alone are not capable of sufficient mutual perturbation.

Recent images of nearby debris disk systems show that some disks possess central gaps comparable to the orbit of Neptune in our solar system, as had been inferred previously based on the observed spectral energy distribution shape (Fig. 2.3).

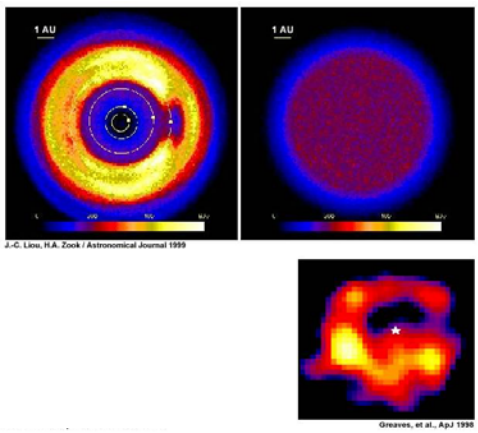


Figure 2.3. A comparison of thermal emission from the dust disk surrounding the star Epsilon Eridani (lower right; Greaves et al. ApJ, 1998) with two computer simulation (Liou & Zook 1999) of how our solar system (upper left) would look to an outside observer; four dots represent Jupiter, Saturn, Uranus and Neptune. Blue represents a relative 'dust void' (resulting from dynamical ejection of colliding bodies from the inner solar system by Jupiter). By contrast, note the even distribution of dust throughout the model solar system having no giant planets (upper right). Epsilon Eridani appears to have an "inner hole" analogous to that found in our own solar system.

The significance of debris disks is that they offer surprisingly easy means for inferring the existence of planetesimals and/or planetary systems. They can be inferred from infrared excesses arising from secondary grains arising from collisions. Their architecture can be inferred both from the shape of spectral energy distributions and from subsequent resolved observations with large, ground-based telescopes (Li & Lunine 2003), and their evolution can be traced from observations of large samples of sun-like stars spanning a wide range of age.

From study of the radial distribution of our own solar system dust, for example, one could infer not only the existence of the asteroid belt and Kuiper Belt, but also the presence of planets with locations and masses, e.g. that the planetesimal populations are dynamically "hot".

2.3.2 Proposed Programs

Observational: UA astronomer and proposal deputy PI Michael Meyer and colleagues from Arizona, Caltech and elsewhere have been granted observing time, as a coherent ("Legacy") program, to observe 345 solar-like stars ranging in age from 3 Myr to 3 Gyr with SIRTF. The goals of this program are to use precise spectral energy distributions (from 3 microns to 160 microns) to (i) constrain the structure and composition of disks; (ii) trace the evolution of gas and dust disk structure and mass over time; (iii) infer the presence of planets at orbital radii ranging from 0.3 to 30 AU. These observations form the basis for addressing several questions key to the planet formation part of the astrobiology roadmap (Goal 1).

Building on the planned SIRTF program (Meyer *et al.* 2001), we will carry-out follow-up surveys with state-of-the-state ground-based platforms aimed at providing spatial information, crucial for breaking potential degeneracies inherent in modeling spectral energy distributions. Meyer, Close, and Hinz will utilize the high resolution MMT-AO system with the ARIES and MIRAC/BLINC cameras (see Module 3 for technical detection) in direct imaging mode to provide the needed observations for our strongest SIRTF detections (~10-30 stars). A similar

capability is planned for the Magellan telescope in the southern hemisphere within the period of performance of this proposal. We also plan follow-up studies at high spatial and spectral resolution with the IRIS (medium resolution mid-IR spectrograph for use with the MMT and Magellan) to look for evolution in dust mineralogy across resolved circumstellar disks. For a similar-sized sample of candidates with weak disk emission, we will utilize the LBTI instrument described in Module 3 to search for remnant dust that could complicate the detection of earth-like planets with TPF (Roadmap Goal 1.2).

Theoretical: Under the proposed NAI membership, Malhotra, Lunine, Kring, and colleagues will develop models constrained by the SIRTF and ground-based data. Our ultimate goal is to connect observational studies of the evolution of planetary systems with the cratering record of our own solar system (under study by the proposed NAI Impacts Working Group) as a tool to understand the effects of solar system dynamics on the evolution of habitable zones.

Constraining collision rates in the terrestrial planet region: SIRTF observations will provide measures of dust optical depth as a function of distance from the parent star via observed excess infrared emission above photospheric levels (*near*-infrared, 10-20 micron, excess emission provides a measure of dust optical depth in the terrestrial planet region). Because the grain population in the disk is 'secondary', dust optical depth is directly related to collision rates between planetesimal-sized bodies. By comparing observed dust optical depths in terrestrial planet zones for sun-like stars of differing age in our SIRTF Legacy sample with those at differing epochs of our own solar system (derived from observations of the Zodiacal Cloud and inferred from cratering histories) we can determine whether collision rates for exo-solar systems are higher or lower than our own. If the typical rates inferred for exo-systems are higher, the frequency of devastating impacts on earth-like planets will likely be higher—diminishing the likelihood that advanced life forms develop.

Presence of interesting organic materials in disks: University of Arizona Theoretical Astrophysics postdoctoral fellow Aigen Li and Lunine have demonstrated with UKIRT (United Kingdom Infrared Telescope Facility) the ability to distinguish the presence or absence of PAH's in disks that are remnants of planet formation around nearby stars (Li and Lunine, 2003; Li and Lunine, in prep). The presence or absence of PAH's seems to be tied to the variation in geometry and hence conditions from one disk to another, and should provide insights into the distribution of organic material present in such disks. The results of these studies for SIRTF-detected disks will be as a boundary condition for the modeling of the distribution and delivery of organics to terrestrial planets based on dynamical models Lunine has pursued with colleagues in Europe (Morbidelli *et al.* 2000) and the University of Washington (Raymond, Quinn, & Lunine 2003). It will also be an input into the chemical studies of complex molecule formation in protoplanetary environments (Module 1).

Planetary System Architecture: Observed spectral energy distributions will provide estimates of the surface density of micron-size dust as a function of distance from the parent star, $\Sigma(r)$ (Li & Lunine 2003). In turn, $\Sigma(r)$ provides the basis for inferring the number of collisions among planetesimals as a function of radius, a quantity that reflects the both the current and past dynamical history of the disk, and the role of giant planets in shaping that history. We plan to combine $\Sigma(r)$ distributions inferred from SIRTF Legacy observations with dynamical modeling

to infer (i) planetary architectures, and (ii) the rate of volatile transport from outer to inner solar system regions.

University of Arizona co-I Malhotra and collaborators will use the configurations of giant planets inferred from SIRTF observations to model the dynamical evolution of planetesimal swarms in these systems. We will employ N-body integrators developed by Malhotra (1996) and Hahn and Malhotra (1999) for the co-evolution of giant planets and planetesimal disks to predict Σ(r) for different initial configurations of giant planets. These Σ(r) predictions can then be compared with those derived from SIRTF observations, thus constraining plausible planetary architectures.

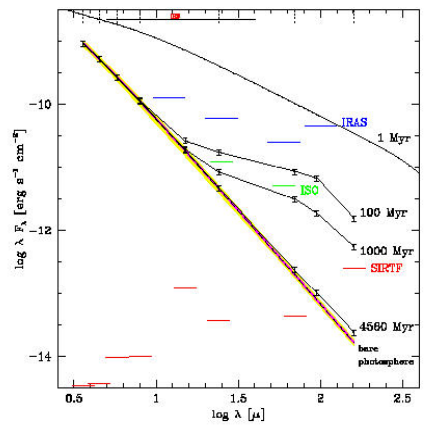


Figure 2.4*:* Model spectral energy distributions for a hypothetical solar system surrounding a solar-like star located at a distance of 30 pc, for ages 4560, 1000, and 100 Myr. Indicated in the figure are the limits of the IRAS survey, and the predicted limits of the Meyer et al. SIRTF Legacy survey observations.

We will compute the probability that planetesimals starting at a given semi-major axis would collide with terrestrial planets on various stable orbital configurations in the habitable zone. For each initial semi-major axis at which planetesimals are initiated in the simulation, based on nebular models, we can associate a particular set of volatile abundances including water and organic compounds (Lunine, Owen, & Brown 2000). Thus we will track, in concert with the probability of collision with potentially habitable planets, the amount of ocean-forming and life-forming materials putative terrestrial planets will gain.

Computation of volatile load of planetesimals will be guided by the results for our own solar system. While detailed models of planetesimal composition do not uniquely define the composition of a planetesimal swarm versus semi-major axis, we are mostly interested in the existence of major volatile species such as water and organics, rather than details such as the chemical form of nitrogen (ammonia or molecular nitrogen) and such other issues. Water-bearing planetesimals include those with water ice or bound water of hydration, equivalent in our own solar system to the icy planetesimals of the outer solar system and of the asteroid belt. Organics can also be predicted based upon what is seen in the major meteorite types as well as what is contained in comets. We thus will label planetesimals "water-bearing" or not, and "organic-bearing" or not, with rough inventories based upon the cometary and asteroidal (meteoritic) analogs in our solar system. This will be sufficient to define the probability of volatile delivery to the habitable zone, as shown by Morbidelli et al (2000). Hence these calculations will serve as input for ongoing calculations by Lunine in collaboration with Sean Raymond and Tom Quinn at the University of Washington (Raymond, Quinn, & Lunine 2003) to predict the delivery of volatiles during large-body accretion associated with the terminal phases of terrestrial planet formation (Chambers & Cassen 2002). While these calculations have been ongoing in parallel using statistical distributions of giant planets, the SIRTF data will give us the first opportunity to use observations to determine locations of giant planets, and hence predict the dynamical environment and delivered volatile inventory to possible terrestrial planets in the habitable zones of those systems.

2.4 Task C: Solar irradiance changes from formation onwards

The next stage of evolution of terrestrial planets after disk dispersal is evolution in the presence of stars that themselves evolve over time as they enter the main sequence phase of hydrogen burning. Stars like the Sun vary in energy output on a range of timescales. This module will, for the first time, systematically quantify such variations.

2.4.1 Overview

The changing output of solar-type stars (total, ultraviolet and x-ray luminosity)—from the time of planet-formation to the current epoch—is needed to understand: (i) the formation and early evolution of planetary atmospheres (t < 500 Myr); and (ii) climatic variations among more mature planets (t > 500 Gyr). Charting overall energy output and cyclic solar activity on sun-like stars spanning a range of ages thus provides crucial insight into how frequently earth-like atmospheres are likely to form and survive, and how frequently exo-earths encounter benign climatic variations.

The solar atmosphere exhibits a variety of magnetic field-related phenomena—powerful flares, cool spots, hot active regions, episodic mass ejections, the warm 10,000-degree chromosphere and the 2 million degree corona, and the sunspot cycle—that we refer to collectively as "activity". The signature of the nonradiative heating in many of these magnetic active areas is easily detected by the enhanced radiation it produces in chromospheric spectral lines, such as the Ca II H and K resonance features near 400 nm. Similarly, evidence for this same kind of activity is readily detected in the spatially unresolved spectroscopic and broadband photometric observations of sun-like stars. In particular, observations of solar-type stars have now demonstrated that activity-related, irradiance variations are a universal property of a normal, solar-type star. Moreover, the level of activity is generally correlated with age. Young precursors to solar-like stars exhibit enhanced activity. This activity then declines with age in single (i.e., non-binary or multiple) stars.

In a study of nearby, solar-type stars, Lockwood *et al.* (1997) find that among stars with activity like the Sun average value, over 40% of the sample exhibited irradiance variations ranging from 0.3% to 1.2%, i.e., 3 to 12 times the amplitude of irradiance variations observed in the Sun (so far)! In fact, the irradiance variability of the Sun appears somewhat subdued for its level of activity (Radick *et al.* 1998). Whether the Sun is unusual in this regard is still a controversial point.

For solar-type members of the much younger, 0.6 Gyr old Hyades cluster, the *mean* level of cyclic irradiance variation is about 10 times the level of present-day solar variations (Radick *et al.* 1998). Thus, we infer that the ~0.5 Gyr -old Sun (end of the Hadean era) typically exhibited irradiance variability at a level that was an order of magnitude greater than what we see now. Even younger sun-like stars in the 100 Myr old Pleiades cluster are characterized by a mean level of magnetic activity that is ~6 times greater than the contemporary Sun. While irradiance monitoring of these stars over long time-scales has not yet been performed (an objective of this module), the predicted level of cyclic irradiance variability is about 2% or 20 times that of the present-day Sun.

2.4.2 Proposed Program

Effort (I): Visiting the epoch when the sun was young: Solar UV radiation plays a dominant role in the chemistry and dynamics of the earth's atmosphere. Moreover, the UV irradiance of the young Sun may have played a critical role in the origin of ozone and free oxygen in the prebiological paleoatmosphere of the earth (Canuto *et al.* 1983). Similarly, the nature of the habitable zones of young planetary systems, including the chemical evolution of young planetary atmospheres, will be impacted by the level and variability of the UV irradiance arising from magnetic field-related activity in the parent stars over both short and long (cycle-to-evolutionary) time scales.

We propose to establish an initial program to investigate the evolution of activity and the associated UV irradiance variations in solar-type stars spanning a wide range of ages. Of course, the UV emittance is not directly observable with ground-based telescopes. Therefore, our approach will rely on the observation of an accessible *surrogate* for UV irradiance in solar-type stars that are members of open clusters of known ages. These are the emission cores of the same H and K lines.

Knowledge of the variability of the Ca II H and K lines can provide an accurate estimate of the variations in the ultraviolet spectrum. Solar-type stars that are members of clusters, such as those shown in Figure 2.5, would be selected for long-term monitoring. The study of cluster members provides a stellar sample that is homogeneous in age and chemical composition. The mean level of normalized Ca II line emission in sun-like stars in clusters of known age is illustrated in Fig. 2.5 (adapted from Walter & Barry 1991).

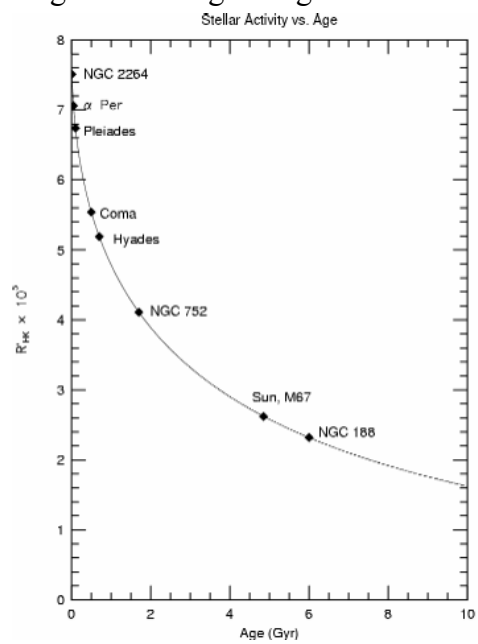


Figure 2.5. ***The mean level of magnetic field-related activity of solar-type stars in open clusters of known age.*** The index R(HK) is the Ca II H&K line emission normalized by the stellar bolometric (total) flux and corrected for non-magnetic (photospheric) contributions to its total strength.

While surveys of activity diagnostics in the visible, UV and X-ray have been performed for several young clusters (see Fig. 2.5), there is little information on the short-term excursions of activity (e.g., flares), which can occur, and no information on the nature of cycles (analogous to the solar cycle) in young (0.1 Gyr), solar-type stars. We would therefore utilize the MMT with the Hectechelle, or the WIYN telescope with the Hydra multiobject spectrograph, as appropriate, for Ca II H and K line observations of solar-type stars in selected clusters during one-night per month for at least 5 years, along with a parallel program of photometric monitoring with a GNAT (*Global Network of Astronomical Telescopes*) automated telescope. The results will yield quantitative insight on the nature of cycle-related activity—both UV and total irradiance variations---in solar-type stars at the young ages that presumably correspond to the early history in the evolution of planetary atmospheres. The most likely clusters for study are, in order of priority, the Pleiades (age ~0.1 Gyr), NGC 752 (1.7 Gyr), and the Hyades (age ~0.7 – 1 Gyr). Together with the M67 (age ~5 Gyr) program discussed in Proposed Effort (II), a complete

picture of the short-term and cycle-related variability of solar counterparts at ages ranging from 0.1 – 5 Gyr will be developed. Because of the development of photosynthesis during the Archean era (age 0.8 – 2.2Gyr) we will place emphasis on this era. We also propose a program aimed at understanding solar activity in the 0.01 to 0.1 Gyr range. Here, our focus will be on establishing the *range* in overall activity levels among solar-type stars during the epoch of planet-building and early planetary evolution. These results will provide critical context for the development of models of exoplanetary atmospheres during the early stages of planetary system evolution.

Effort (II): Placing the Mature Sun in Context: Our knowledge of the full range of solar variability that may occur is extremely limited. Given that individual solar cycles are different in form, amplitude and length, and because accurate solar data have been available only for the most recent cycles, there is no direct way of understanding *long-term* solar variability.

The observation of solar-type *stars* however, can be productively exploited to overcome the temporal confines of the solar database, thereby revealing the potential range and nature of *solar* variability over time scales that are simply not accessible to the modern solar database of only a few decades. We therefore propose to utilize the upgraded MMT with the Hectechelle multi-object spectrograph, along with an automated photometric telescope, to assemble a unique data set consisting of long-term observations of cycle-related, magnetic activity and associated irradiance variability in solar-type stars at various stages of evolution. The results will vastly increase our knowledge of the potential range of solar cycle-like variability on both short (~decades) and evolutionary time scales, from the very young Sun to the contemporary Sun. The resulting data can then be utilized as input for models of global climate change and for models of planetary atmospheres at various stages of evolution. In essence, we will delineate the nature and potential range of the joint variability of luminosity and activity experienced by "mature" planetary systems (including our own) bathed in the ambient and variable radiation fields of their parent suns.

As a first step toward understanding the nature and full range of variability of the contemporary Sun, we have completed a survey of the level of chromospheric Ca II H&K line core emission in the solar-type stars in the galactic cluster M67. This cluster is an especially appropriate object of observation since it is approximately the same age (about 5 Gyr) and has the same chemical abundances as the Sun. Our working hypothesis is that a single `snap-shot' of a large sample of solar analogs reveals the potential range of *solar* chromospheric activity. In this way, we immediately obtain information on the potential *long-term* variability of the Sun that would not otherwise be possible (or practically feasible) with the modern solar Ca II data-base of only two decades. This is especially important given that the amplitude of long-term, solar and stellar variations in *brightness* are correlated with cycle variations in *chromospheric* emission (Hudson 1988; Radick 1991). In view of the fact that the Sun is the engine that drives climate on the Earth, any variation in the solar `constant' must be taken into account in the investigation of the long-term behavior of the global climate.

In the M67 sample, there are 74 solar-type stars in the M67 sample with spectral types of G0 V - G9 V), corresponding to a range in mass of 1.10 - 0.95 solar masses (following VandenBerg & Bridges 1984). Among these stars are 21 "solar-twins" with intrinsic B-V colors between 0.63

and 0.67, which is the range of colors that has been quoted in the literature for the Sun as a star (VandenBerg & Bridges 1984, see their Table 2).

The results from the M67 survey of chromospheric activity in solar-type stars are encapsulated in the accompanying histogram (Fig. 2.6). The abscissa gives the H and K index, R, which is the flux in the Ca II H and K cores divided by the stellar bolometric flux. The R-values have been corrected for a non-chromospheric (photospheric) contribution (following Noyes *et al.* 1984). We display the distribution of this index for 74 solar-type stars in M67. The contemporary solar cycle is also shown, based on data obtained by W. C. Livingston (NSO/Kitt Peak) at the McMath-Pierce Solar Telescope facility of the National Solar Observatory on Kitt Peak from 1976 - 1994.

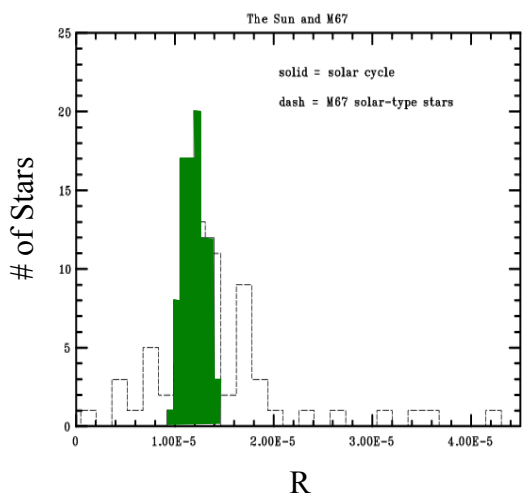


Figure 2.6. ***The solar cycle and M67 solar-type stars with masses in the range of 1.10 – 0.95 solar masses.*** About 18% of the solar analogs (colors 0.60 < (B-V) < 0.76) exhibit especially quiescent levels of emission while about 32% display enhanced levels of activity compared to that at solar maximum. Of these, about 5% are an extreme state of activity.

The broader distributions in chromospheric Ca II strength for both the M67 solar-type stars, compared to that of the Sun during the contemporary solar cycle, suggest that the potential excursion in the amplitude of the solar cycle is greater than what we have seen so far. The stars with R-values noticeably less than solar minimum may be in a prolonged state of quiescence analogous to the Maunder-minimum episode of the Sun during A. D. 1645-1715 when visible manifestations of solar activity vanished. This period corresponds to a time of reduced average global temperatures on the earth known as the ``Little Ice Age'' (Foukal & Lean 1990).

Among the solar-type stars, about 18% are characterized by R values that are less than that estimated for the Maunder Minimum while approximately 32% exhibit R indices which are greater than that found at solar maximum. The Maunder-minimum value for R is derived from data given by Baliunas & Jastrow (1990) who, in turn, base their estimate on observations at the centers of solar supergranulation cells where the magnetic flux density is small.

In summary, we find that approximately 32% of the solar-type stars are in an enhanced state of magnetic activity while about 18% are at a significantly lower level--perhaps in a Maunder-minimum state. This could imply that the Sun spends nearly 50% of the time in a state of magnetic activity unlike what we have directly observed! Approximately 5% of the sample is in an ``extreme state'' of activity that could produce cyclic irradiance variations in the range of approximately 0.5% - 1% (i.e., 5-10 times the current solar levels), as inferred from the mean

relations of cyclic photometric variation versus mean chromospheric activity given by Radick *et al.* (1998). About 24% of the M67 solar counterparts are in an ``enhanced activity'' state with an estimated amplitude of irradiance variability of ~ 0.2 %, or two times that of the Sun.

Given the positive correlation between magnetic activity and brightness changes, our results suggest that the total solar irradiance could change by more than the 0.1% currently observed! This could, in turn, have significant implications for climate change over century-long time scales. In fact, a change of only 0.22-0.55% has been estimated to lead to a change of 0.4-0.5 degrees C in global mean temperature (Wigley et al. 1990).

These intriguing results pose crucial questions. In particular, is the stellar distribution in the figures really the result of the modulation of activity by cycles analogous to the solar cycle, or are the *relative amplitudes* of the cycles actually similar with the differences due only to differences in the *mean level* of activity among solar-type stars? Even more fundamentally, is the distribution of chromospheric H&K emission strength arising from solar-like cycles at all? The only way to address these critical issues is to obtain regular observations of the M67 ``Suns'' over a period of several years.

We propose to implement a long-term program of H&K observations during each bright run during the M67 observing season (about Dec. - Apr.). Specifically, we would utilize the MMT with the Hectechelle one-night per month for at least 5 years and preferably 10 years (about the length of the sunspot cycle).

We will establish a parallel program of photometric monitoring using an automated telescope in order to obtain data on the associated luminosity variability of the program stars. The telescope is a facility of the Global Network of Automated Telescopes, Inc., in Tucson, Arizona.

The results will enable us to build a statistical picture of all the modes and amplitudes of the cycle variability of solar-type stars and, by inference, of the Sun itself. Of equal importance, they will provide valuable input for the construction of global climate change models.

Module 3: Characterization of Planetary Systems

Team: R. Angel (Lead), A. Burrows, L. Close, P. Hinz (Deputy), J. Lunine, M. Meyer, and N. Woolf

3.1 Introduction

Our solar system may be only one many planetary systems in the neighborhood of our sun capable of sustaining life. We propose to further our knowledge of where planetary systems exist in the solar neighborhood, including the nearest star formation regions. We want to discover the range of planetary systems, the frequency of types, and study how likely they are to be abodes for life outside our solar system. The work is directed towards Goal 1 of the Astrobiology road map, and in particular towards goal 1.1, though it also impinges on goal 1.2.

We also plan to study the spectrum of the whole earth observed from earthshine on the moon. We want to discover the spectral characteristics of those regions not well studied, and to observe the time variation of features to understand the problems and issues in interpreting the spectrum of earth as a whole. This work is directed towards goals 1.2 and 7 of the Astrobiology road map.

3.2 Planet Searches

Jovian planets are the alpha and omega of planetary system evolution. They control whether terrestrial planets form, the abundance of volatiles on these planets, and the frequency of violent impacts on them after formation. We want to image giant planets directly, to discover planetary systems of kinds that cannot currently be found.

We are looking for gas giant planets, and particularly for a change in the frequency of these planets beyond the ice line. We take this to be at a distance $5(L/Lsun)^{0.5}$ AU from a star. We expect such a change if "radial velocity planets" are a minor group compared with "solar system-like planets" with similar orbital parameters. Our proposed techniques are:

1. Directly image massive planets orbiting nearby 0.1-10 Gyr old "Sunlike" stars. This challenging task will be accomplished utilizing two complementary and novel imaging techniques (3 color simultaneous differential CH_4 imaging and nulling interferometry) with Arizona's Monolithic Mirror Telescope (MMT) and Large Binocular Telescope (LBT);

2. Probe the physical properties of these planets and systems through photometry and spectroscopy. Our goal will be to use the photometric data to infer the luminosities of planets. Combined with the known age of the system, this leads to mass estimates. By comparing synthetic spectra of giant planet atmospheres with observations of the planets we can constrain the planet mass. These data, combined with the orbital information gathered and dynamical simulations outlined in Module 2, will enable us to assess the potential of systems to have terrestrial planets with volatiles.

3. Through this we will help lay the technical and theoretical foundations for successful surveys for life bearing worlds to be undertaken with the Terrestrial Planet Finder and its precursor missions.

We also want to learn about the potential for planets with a water-atmosphere boundary. In Jovian planets we expect to find parts of the atmosphere where water is present in droplet form. In some lower mass planets we may find a water layer sitting on a rocky core. This potential for "super-earths" needs exploration, and we will start moving towards it by modelling planets down to 0.1 Jupiter masses.

In addition to searching for other planets by learning about the frequency of potentially habitable nearby planetary systems, we want to make more studies of the one planet we know to be both habitable and inhabited - Earth. We want to learn more about the problems of observing the changing spectrum of the earth using earthshine and the issues in interpreting a crude spectrum such as we would expect to get from Terrestrial Planet Finder.

Our efforts here are part of a long-term strategy to insure success (or place meaningful limits) in the search for life beyond the solar system. We were first in proposing schemes for the detection of terrestrial planets in the mid-IR. We were first to examine the requirements for an adaptive optics system to find planets around other stars. We proposed the re-direction of the search for Jovian planets towards young planets in our NRA study - "Self-Luminous Planet Finder". We will continue to develop novel ideas to solve daunting problems associated with the search for the origins of stars, planets, and life. Support through this program will insure that we focus our efforts in areas that are most likely to be interesting to astrobiology.

3.3 Planet Searches at the University of Arizona

The University of Arizona is well suited to carry out astrobiological inquiries into massive planets and their influence on extra-solar planetary systems. Arizona arguably has one of the strongest interdisciplinary synergies between telescopes, instrumentalists, observers, planet modelers, planetary system modelers and TPF experts.

The synergy from these interdisciplinary groups will enable an astrobiology program at the University of Arizona to make breakthroughs into studies focused on the nature, frequency, formation, and evolution of planetary systems where massive gas giants point to the presence of habitable terrestrial planets.

3.4 Science goals

In this module we propose to explore directly the frequency of giant planets around other stars near the Sun, and relate this to the conditions in the so-called “habitable zone,” where life might exist if solid (terrestrial-type) planets are present. We plan to combine the observations of Hinz, Angel, Close, and Meyer and TPF studies of Woolf, Lunine, Angel, and Hinz with the atmospheric and dynamical modeling expertise of Burrows and Lunine to address the following list of questions:

1. What is the lowest mass planets that we can discover through direct imaging, both for star formation regions less than 10^7 years old, and for nearby stars where the answers are better known. Based on these observations we can start to answer:

2. What is the frequency of giant planet formation and, in particular, how often do Jovian planets form in a planetary system (*i.e.*, compared to higher mass sub-stellar objects)? Do the spectral properties of these objects fit the current models? What are their masses? Do follow up observations suggest circular or highly eccentric orbits? Given this newly characterized distribution of "wide" (~3-50 AU) giant-planet objects, combined with the known "tight" (0.05~3 AU) radial velocity/transit planets, we then ask:

3. What are the habitable zones for the new systems compared to the radial velocity systems?

4. What are the spectral signatures of terrestrial planets orbiting at various thermal environments around stars? What are the characteristics of life and habitability signatures on "super earths"? To what extent will biospheres develop obvious biosignatures? To what extent will there even be habitability signatures? What spectral evidence do we search for to identify acquired volatiles? What do young terrestrial planets in their Hadean era – up to 5×10^8 years old – versus older terrestrial planets in Archean versus Proterozoic and later eras – 10^9 to 10^{10} years – look like? How might habitability be mimicked by terrestrial planets undergoing unstable, dynamical episodes of atmospheric evolution or loss or ice-ball episodes? Finally, to broaden the search for habitable places in the cosmos, we ask:

5. Given the properties of detected giant planets (spectral type, atmospheric composition, temperature, mass, radius, semi-major axis, stellar type and age of parent), what is the full range of habitability possibilities around these objects? That is, might the giant planets have habitable moons, or even be habitable themselves? Is there a biochemical distinction between habitable moons with an ice layer over their water and ones with a water-atmosphere boundary? Ultimately, regardless of whether habitable moons exist around giant planets, we wish to characterize their spectral properties both to understand the range in giant planet atmospheric compositions, and as a pathfinder to doing the same for terrestrial planets. Hence:

6. What key spectral features might be detected in next-generation images of extra-solar planets and what are the key properties we seek in such giant planet spectra?

3.5 Observational Detection of Other Planetary Systems

In this section we outline the facilities and observational programs planned to further our knowledge of the makeup and habitability of other planetary systems. We describe two specialized techniques planned for detecting companions within the region of expected planet formation (1-40 AU): simultaneous differential imaging (section 3.5.2) and differential imaging (section 3.5.3). Simultaneous differential (Sim. Diff.) spectral imaging at 1.6 μm is expected to be most useful for finding and characterizing young planetary companions. Nulling interferometry will be important at 5 μm to detect older (and thus cooler) planets.

3.5.1 Facilities for Extrasolar Planetary Searches

Arizona has pioneered both the development of large-aperture telescopes and an implementation of adaptive optics (AO) most suited to planet searches. Because of the difficulty in detecting faint planetary companions next to bright stars in observations limited by the atmospheric blurring known as ``seeing", techniques to control the wavefront distortions caused by the earth's atmosphere known as ``adaptive optics" have been developed. Corrections to the wavefront are achieved by monitoring the distortions many times per second and using a deformable mirror whose shape is altered on similar timescales. By integrating the correcting element of the AO system into the telescope itself (as its secondary mirror) both the sensitivity and the image of the sharpness can be maximized.

The 6.5 m MMT (in which Arizona is a 50% partner) is the first realization of a large aperture telescope coupled with a deformable secondary. Figure 3.1 shows an image demonstrating the successful use of the MMT AO system to create ultra-sharp images by correcting the atmospheric turbulence. The system allowed images to be made with 40% of the object's flux in a diffraction-limited core of size 0.050" at 1.6 μm. At longer wavelengths the MMT achieves even higher amounts of light in the core ~98% with a FWHM = 0.32" at 10 μm. Concentrating the blur of these point sources of light is central to optimum detectability. These angular scales correspond to separations of 0.5 AU and 3.2 AU respectively between a host star and any potential planetary companions.

At longer wavelengths (>2.5 μm) the MMT AO system is uniquely sensitive, due to the lower background light it emits at infrared wavelengths compared to conventional AO systems. This is because it uses a deformable secondary mirror for wavefront correction (Brusa *et al.*, 1999) which has the advantage of eliminating ~8 warm, dusty, optical surfaces from the AO system design (Lloyd-Hart, 2000). The elimination of these extra optical surfaces (that are required of all conventional large telescope AO systems – *e.g.*, Keck, VLT, *etc.*) allows the MMT AO to have much lower scattered light, and lower thermal emissivity, while increasing the system throughput. Conservative estimates of this suggest that 6.5 m MMT AO system in conventional imaging mode will be ~1.0 times as efficient as the Keck (10 m) AO system at H (1.6 μm) and 2.28 times as efficient at N (10.5 μm) despite having a smaller primary mirror (for more on the MMT AO system see Lloyd-Hart, 2000; Wildi *et al.*, 2003). Arizona is also a partner in the Magellan 6.5 m southern telescopes, and we expect to create a similar adaptive

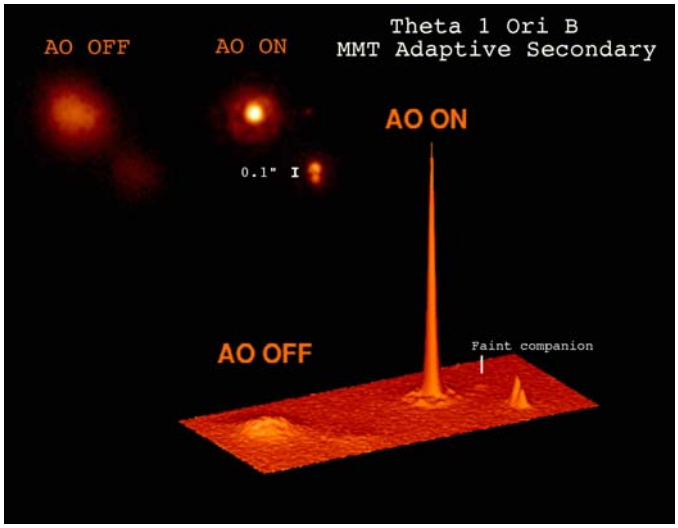


Fig. 3.1. Demonstration of the MMT adaptive optics system to create diffraction-limited images at 1.6 μm. We see that with the AO correction "OFF" faint companions are impossible to detect (upper left), whereas with AO "ON" all four stars (in the theta 1 Ori B group) become clear. Adaptive optics correction is the first step in being able to directly image extra-solar planets.

secondary system for one of these telescopes.

Arizona is also a 25% partner in the Large Binocular Telescope, a 2 × 8.4 m telescope with uniquely sensitive planetary detection capabilities. NASA has already recognized this in its funding of the LBT Interferometer (LBTI) as part of the Navigator Program. The development of the LBTI is being funded to further technological development for the Terrestrial Planet Finder Program, through the demonstration of nulling interferometry. We want to use the already-funded facilities of this NASA program to make surveys for extrasolar zodiacal dust (module 2) and giant planets focused on the astrobiological issues. The secondary mirrors of the LBT will, like the MMT, be deformable for AO wavefront correction. The increased collecting area as well as greater angular resolution of the LBT will allow us to study planetary systems found with the MMT in greater detail as well as extend our searches to fainter (and thus less-massive planets).

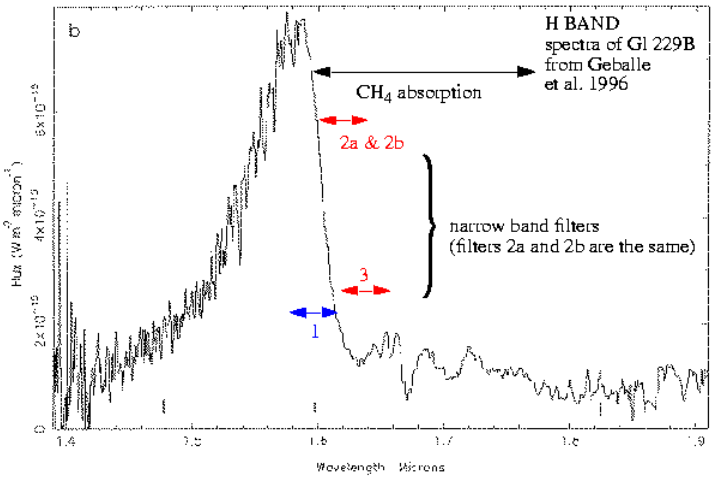


Figure 3.2. All planets between 1300-400 K will have strong CH_4 absorption. We have built an adaptive optics instrument (a 3 color Sim. Diff. Imager in the ARIES camera) with filters 1, 2a, 2b, & 3 on either side of the CH_4 absorption. Subtracting images made in these filters will remove the star but leave the planet's image to be detected.

3.5.2 Three Color Simultaneous Differential Imaging

We have developed a new technique that is a variant of the simultaneous differential imaging technique of Rosenthal *et al.* (1996), Racine *et al.* (1999), and Marois *et al.* (2000). The technique is designed to detect a companion close to its parent star by taking advantage of the difference in brightness of the planet at two closely spaced wavelengths. This allows one to achieve a gain in limiting sensitivity over normal imaging by ~ 20000 times. The 3 color technique is photon-noise limited for separations of >0.2". For the first time massive planets in the range 5-13 M_J, at distances 5-30 AU from young (0.1-1 Gyr) Sun-like stars, within 50 pc of our solar system, can be directly detected and imaged.

3.5.2.1 How three color simultaneous differential imaging works

The simultaneous differential imaging works by means of creating 4 images of the same star in 3 different wavelengths simultaneously. As Fig. 3.2 shows we plan to have 3 different filters that will sample either side of the CH_4 absorption feature at 1.61 µm. A subtraction of these filters will remove the starlight but reveal the massive planet. It is important to note that all massive planets from 1-13 M_J and from 0.1-5 Gyr have strong CH_4 absorption at 1.62 µm (Burrows *et al.*, 1997). Therefore, differential CH_4 imaging is a powerful technique to detect massive planets over a wide range of ages and masses.

Early experiments with CH_4 differential imaging were made at the CFHT 3.6 m telescope with the MONICA 256 x 256 IR camera and the PUEO AO system (Racine *et al.*, 1999). These experiments proved that gains of ~50 in the sensitivity were obtained when using 2 colors and subtracting images at 1.5 µm from images at 1.7 µm. This is, however, nowhere near the improvement needed to reach the photon-noise limit of a deep exposure ~400x more rejection of the primary's starlight is required. To get this extra rejection of the primary's contaminating "wings" requires a more complex subtraction process less prone to systematic errors between the "out" and "in" CH_4 images. To achieve a higher rejection we will use 3 colors and 4 images (instead of the previously used 2 colors and 2 images) and will obtain a final image by differencing the differences between the pairs of images. This in effect improves upon previous techniques by removing second-order systematics resulting from the wavelength dependence of the speckle pattern.

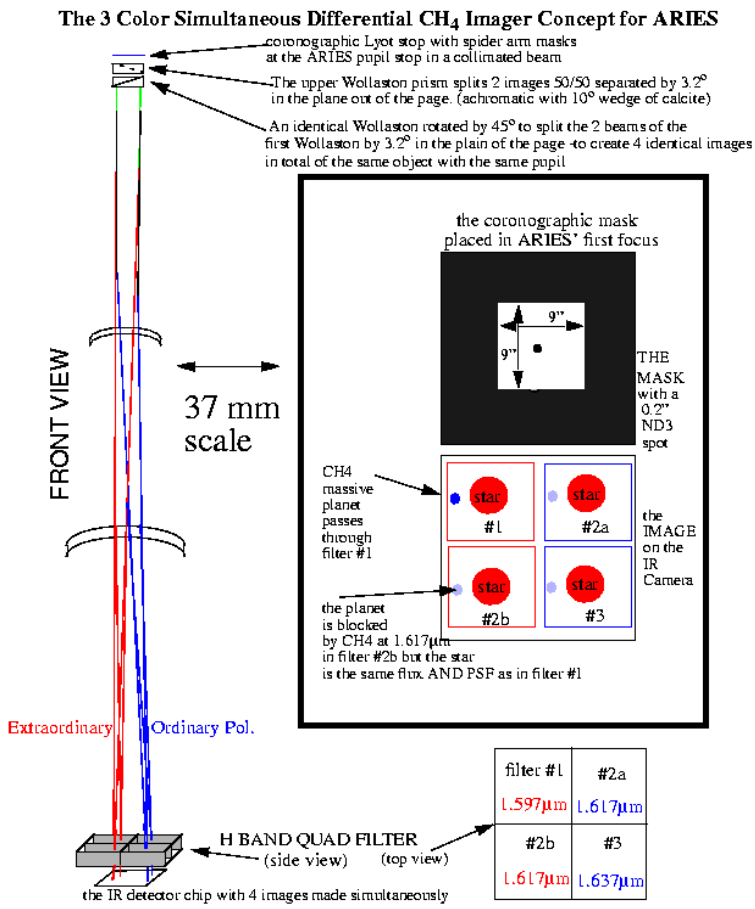


Figure 3.3. A schematic diagram of the instrument built for the MMT adaptive optics telescope. This instrument (hosted inside the ARIES infrared camera) is operating in the lab. It allows for the direct detection of gas giants for the first time.

3.5.2.2 Status of 3 color simultaneous differential imaging

The differential imager instrument has been tested cold (77 K) in the ARIES science camera dewar for use with the MMT AO (see Figure 3.3; Freed *et al.* 2003). Our first telescope observations could occur in May 2003. However, our lab data already show that our instrument is performing as expected (at the photon-noise limit). Based on laboratory experiments we believe we can detect planets 10,000 times fainter than the primary star at just 0.4" from the star (10 AU if the star is at 25 pc from the Sun) in only 5 seconds of integration at the MMT. In 2 hours of integration at the photon limit we should detect planets 100,000 times fainter than the primary at the MMT at the 10σ confidence level. This is >1000 times more sensitive than what has been possible before from any space or ground based telescope at 0.4" separations (Close 2000). Based on these measured lab sensitivities and the planetary models of Burrows *et al.* (1997) we show in Figure 3.4 our predicted sensitivities to extra-solar planets for several different primary star types and masses.

3.5.1.3 The proposed 3 color Sim. Diff. surveys

We propose to capitalize on this sensitivity and efficiency to carry out the first large, deep, 3 color differential CH_4 (methane) AO survey of of nearby stars. The survey will characterize companions about both nearby mature stars and younger stars.

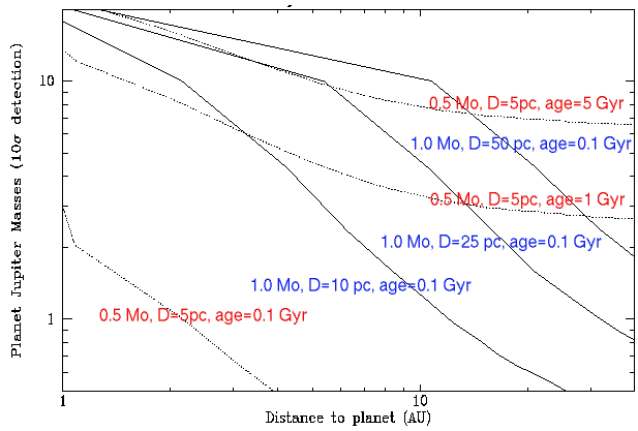


Figure 3.4. The sensitivity of the 3 color Sim. Diff. Imager to planets (at a very high 10 σ level of confidence) orbiting different primary star masses (0.5 or 1.0 solar masses (Mo)), ages (0.1-5 Gyr), and distances (5-50 pc). This assumes 2 hours of integration per star at the MMT 6.5m telescope.

Survey #1: MATURE STARS: A Complete survey of all 100 known (from NSTARS- Henry *et al.*, 1998.5) northern hemisphere stars between 0.5 and 1.5 M_{Solar} within 10 pc. In two hours of integration we will be sensitive down to planets of magnitude H = 26 (corresponding to 5 M_J surrounding 5 Gyr old stars at 5 pc from the Sun). We will obtain deep two hour images of the nearest stars with DEC > -25° to extend massive planet searches down to planets of H~26, ~100 times deeper than has been attempted before. We will detect planet masses of 7 M_J at 5 AU around a 5 Gyr star at 5pc. In general, this photon-noise limited survey will be complete for older ~1.0-10 Gyr old (mature), massive planets of ~5-80 M_J with semi major axis of ~5-90 AU.

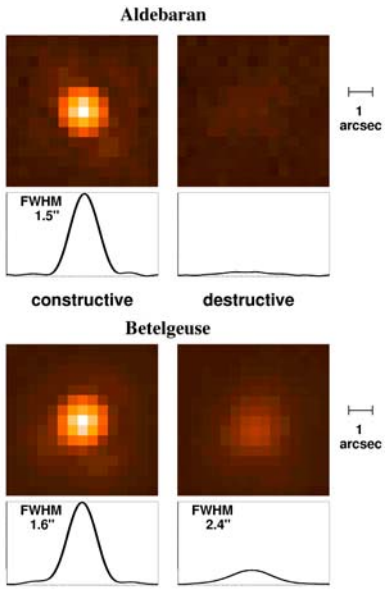


Figure 3.5 The first demonstration of nulling interferometry with the Multiple Mirror Telescope. The star Aldebaran is effectively suppressed in the right top image by destructively interfering light from two telescopes. In contrast, when the star Betelgeuse is suppressed an extended image remains, which is a direct image of the dust nebula surrounding the star. The nebula is not suppressed since it is much larger than the stellar disk of Betelgeuse.

Survey #2: YOUNG STARS: Image all 60 young (<1.0 Gyr; age based on CaII H and K emission survey of Soderblom *et al.* (1994)) stars that are nearby (D < 25 pc). We should be sensitive to 3 M_J objects at 5 AU from a 300 Myr old G2V star at 5 pc in two hours. Although there may not be as many of these young nearby stars, they will certainly be good targets for direct detection of lower mass planets. The radial velocity planet confirmed around ε Eri (after 15 years of radial velocity observation is observed to have mass ~4 M_J (assuming a nearly face-on orbit), current separation ~0.5", D = 3.3 pc, age ~ 700 Myr) would be relatively "easy" to image in two hours and is a good example of a potentially accessible planet for detailed study.

3.5.3 Nulling Interferometry Detection of Giant Planets

Nulling interferometry is a technique which is central to NASA's plans to look for life around nearby stars, through the Terrestrial Planet Finder mission. It allows the detection

of faint circumstellar material or object in the presence of the much brighter stellar emission. We first demonstrated nulling interferometry to be capable of directly imaging faint circumstellar environments (Hinz *et al.* 1998) using two of the six mirrors of the old Multiple Mirror Telescope (now refurbished as a single 6.5 m telescope). The two pupils were overlapped so that the starlight combined out of phase, canceling out in the image. Light from a source elsewhere in the image does not combine out of phase and can thus be seen even if it is intrinsically much fainter than the nulled out star. The results of this first test are shown in Figure 3.5.

With the deformable secondary, suppression factors of 10,000 will be possible while still maintaining optimum sensitivity in the thermal infrared (Hinz *et al.* 1999). This combination will allow detection of very faint circumstellar objects and structure.

3.5.2.1 Status of nulling interferometry with the MMT and LBT

We have completed a nulling interferometer for use with the 6.5 m MMT and Magellan. The instrument was first tested in January 2003. The instrument is designed for use both at 10 μm wavelength (where we expect to use it for detection of zodiacal dust, as described in module 2) and at 5 μm for detection of giant planets.

The Large Binocular Telescope, currently near completion on Mt. Graham in Arizona, will be a uniquely powerful nulling interferometer. The 8.4 m primaries, center-to-center separated by 14.4 m, on a common telescope mount with deformable secondaries all contribute to form an interferometer which is sensitive in the infrared and capable of deep stellar suppression. Full interferometric operation of the LBT is planned by October 2005. We are currently building the nulling interferometer for the LBT (NIL) which has benefited from our experience with nulling on the MMT. Nulling is a technique most easily performed in the thermal infrared (4-13 μm). Here the noise source is different than in the near infrared (1-2 μm). Through nulling, scattered starlight and speckles associated with it are reduced to well below the noise level associated with the background light contributed by the sky and the warm telescope mirrors. For this situation the MMT AO system is advantageous, not only for the better PSF it produces, but because it does not introduce any extra warm mirror surfaces which glow in the infrared, adding extra background light. This allows us to have a very sensitive system. Other interferometers will have many warm reflections prior to combination of the light. Extra mirrors are needed both for conventional AO systems and for movable delay lines required in interferometers which are not mounted on a common structure as is the LBT. We estimate the sensitivity of the MMT nulling interferometer to be able to detect a 200 microJansky source at 5 μm and a 900 microJansky source at 10 μm for a 1000 second integration (Hinz *et al.* 1999). The LBT will improve on this, detecting 30 microJansky sources at 5 μm and 150 microJansky sources at 10 μm. We relate these sensitivity numbers to detectable objects below.

3.5.3.2 Nulling survey for detecting giant planets

Surveys undertaken with nulling both at the MMT and the LBT will have two separate objectives related to extrasolar planetary systems: detecting zodiacal dust disks at 10 μm, where they are relatively bright (which is addressed in module 2), and detecting extrasolar giant planets. Direct detection of a companion is more feasible in the thermal infrared than the visible or near infrared, since the planet's thermal emission in the infrared is brighter, in comparison to the

stellar emission, than the amount it reflects in the visible or near-infrared. However, by looking for a planet in the infrared where it is emitting its own radiation rather than the reflected light from its star, the detectability of a giant planet is sharply dependent on how old it is, and thus how much it has had a chance to cool.

We have used the models by Burrows et al. (1997) to determine planet detectability. Figure 3.6 shows an interpolation of the Burrows *et al.* predictions for a 5 Jupiter mass object at 10 pc from an age of 0.1-1 Gyr in the N, M, and L' bands along with the plotted MMT sensitivity limits. From these it is possible to derive mass limits for detection with each instrument. The photometric sensitivity for the MMT allows us to detect 4 Jupiter mass planets 0.5 Gyr old, 6 Jupiter mass planets 1 Gyr old, and 15 Jupiter mass planets 5 Gyr old for stars 10 pc away. For the LBT the numbers are 1, 2, and 5 Jupiter mass objects for the respective ages. These numbers are expected to be only mildly dependent on the planet-star separation. The closest separation nulling can sense is determined by the separation of the apertures, and is 0.10" for the MMT and 0.027" for the LBT in the L', corresponding to 1 and 0.3 AU, respectively, at 10 pc. The outer limits are set by detector area and are expected to be 130 and 35 AU respectively.

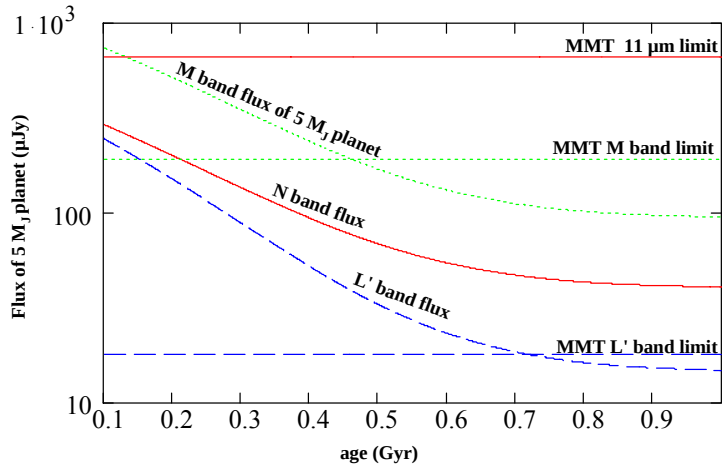


Figure 3.6. Detectability of planets in the N (11 μm), M (4.8 μm), and L' (3.8 μm) bands at the 6.5 m MMT. The fluxes, from Burrows et al. (1997), are for a 5 Jupiter mass at 10 pc. The planet is most readily detectable in the L' band

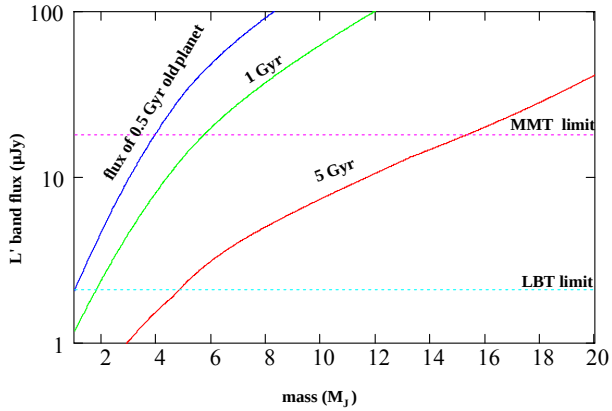


Figure 3.7. Minimum planet mass detectable at L' for the MMT and LBT. Fluxes are for planetary systems at 10 pc from Burrows et al. (1997).

In support of this proposal we will conduct nulling surveys at the MMT complementary to the differential imaging survey outlined above: first observing a volume-limited sample of 100 nearby stars and then extending our approach to the nearest 60 young solar-type stars. Follow-up observations of companions detected with the MMT will be carried out with the LBT, which will have the ability to obtain low-resolution spectra of any object detectable with the MMT.

Direct detection of planets is not a mature field. Although much effort has gone into developing a variety of techniques, no one approach has emerged as uniquely successful in detecting faint substellar companions. We have outlined a multi-faceted approach for the direct detection of extra-solar giant planets, each searching a specific range of parameter space in complementary ways. In this way, we hope to provide observational constraints that will help answer aspects of the fundamental questions: where, when, and how frequently giant planets form around solar-type stars.

3.6 Giant Planet Modeling

In the last seven years, more than 100 extrasolar giant planets (EGPs) have been discovered by precision radial-velocity measurements of the telltale wobble of their central stars, and their discovery constitutes a major turning point in both planetary science and astronomy. The unanticipated variety of these extrasolar planetary systems (most completely unlike our own) has upended conventional wisdom concerning the formation of planetary systems and is drawing an increasing fraction of the world's astronomers into the technological, theoretical, and observational programs designed to study and understand them in more detail.

Although the radial-velocity programs have launched the modern study of EGPs, it is only by making *direct* measurements of the planet's spectrum that its physical characteristics can be fully discovered. Hence, the next stage after the initial ground-breaking Doppler campaigns in the study of EGPs will be their direct imaging and spectroscopic measurement. The theoretical modeling of EGP atmospheres is important for at least two major reasons. First, since the emergent spectra of EGPs are determined by the chemistry and physics of their outer atmospheres, when direct detection of EGPs is achieved and spectra are obtained, theoretical models will be essential in the interpretation of the data and in the extraction of essential physical information such as radius, gravity, temperature, and composition. Then in turn the verified characteristics can be used to determine masses of planets that do not have a radial velocity signature. This is the essential key to exploring the nature of planetary systems.

Second---and this is perhaps the more pressing of the two---theoretical spectral models are important in guiding current and upcoming direct EGP searches. Observers need to know which regions of the spectrum provide the greatest chance for detection, while avoiding those regions in which attempts are likely to be futile. The two observing techniques outlined above illustrate this; both the methane absorption and 5 μm feature are well modeled by our current spectra, and are providing input for estimated sensitivity in both cases.

We (Sudarsky, Burrows, and Hubeny 2003) have already conducted an extensive exploration of spectral and atmospheric models of irradiated extrasolar giant planets. Our central purpose has been to provide a map to observers, as well as a comprehensive view of the theoretical possibilities. We have investigated the dependence of the phase-averaged emergent spectra on orbital distance, stellar type, the presence or absence of clouds, cloud particle sizes, surface gravity, and the inner flux boundary condition. Included are calculations for irradiated brown dwarfs, specific known EGP systems, and a generic sequence around a G0V star. The hot planets give information that helps the understanding and modeling of those planets which will be in potentially life bearing planetary systems.

There are many overall, as well as specific, conclusions resulting from the current state of modeling. Some of the more salient points are the following:

1. The planet-to-star flux ratio is a very sensitive function of wavelength and orbital distance.

2. EGP band fluxes are not strictly monotonic functions of orbital distance, nor are the Bond and geometric albedos.

3. EGPs fall naturally into classes due to qualitative similarities in the compositions and spectra of objects within several broad atmospheric temperature ranges.

4. The mid-infrared region of the spectrum from 10 to 30 μm has a favorable planet-to-star flux ratio, even for distant EGPs.

5. Due to Rayleigh and/or grain scattering, the optical spectrum of an irradiated brown dwarf can be very much brighter than that of a brown dwarf in isolation, even when its near- and mid-infrared spectra remain relatively unaffected.

6. Fluxes in the *Z*, *J*, *H*, and *K* bands of an EGP in a long-period orbit can be enhanced above baseline levels (normally determined by stellar irradiation and scattering alone), if the planet is either young or massive.

7. There is a *relatively* bright feature within the 3.8 to 5 μm wavelength region for all irradiated EGPs, and in particular for the more distant EGPs (such as 55 Cnc d, ε Eridani, 47 UMa c, Gliese 777A, and υ And d).

8. As a result of the progressive decrease in atmospheric CO abundance, the center of the 3.8 to 5.0 μm feature shifts systematically from shorter to longer wavelengths with increasing orbital distance (or decreasing stellar luminosity).

9. Rayleigh and grain scattering elevate the optical and near-IR fluxes, but grain absorption depresses the mid-infrared fluxes (in particular in the ``5-μm" band).

10. Increasing surface gravity slightly decreases the flux shortward of ~2.2 μm, but also slightly increases it longward of ~2.2 μm. The larger the gravity the smaller the peak-to-trough variations throughout the spectrum.

11. The Na-D and K I resonance doublets are prominent features of the hottest, close-in EGPs (such as 51 Peg b, τ Boo b, HD209458b), but quickly wane in importance with increasing orbital distance.

However, much remains to be done. At low effective temperatures (< 400 K), water vapor condenses into water clouds in both irradiated EGPs and brown dwarfs. The formation of clouds of this sort is one of the first markers of planet-like behavior as seen in the Solar System are familiar and ties the physics of our nine planets and their moons with that of extrasolar giant

planets and brown dwarfs. Objects less massive than ~20 M_J will in a Hubble time or less achieve sufficiently low effective temperatures (T_{eff}) for water clouds to be manifest. Such objects could exist either as free-floaters in the ISM and be detectable by SIRTF, JWST, and TPF or could be bound in orbits around nearby stars. The latter ``planets" are subject to irradiation and will have distinctly different spectra and colors.

In this proposal, we will extend the theory already developed for the known EGPs and brown dwarfs to lower T_{eff}, gravities, and masses to include the onset of water cloud and ammonia cloud (T_{eff} < 200 K) formation. This will include objects as small as 0.1 M_J. The technology to do this is already in hand. It will be used to make predictions in support of our local observational efforts, as well as those of the international community via the variety of telescopes now being designed for the direct characterization of planets outside our solar system. The study of the lower mass objects will start the exploration of the character of "super earths", Uranus-Neptune mass objects at greater irradiance.

3.7 Terrestrial Planet Finder Preparation and the Earth spectral signature

Ultimately spaceborne platforms will be built that are capable of detecting, and collecting spectra of, terrestrial planets. We currently do not even have adequate information about the spectrum of the earth, or its time variability. We have already made observations of lunar earthshine (Woolf *et al.* 2002) as shown in Figure 3.8. We discovered that our modeling had missed a most

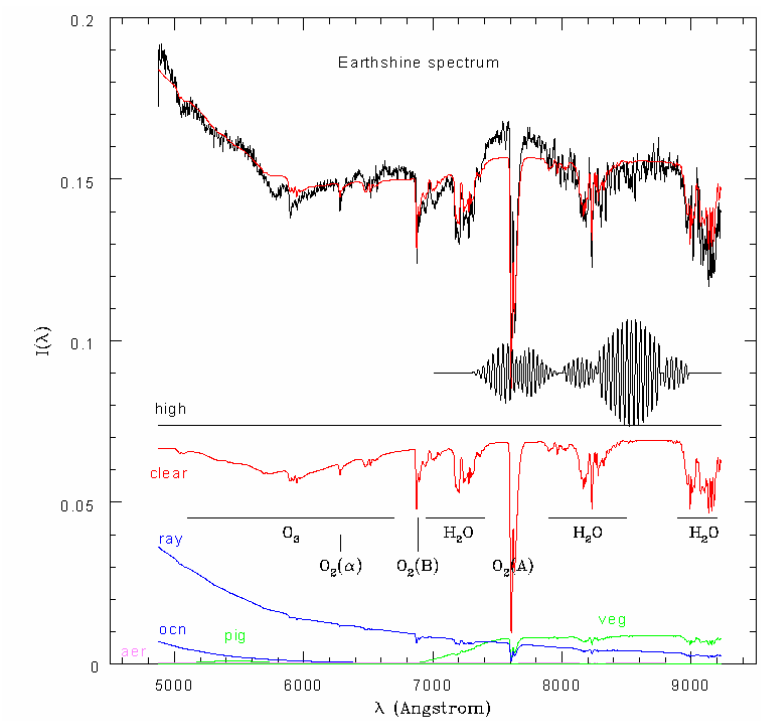


Figure 3.8. The earth's integrated reflectivity versus wavelength determined from lunar earthshine. The blue "ray" curve is the extracted Rayleigh scattering component. The green "veg" curve is the extracted vegetation signature, though there are indications in the 7500-8000A region that this component is stronger, and with a different signature than we have used in the analysis.

significant feature - the Rayleigh scattering of the atmosphere. This is an essential tool to interpret the molecular spectral features in the atmosphere. Without it we cannot determine whether features are strong because of the abundance of the molecules, or because of collisional broadening. Unsaturated features, that have for example allowed us to infer the presence of water and CO_2 on Mars, are too weak to be visible in spectra we expect from external planets.

The Rayleigh scattering would be straightforward to interpret as atmospheric pressure in a clear atmosphere, but in the presence of cloud the results are more complicated. We would like to model the effect of clouds, and make observations to see how the Rayleigh scattering changes with time, using weather maps to tell whether we have the story correct.

The vegetation red edge only measures land plants because seawater has a very strong absorption in this spectral region. The signature is not expected to be unique, as e.g. bacteriochlorophyll would produce a red edge beyond 1 micron. However the inefficiency of biological processes, and the susceptibility of land plants to overheating, makes it seem plausible that oxygenic photosynthesis land organisms on another planet will also have a red edge in this general wavelength region. We would like to study this issue further, by collaborating with oceanographers at other NAI modes to compare the spectral characteristics of green algae, red algae and brown algae. We want to know whether their absorption at ~ 8000A differs and makes the green algae less susceptible to desiccation and so selected the green algae to be the ancestors of land plants.

The spectral region from 1-2.5 μm is not yet known in a whole earth spectrum, and we propose to observe it on the VATT telescope on Mt. Graham, where we now have an appropriate spectrograph. (An earlier request for time on IRTF for this work was denied). We think that there may be some indicators of the kinds of bare rock and sand in Earth's desert regions in this part of the spectrum.

We are also interested in finding about the variability of the Earth spectrum, and the visible spectrum is highly time variable. We will find if it impinges on analysis of results if the TPF mission is designed for the visible spectral region. Fortunately, we already have a good whole-earth spectrum of the mid IR region from TES spacecraft, and this will have less variability.

3.8 Summary of Module 3

Products of the observational effort will initially be discoveries of >Jupiter mass objects around nearby Sun-like stars ($0.5 < \text{mass} < 1.5M_{Solar}$) and younger. Our first survey of 100 nearby (1-10 pc), mature (1-5 Gyr old) stars will be sensitive to massive planets of 5 M_J at r > 5 AU at 5 pc. Our second survey of 60 younger (<1 Gyr - CaI selected objects) will be sensitive to massive planets of $3M_J$ at r > 5 AU at 5 pc. Each massive planet detected will have photometric and spectroscopic follow-up observations to characterize its mass, temperature, luminosity, albedo, composition, and surface gravity. From our final "catalogue" of massive planets we will distill the frequency of massive companions as well as the semi-major axis distributions and mass distribution of these objects (taking into account the selection bias of our well designed samples). Our goal is not just to observe single planets but to determine the characteristics of planetary systems.

Products of the theoretical effort will be: giant planet spectra, effective temperatures and radii *vs.* time, predictions for terrestrial planet spectra as a function of planetary system configurations and conditions in which they are embedded, new designs for platforms to detect and characterize giant planets and terrestrial planets.

The products of the earthshine studies will be learning how to interpret low-resolution noisy spectra of an earth-like planet. We expect in the process to generalize these results to planets that are much less like earth.

4. Management Plan:

The management of an Astrobiology Institute node is similar in many respects to that of other Interdisciplinary Programs at the UA in that it involves faculty in several departments working on different aspects of the project but needing to interact frequently and efficiently with colleagues. The goal is to ensure proper coordination while maximizing the opportunities for the individual investigators to develop their ideas. The key to success will be communications. With the proper level of information flow and communication, coordination of the diverse science and educational elements will be straightforward. Therefore the main emphasis of the project management efforts will be facilitation and coordination of the numerous individual participants.

4.1 Project Organization

This proposal brings together, in a cooperative effort within the Laplace Center, a wealth of knowledge, experience, and expertise. The figure below describes the organizational structure and key personnel of our team:

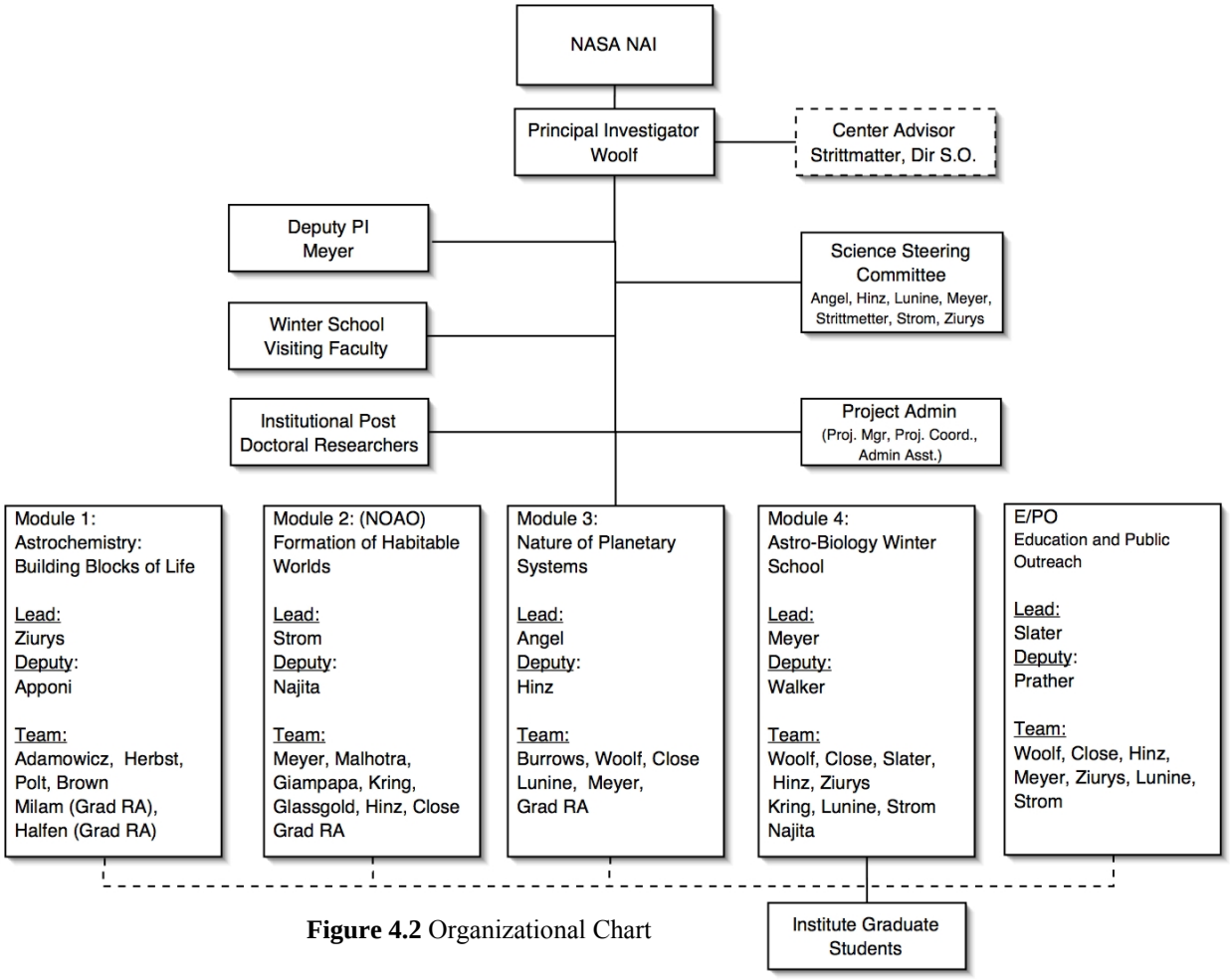


Figure 4.2 Organizational Chart

4.2 Program Management

We propose a strong management structure that will enable us to track the diverse research activities of our collaboration, yet maintain flexibility in allocating resources The overall responsibility for the effort will fall to the Principal Investigator, Neville Woolf. Professor

Woolf will be the primary arbiter, responsible for the fulfillment of the core science, technical, and programmatic goals. The management team will be augmented by strong Module Leaders who will take responsibility for meeting program objectives within each module. The program management efforts will be led by Thomas McMahon an experienced administrator of major science programs such as the NASA funded, Large Binocular Telescope Interferometer. It is our intension to provide a comprehensive tracking and reporting structure with minimal project cost impact.

4.3 Module Leaders

Lucy Ziurys, Director of the Steward Observatory Radio Observatories, will be the lead for Module #1 focused on astrochemistry. Ziurys will be assisted by Aldo Aponi as her deputy.

Steve Strom, Associate Director for Development for the National Optical Astronomy Observatories, will serve as the leader of Module #2 focused on the formation of habitable planets with Joan Najita as his deputy.

Roger Angel, Director of the Center for Astronomical Adaptive Optics, will be the lead for Module #3 focused on the detection and characterization of extra-solar planetary systems with Phil Hinz as his deputy.

Michael Meyer, Deputy PI, will be the lead for the Astrobiology Winter Institute, which will focus on educating a new generation of scientists in the field of Astrobiology.

Tim Slater, Director of the Science and Mathematics Education Center and Associate Professor of Astronomy will lead the education and public outreach program

Module leaders will be responsible for overseeing the activities of each module, organizing internal team meetings, and providing bi-annual progress reports to the PI. Module leaders will help organize the activities of several members of the contributing scientific staff including faculty, post-doctoral research associates, and graduate students.

As you can see, our program team members are rich with strong institutional and personal heritage in theoretical and experimental astrophysics and astrobiology. This team is well balanced and suited for what we propose.

4.4 Science Steering Committee

A Science Steering Committee will be constituted from the Module leaders and other co-investigators of this proposal. The charter of the committee will be to:

- Assist the PI in the administration of program
- Coordinate the research efforts
- Review and evaluate progress toward the stated science and educational goals
- Facilitate institutional and inter-institutional communications
- Provide a forum for new research paths
- Provide a communications path to other NAIs

The Science Steering Committee will meet quarterly and provide comments, suggestions, and concerns, directly to the Principal Investigator.

4.5 Reporting and Meetings

4.5.1 Monthly Status Meetings

Communications and information management are critical to the success of this program. We plan to hold face-to-face, full team meetings every month for a tightly focused exchange of

information. The agenda will be circulated one week in advanced (advertised throughout the NAI) and honed immediately prior. The Team meetings will be structured to keep team participants informed concerning the wide-ranging activities of the node, but to avoid "meeting-creep". Our goal is to DO astrobiology, not merely talk about doing astrobiology! At these meetings, Module Leaders will update the team concerning progress toward major milestones (outlined in Figure 4.2). We will encourage team members at other institutions, as well as members of other NAI nodes to participate via electronic conferencing.

4.5.2 Annual Meetings

We will hold annual team meetings as well as hold a full node meeting at the annual general meeting of the NAI. These meetings will be staggered by six months and take the place of the monthly meetings at those times of the year. The main goal of these meetings will be to review the biannual reports of the Module Leaders, to record difficulties in meeting outstanding milestones, identify possible solutions, and to provide for the mid-course corrections of the main science goals for each module. We will make extensive use of the internet with web sites to disseminate documentation and information to those participants not present for our team meetings. This plan allows for flexibility in re-allocating resources within the baseline plan.

4.5.3 Reporting

The PI will report to the Director of the NAI through annual reports and these reports will be made available to the heads of all participating departments, and the Dean of the College of Science at the University of Arizona, as well as the Director of the National Optical Astronomy Observatories.

4.5.4 External Review Board

We plan to constitute an external review panel made up of:

1. The Dean of the College of Science, Department Head, or Director of NOAO;
2. PI of another NAI node;
3. Senior staff member of the central NAI Ames Institute;
4. Outside expert on Education and Public Outreach.

This panel will be invited to our annual team meeting every other year and will provide a report to the PI and the Director of the NAI.

4.6 Tracking

A significant effort of the management team will be the tracking of project finances as well as programmatic goals and accomplishments. The experienced management team possesses a complete suite of tracking, reporting, and implementations plans that have been developed for other NASA programs. Leveraging the existing infrastructure is how we can maintain a small workforce that is focused and highly effective at project oversight.

4.7 Schedule

As indicated in Figure 4.2, we plan a range of activities throughout the period of performance for this proposal. We have identified key milestones (at the rate of 2-3 per year) for each program element and included those in the chart. While each Module stands alone from the rest in terms of schedule and deliverables (avoiding complex dependencies), results from the individual investigations complement and extend the overall impact of each other creating a whole that is

greater than the sum of its parts. The schedule plan also maintains needed flexibility to deal with mid-course corrections suggested by the science steering committee or oversight board. Our schedule plans for significant accomplishments over the period of performance for this proposal that directly address several specific goals outlined in the revised roadmap for the NAI.

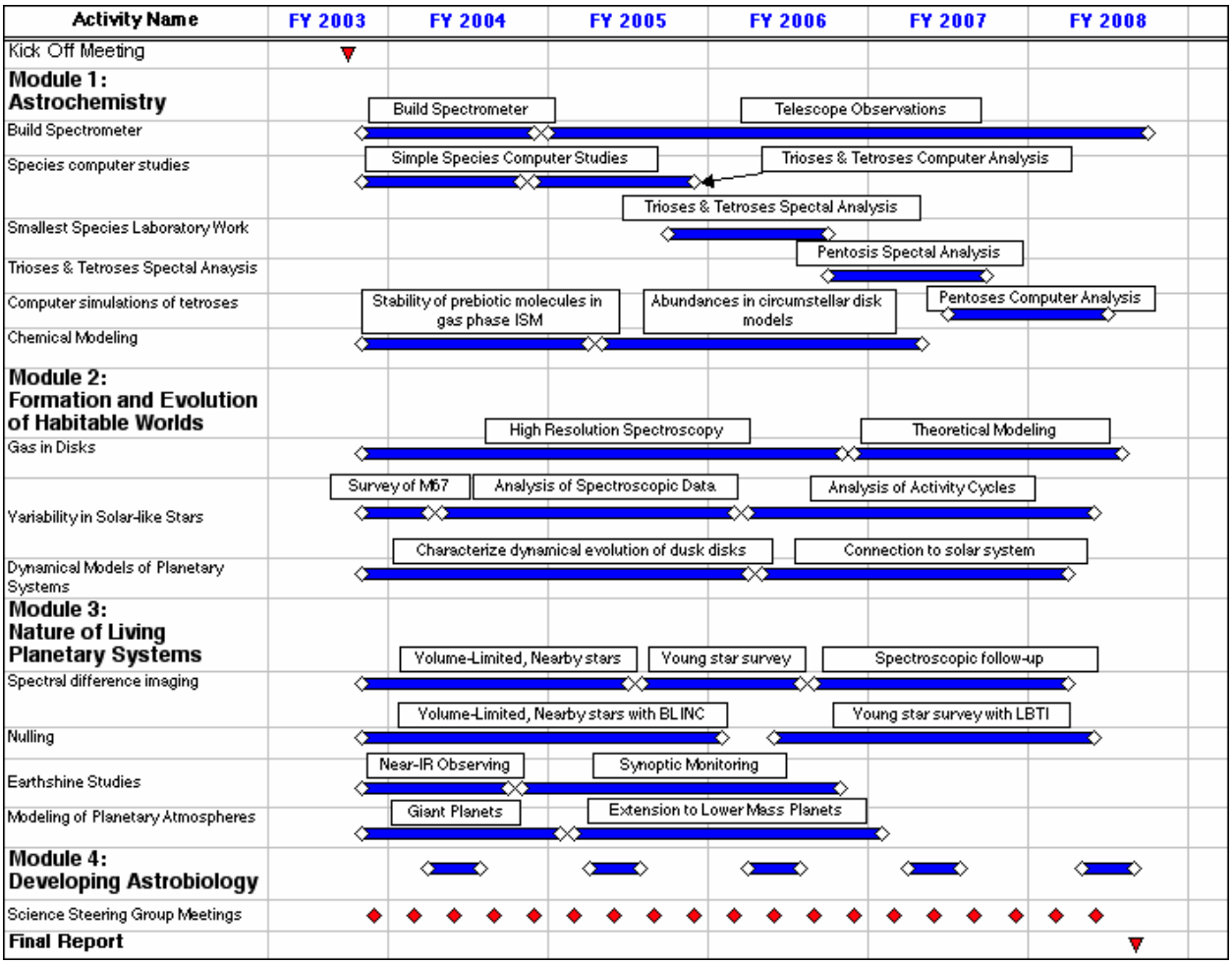


Figure 4.2. Project Schedule

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6.0 Module 4 Developing Astrobiology: Interaction at the Boundaries

Team N. Woolf (Laplace Center, Director), R. Angel, M.Meyer (Winter School, Lead) , P. Strittmatter, S.Strom, L. Ziurys, L. Close, J. Lunine, J. Najita, C. Walker

6.1 Introduction

This module describes how we plan to strengthen astrobiology as an academic discipline and to provide opportunities for students to develop their interests in the field despite the barriers often presented by existing departments. It also contains an educational and public outreach component (EPO).

Despite the fact that the research funding for this proposal is restricted to the work of the three modules, we see a need for a far wider research goal in Tucson. First there are the many astronomical organizations present in Tucson, and a number of them will have their own astrobiology work, which should be coordinated. Then also there is a continuing need, and already activity in interdisciplinary research across the fields of the physical and biological sciences throughout the University of Arizona.

As the National Academy COEL report noted " Astrobiology spans a much larger volume of intellectual and capital resources than the NAI itself. NASA should emphasize the broad base of national scientific capability in astrobiology, ... and not just the institute itself." And there is a need for "enhancing scientific collaborations with non-NAI scientists. To date, NAI has not adequately fostered such collaborations."

At the University of Arizona there are many activities that need to be brought together as part of astrobiology. For example, the Tree-Ring Laboratory has a program in predicting global climate change.(astrobiology goal 6) In biochemistry there is a strong program in organo-metallics that has in the past been used by a different astrobiology node. We need to bring the programs all under a supporting umbrella center to benefit by the interactions.
Only then will we see how to use judicious placement of new faculty hires to fill in key gaps. To achieve our long range goals will take far more than the resources directly discussed in this proposal. It will be necessary to use help wherever it is available. Some help will come from faculty and research associates with alternate funding. Many of them will be in the College of Science at the UA. Some will be in the College of Medicine at UA, and likely other colleges. There will be colleagues outside UA, as for example the NOAO. It will be necessary to meet with many research workers and create an inventory of people resources. It will also be necessary to create an inventory of monetary resources, both for research and teaching. We expect that private fundraising will be required.

6.2 The Laplace Center

Our goals are for the Laplace Center to be the focus of an Interdisciplinary Program (IDP) at the University of Arizona. It will be run by a Director (initially Woolf) and will provide coordination of the entire Astrobiology node (NAI and non NAI work) in Tucson. Members of the IDP will initially be drawn mainly from the current departments of Astronomy, Chemistry (and Biochemistry), Planetary Sciences and from NOAO; it will be open to faculty members from other departments, and there will be an active attempt to recruit them. The UA has a successful

record in multi-departmental, multi-college IDPs, including those in Applied Mathematics and in Neurosciences. IDP programs of study will be developed so that students can earn academic qualifications within the IDP including minors in any of the departmental specializations or vice-versa. The initial focus will be on departments within the College of Science and the Winter School. As opportunity arises, other activity that belongs with astrobiology will be incorporated.

If there are people to do the work, and the needed funds are available we will incorporate the work in the center. If the work seems worthwhile, but the needed resources are not available, the center will try to help find funds. It will develop an inventory of resources. If good research workers are available, and are doing work that fills in gaps, we will try to find employment niches for them. We shall deliberately seek to bring in new faculty members responsive to astrobiology. If the work is astrobiological, the Laplace Center will try to help it.

Fund raising activities will probably be needed. There are possibilities of working with foundations and those who have available money and special interest in astrobiology. The most obvious needs are to find additional funds for outreach, research beyond that funded by NAI and for scholarships for academically deserving students, both graduate and undergraduate. It is clear that current fund-raising activities at UA have not yet tapped the potential for supporting science.

The Laplace Center will also coordinate interchange of faculty with other members of the NAI to develop understanding of issues in all areas of the roadmap. Some of this interchange will focused on the Winter School, which will also be coordinated by the Laplace Center. Students will not only participate in formal class work and seminars on astrobiology, but will be actively involved in both the astronomical, instrumental and laboratory activities.

6.3 The Winter School

A novel and major item in our proposal is the development of a Winter Astrobiology School at the UA, where we would for one complete semester each year train 20 graduate students (10 from outside the UA) in astrobiology. The Winter School will be organized by Meyer (Deputy PI) with assistance from C. Walker who has experience in developing courses on life in the universe and interdisciplinary graduate programs. His participation will be supported as part of his normal teaching load through the department of Astronomy. We would also bring in visiting faculty to complement our local expertise, especially in areas such as molecular biology of organic molecules, the physical chemistry of life, and artificial and model life, where we do not have enough internal breadth. Over the five years of this proposal we would train 100 students, and make significant strides to develop a broad astrobiology program at the UA and to provide well-trained scientists for astrobiology endeavors at other institutions.

The goals of the winter school are to have two graduate courses for credit that last the complete winter semester. The courses will be broad reaching across the boundaries in astrobiology. Typically there would be one course that was focused on areas of astronomy and planetary sciences which covered origins astronomy and the search for life and a second course which covered molecular biology, fossil and genetic evidence and the origin of life. Meyer and Kring have already developed an interdisciplinary course on the origin of stars and planetary systems.

It is planned to offer 10 scholarships to the best U.S. graduate students who wish to come from outside UA. They would each be given a home in their main discipline. Another 10 students from inside would be admitted, with an initial limit of no more than 3 from any department at UA, and a goal of no more than 2. There would be two to three visiting faculty brought in both to interact with UA faculty, and to help with teaching the second course.

The scholarships would provide travel funds to students from outside UA, homes in departments, and stipends as research/teaching assistants. Credit would be offered for the courses, but the students would be responsible for securing credit (if possible) at their home institution. Every student completing the course satisfactorily would receive a certificate of training is astrobiology.

The logistical sequence will be to arrange for the visiting professors, and securing them accommodations during the previous summer. Also internal faculty roles and lectures will be arranged. Then the opportunities for internal and external graduate students will be advertised in the early fall semester. Applications will be required by the start of October, and students notified of decisions by 1 November. Plans for special experiences, e.g. astronomical observing, geological field trip, biological lab experiences will all need planning during November. External graduate students will be helped to find accommodation during November. Thus the Winter School can start in January without living problems hanging over faculty or students.

Special mentoring will be needed to help students cross the discipline boundaries. The students will be divided into groups of ~5 with moderately common background. Mentoring will need to be done by a professor in the discipline new to them. The student groups will also need help and encouragement from within their discipline.

Each year at the end of the Winter School, the faculty and students will be asked to evaluate the process and suggest modifications. The faculty will then plan for the next Winter School, with the collected evaluation results of the suite of previous Winter Schools to develop revisions to the plan.

6.4 NAI Astronomy Focus group meetings

The first meeting of a potential astronomy focus group occurred at the NAI meeting this spring. We propose to help develop an annual meeting for this group. We suggest alternating annual meetings between the Winter meeting of the American Astronomical Society, and a purely focus group meeting here in Tucson. This would allow both an opportunity for proselytizing among AAS members, and on alternate years to be more focussed towards cross-disciplinary topics. We will suggest that other discipline centerd focus groups may do likewise.

6.5 Astrobiology Language and Culture

The study of astrobiology can be seen as the culmination of the process of uniting all the sciences. The great gulf between physical sciences and life sciences started to fill in with the development of molecular biology, but still the parts are held apart by lack of a compelling theory for the initiation of life on Earth, and a demonstration of the existence of life beyond earth. This is the role of astrobiology.

Yet it is hard to change a culture. To move astronomy towards biology, it has to forget its emphasis on great distances, large sizes, huge luminosity and symbolic mathematical demonstrations etc. It needs to return to the idea that the original purpose of astronomy was to find the place and role of humans in the universe. To move biology towards astronomy, it has to remove the emphasis on classification within a system that excludes objects and systems without nucleic acids. It has to focus on the need to put life into a context. The very difficulty of developing a definition of life - the problem in separating the living from crystals and fire is a sign that there is no absolute boundary. Life and the non-living are a continuum, and at present that continuum is extended through other disciplines, but with huge gaps that relate to the origin of life.

Then there is the problem of language itself. At the 1997 International Origins conference in Estes Park, communication between disciplines was at low ebb. There were a series of talks about astronomy, where astronomers only talked jargon to astronomers, ignoring that people from other disciplines were present, totally ignoring the problems of communicating with biologists. And the talks ended with a discussion by a molecular biologist of variations in the form of ATP-ase and discussions about its origin that were totally obscure to all astronomers present, most of whom had not even heard of ATP! Somehow jargon must be discarded where possible and accepted across the board where it is essential, as with ATP. The barriers must be crossed, not only for students entering the field, but also for the benefit of practitioners. Hopefully the process can be worked through in face-to-face meetings, and that is a special strength in developing a broad program in one place. Across-distance communication is, at this time, more likely to end in long unintelligible monologues.

This is a significant issue for NAI's goal to be a virtual institute. There are limits to remote communication at the best of times, probably from the loss of fine body language indicators. The benefits of working day-to-day and face-to-face with those in other disciplines is that we can hope to find ways of presenting material to each other that works, and can be carried into the virtual institute. We hear that astrobiology students at U. Washington developed their own glossary. Perhaps that could act as a cross-disciplinary starting point.

To change the culture of disciplines will also require us to build new habits of meetings, new habits of whom we talk to and how we talk, new habits of how we evaluate research and teaching. Every step will need careful planning and timing, and reflection on the results. Every step will need communication so that people do not feel left out. And regardless of how we do it all, there will be hard feelings of those who feel that they belong to the old way that things were done, and they will resist the change. Nonetheless it is essential to proceed with this development, and the spirit of the time will support the activity.

6.6 Communication Aspects of the Virtual Institute

We see the process of interacting with others at a distance as a two-fold issue. First there is helping NAI with its goals of using visual image communication to be a virtual institute. There is to be a sharing of talks, and an increased interaction in meetings of people who are physically apart. We will help that.

But we also see the benefit of face-to-face interaction. We plan to send our research workers out to other NAI centers and to organizations that are not centers. And we plan to bring in visiting professors, both for our Astrobiology Winter School, and for seminars. And we need to arrange the seminars so that they are spread among the science buildings on campus so that we get all groups into the habit of crossing the ground between the buildings.

One tool that we have tried in astronomy, and which works is an *Annual Internal Symposium*, where we can expose all of us to the broad range of astrobiological work already proceeding in Tucson. During the Winter School there will be a Tucson Astrobiology Internal Symposium, with talks and poster papers. Even the discovery of all the ongoing relevant work will require the help of a number of deans and department heads, so that we can invite all appropriate people to talk and listen

6.7 Advanced Education

6.7.1 Astrobiology Graduate Minors

At the present time we do not see an advanced degree major as appropriate in Astrobiology. Every worker in the field needs a strong background in at least one of the current disciplines. We do however need a graduate minor for future workers in the field. The requirements for a graduate minor in the various departments in the College of Science currently need coordinating. It should be possible for a graduate student in any of the disciplines to get an astrobiology minor by taking appropriate courses that are agreed between the departments. As stated above the IDP will arrange for astrobiology minors to be generally available.

6.7.2 Advanced Undergraduates

Those undergraduates considering going to graduate school to become astrobiologists will be permitted to attend the internal symposium in their final two years. Students in their Junior and Senior year with grade point average above 3.5 will also at their request be assigned a mentor to meet with them at ~ 2 week intervals to discuss their work, encourage their reading, alert them to interesting talks, and generally give them advice and encouragement.

We consider that, at the present time, the best preparation for astrobiology is a strong focus in one of the disciplines that make up the field. Thus astrobiology courses for advanced undergraduates are not recommended at this time. The faculty that plans Winter School will reconsider that issue as we get more experience of the courses and students.

6.7.3 General Education for undergraduates

We will ensure that astrobiology components are available in the UA General Education programs in Natural Sciences—that is science for non-specialists. Astrobiology is a good topic for capturing interest as a general science education topic for undergraduate students in non-science fields. Much of the student enrollment Natural Sciences is in the Astronomy and Planetary Sciences departments, which together provide instruction for over 1,500 students per semester. The Laplace Center will ensure that suitable astrobiology materials are available for these and similar courses in Geosciences, the Tree Ring Lab, Ecology and Evolutionary Biology.

6.7.4 Public Activities

There are many professional astronomers in Tucson and they provide talks at the Steward Observatory Public evenings every month. And there are student and public groups such as the Students for the Exploration and Development of Space, and the Tucson Amateur Astronomical Society where talks, and assistance would be very welcome. The Laplace Center will keep a list of public speakers for astrobiology talks. There are also opportunities for programs on local radio and television, including both public television and radio stations in Tucson.

6.8 Summary

Our goals are to develop a very broad astrobiology research center in Tucson for which the NAI program becomes the initiating momentum. The work will have a focus at the Laplace Center. This will also become the center for teaching and outreach activities. The priority will be focussed on the research because the research is the process that energizes the teaching and outreach.

We see the language and culture barriers between disciplines as major difficulties and will try to break them down with frequent face to face meetings. We believe this will allow us to be helpful to the whole virtual Institute.

The Laplace Center will be started as a UA Interdisciplinary Program (IDP). It will be the focal center for organizing research and teaching, and a cross discipline and department communication center. We will develop a broadly based research program, beyond the boundaries of the NAI sponsored research. We will develop an inventory of local people and monetary resources. We will explore fund raising opportunities.

We propose to hold a biennial NAI astronomy focus group meeting in Tucson, with alternate year meetings at the American Astronomical Society winter meeting. We will also encourage other disciplines to have similar meetings.

Graduate teaching will be focused on an Astrobiology Winter School where two courses will be offered, and ~ 10 graduate students for outside UA given scholarships each year so that they can attend. There are already graduate minor programs in astrobiology in some departments and we will seek to expand this option.

Advanced undergraduates (gpa >3.5) will be allowed to audit the internal symposium, and will be mentored, but we recommend development of competence in one discipline first. We already have a non major general education course offering, and will expand it in so far as demand requires.

6.9 Education and Public Outreach

Team T. Slater (Lead), E. Prather (Deputy), N. Woolf, L. Close, P. Hinz, M. Meyer, L. Ziurys, J. Lunine, S. Strom

A particularly innovative part of this proposed project is for a significant and integrated education and public outreach component. EPO Lead T.F. Slater's primary area of scholarship is the teaching and learning of astronomy. He is the director of the UA Science and Mathematics Education Center and an associate professor of astronomy at UA. His astronomy education research group focuses on identifying students' misconceptions in astronomy and designing effective instructional materials to improve student understanding, both in formal courses and for museums and informal science centers. This unique research group has earned national recognition in the area of astrobiology education by conducting seminal research that systematically studying student beliefs and reasoning difficulties regarding the search for life in the universe (viz. Offerdahl, Prather, and Slater, 2002). This work serves as the foundation for a new laboratory activities manual for undergraduate astrobiology courses for non-science majors (Prather, Offerdahl, and Slater, 2002).

Because a comprehensive program to improve the public's understanding of the this interdisciplinary science requires targeting schools, this research group works directly with secondary school teachers on improving their understanding of the myriad of underlying concepts surrounding the search for other worlds. Through the support of a NSF teacher enhancement award, they have worked with nearly 100 high schoolteachers over the last four summers at the Toward Other Planetary Systems Teacher Leadership Workshop, conducted in Waimea, Hawaii (Meech, Slater, Mattei, and Kadooka, 1999). In addition, to providing numerous workshops at professional conferences for teachers, a NASA education award supports the development and delivery of an Internet-delivered, distance learning course, Astrobiology for Teachers, that more than 100 teachers have already successfully completed (Prather and Slater, 2002). This distance-learning course now constitutes one of the foundational courses provided by the National Science Teachers Association (NSTA) Professional Development Institute. Taken together, a basic research program along side a comprehensive teacher education program uniquely positions this research group to make meaningful and innovative contributions to the present proposal.

Doctoral candidates supported by this program will work on three efforts in the area of education and public outreach. The first effort is to conduct systematic studies of the understanding and beliefs about the scientific search for other worlds held by K-14 students, teachers, and the general public. In addition to contributing to the scholarly literature base of science education, a detailed understanding the existing notions and attitudes people have about this interdisciplinary science are crucial to designing the most effective education and public outreach programs. This will be accomplished through a systematic series of surveys, interviews, and carefully monitored instructional interventions. The results will be disseminated through journal articles and presentations at professional education conferences.

The second effort is to help other graduate students and university faculty supported by this program to become informed about the reasoning difficulties K-12 students, teachers, and the general public have with understanding the search for other worlds. If research scientists have an appreciation for the specific parts of this science that people find difficult, they will be better able to communicate the exciting results and enhance the public's attitudes toward supporting this endeavor. This will be accomplished by conducting frequent workshops and contributing scholarly papers at professional science conferences, as well as regularly

contributing to the program's seminar series. In support of these efforts, the team will undertake the creation and dissemination of an astrobiology public speakers toolkit and a dynamic FAQ (frequently asked questions) on the World Wide Web.

The fourth effort is to provide professional development programming to secondary teachers across the country in implementing high quality curriculum materials that support the interdisciplinary themes and aims of astrobiology. In particular, NAI consortium supported curricula materials that have national appeal and infrastructure, such as the Voyages Through Time Interdisciplinary Science Curriculum. This institute's EPO team is uniquely qualified and positioned to engage and succeed in such a national effort because of their extensive experience in teacher training and their scholarly track record in education and public outreach.

Taken together, these efforts will result in the following as part of the education and public outreach plan:

Referred publications and conference proceedings that contribute to the science education literature base;

Effective analogies and instructional strategies participating scientists can use to help the general public understand and value the search for other worlds; and a better

Appreciation by scientists of the importance of their being actively involved education and public outreach endeavors.

Most importantly, these collective efforts will benefit all community members of the National Astrobiology Institute consortium

In addition to scholarly work in the area of astrobiology educational research and innovative curriculum development, this project supports the scientific research efforts of graduate students working in support of the overall scientific goals described at the beginning of this proposal. As in many institutions, the education of graduate students working along sidepost-doctoral fellows, researchers, and professors is a fundamental and integral part of this proposed project. These doctoral students will pursue newly approved minors at the University of Arizona in biology, chemistry, and planetary sciences, which directly align with and support the overarching themes of this proposal.

Dr. Slater is also working with Discovery Park, a science education center located in Safford, Arizona at the foot of Mt. Graham. His goal is to use the center and the proximity of major astronomical facilities on Mt. Graham to extend the outreach program to parts of rural Arizona and to the San Carlos Indian reservation. The Laplace Center will provide material and support to these efforts.

In addition Dr. Don McCarthy has, for many years, run a very successful Astronomy Camp for high school and university students as well as for teachers using the astronomical facilities on Mt. Lemmon and incorporating components of geosciences and tree ring studies as well as

astronomy. If selected, the Laplace Center will seek to expand this activity and to ensure that suitable materials on astrobiology are available for each of the above program elements.

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BIOspheres of Mars: Ancient and Recent Studies

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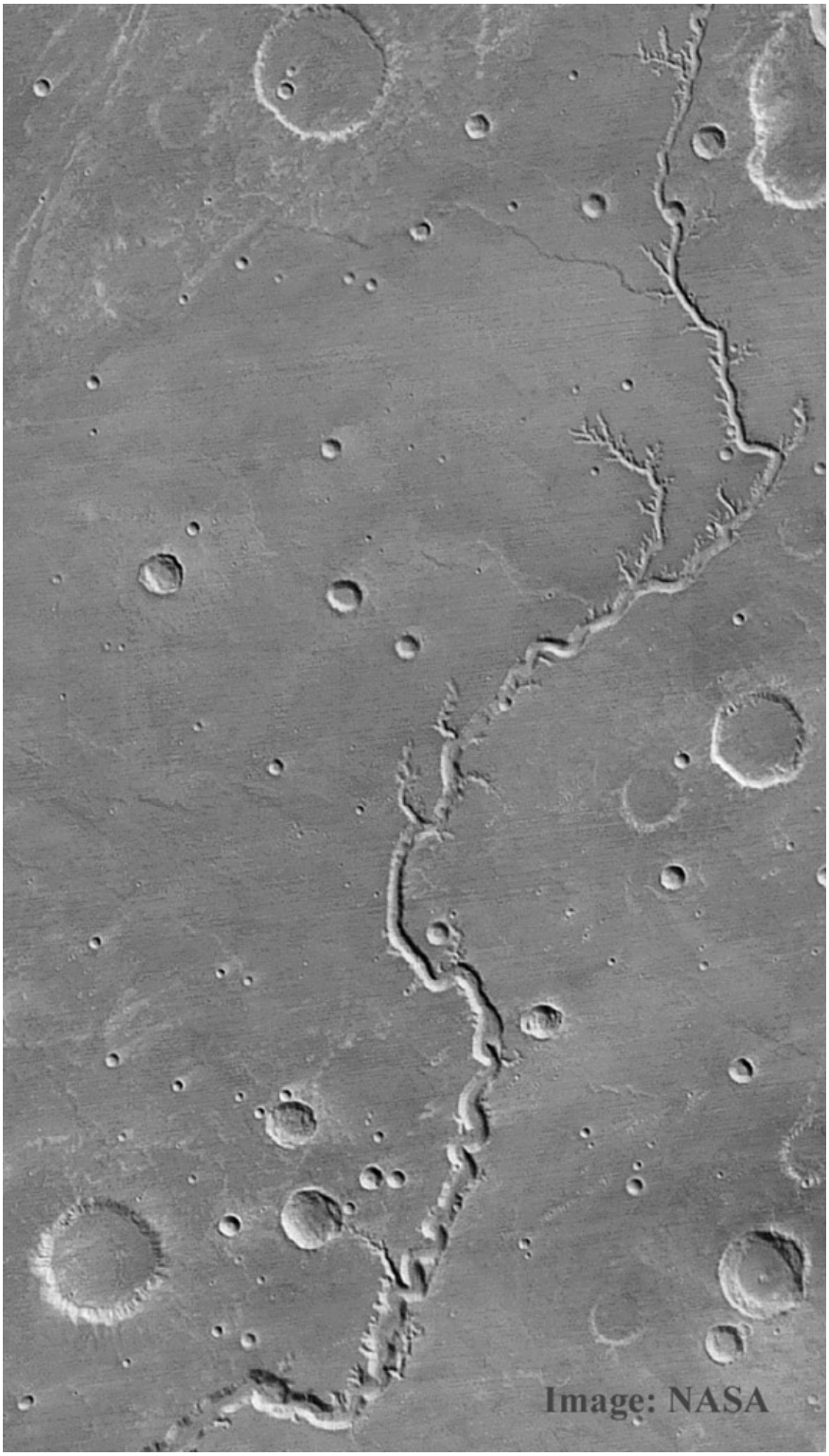
Bio M A R S



A proposal in response to Cooperative Agreement Notice 02-OSS-02
to become a member of the NASA Astrobiology Institute

- BIOMARS -

BIOsphere of **M**ars: **A**ncient and **R**ecent **S**tudies

Submitted by:

The Regents of the University of California
The Center for Integrative Planetary Science*
University of California Berkeley
March 6, 2003

* Unit will administer award

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EXECUTIVE SUMMARY

Overview: Mars is an exciting, and comparatively accessible target for astrobiological studies aimed at detection of current or past extraterrestrial life. We will analyze the evolution of the Martian hydrosphere and surface topography to understand the history of water distribution and investigate atmospheric processes that may have contributed to a UV shield. Our goal is to identify the types of sites on Mars that experienced long-term fluid flow and may be, or have been, conducive to life. We will characterize biomes that develop in analogous Earth environments, conduct experiments to determine limitations for life in these habitats, and identify features that constitute indicators of life. We propose robot-based sampling and *in situ* analyses of terrestrial sites so as to develop methods for dealing with the challenges of remote geomicrobiological investigations. Our work will provide constraints for selection of optimal sites for future Mars exploration and methods for sample analysis, and ultimately will be relevant to the question 'did life evolve elsewhere in the universe'.

Habitat constraints from Mars and modeling: Early microbial life probably originated on Earth in environments characterized by redox disequilibria. Habitats may have developed on Mars in redox gradients between reduced basaltic rocks and oxidized fluids and/or gases. Element cycling driven by fluid flow through such redox gradients could underpin (or could have underpinned) a substantial biosphere. We will analyze Mars planetary evolution to develop models for the timing and scale of hydrosphere development and subsurface water circulation. We will couple these hydrosphere models to geomorphological models based on terrestrial field site analyses and experimental geomorphological studies to allow detailed interpretation of Mars surface features. This will permit analysis of the history, form, and timing of fluid flow events that shaped the planetary surface and determination of the factors that control them. In parallel, we will explore atmospheric processes that could have contributed to a UV shield and conduct spectroscopic studies to constrain Mars surface mineralogy.

Habitat constraints from Earth: Studies of chemoautotrophically-based ecosystems will focus on terrestrial aqueous environments in basaltic rocks similar to those at the Martian surface. Hydrology, geomorphology, spectroscopy, and geomicrobiology research will begin at sites where groundwater discharge in basaltic andesites or basalts is generating channels with features similar to those on Mars. These springs appear to offer the best chance of sustained water flow and protection from UV radiation. Initially, our studies will be conducted at cold and warm springs associated with basaltic rocks in dry, cold desert environments in Oregon and Idaho. We will refine our choice of study sites as our understanding of Mars' surface improves.

Abundant, redox-active species such as iron and sulfur represent potential energy sources for possible Martian life at springs in basaltic rocks. Recent microbiological studies, geochemical calculations, and experiments indicate that the kinetics of both Fe-silicate and Fe-sulfide mineral dissolution reactions are fast enough to sustain significant biological populations. We will characterize currently poorly understood microbial habitats in the near subsurface in terms of their population structure, aqueous geochemistry, mineralogy, and isotopic signatures in order to determine the form of the record life might leave in similar Martian systems.

Laboratory constraints for habitat development and biosignatures: Results of *in situ* analyses of terrestrial ecosystems will be paralleled by laboratory-based studies that will explore the ranges of temperature, concentration, and pH consistent with life in the these habitats. Biochemical analyses will explore the factors that set these limits. We will analyze the structure, elemental and isotopic composition, microstructure, morphology, and distribution of minerals generated by, or impacted by, life in basaltic-rock hosted systems so as to develop and test potential new biosignatures. Parallel inorganic experiments will be conducted in order to resolve non-biological features and to examine changes in mineralogical biosignatures with time. As yet unstudied isotopic characteristics of Martian meteorites will be determined in order to provide baselines for isotopic biosignatures. Similarly, work on the isotopic evolution of the atmosphere will establish the magnitude and form of non-biological isotope fractionations. Application of state-of-the-art methods for analysis of Martian, and Mars-like rocks will yield procedures that will be useful for future analysis of samples returned to Earth or encountered during remote analysis on the Martian surface. Our results will contribute to selection of sites on the Martian surface with the highest potential for future detailed in situ investigations.

Our team: Our goal is to create a highly interactive, focused NAI team to address a well-defined set of problems. The necessary interactions will be facilitated by close geographic proximity of most team members. Five of the 10 scientific team members are at UC Berkeley, one is at the SETI Institute, and one in Palo Alto. The three other CoIs have essential expertise for study of difficult to cultivate Fe-oxidizing neutrophiles and in situ measurements. All three non-Bay area CoIs have collaborated with the PI on a NASA-funded seed project preliminary to this proposal. Communication between all CoIs will be promoted through work on common sites and processes, shared goals, and virtual (internet-based conferencing) and traditional group meetings.

Our group includes members with strong, integrated field and laboratory-based research programs and experience with study of a diversity of natural environments. Several team members are expert in the development and deployment of state-of-the-art analytical methods (e.g., isotopic analyses, microsensor measurements) to interdisciplinary problems. Our group also includes a robotics engineer and scientists familiar with ancient and recent Mars planetary history. The NAI support will be essential to facilitate the new interactions between hydrologists, geomorphologists, geomicrobiologists, chemists and engineers that are needed to meet the project goals.

Education and pubic outreach: The topics of life on Mars, life in extreme environments, and extraterrestrial exploration easily capture public attention. Our group will use the broad appeal of these subjects to create educational materials designed to foster interest in science, especially geology, chemistry, and biology. Educators from the University of California Berkeley's Lawrence Hall of Science (LHS) will work closely with the BIOMARS team to develop, field-test, and implement materials that incorporate key project concepts and emphasize the interdisciplinary nature of space exploration. LHS is a public science center that is world-renowned for development of high quality middle and high school science curriculum materials based on current research and understanding of how students think and learn. Educational materials will be disseminated through the use of the LHS infrastructure, and its well-established national and international network of educators.

Summary of Personnel

Principal Investigator:

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Co-Investigators:

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RESEARCH, TRAINING, MANAGEMENT

1) INTRODUCTION

Is there, was there, life on Mars?

Life may take many forms. However, all life forms require a bioavailable source of energy. The range of energy types utilized by modern life on Earth is vast. However, the range of abundant energy sources available to early or primitive current life on Mars is probably more restricted. Disequilibria between reduced components of Martian rocks or hydrothermal fluids (e.g., H_2, Fe^{2+} and S^-) and oxidized constituents of the soil (Foley et al. 2001; Bell et al. 2000), dust, and atmosphere (e.g., Fe^{3+}, O_2) provide specific targets.

Observations of Earth systems reveal that energy utilization causes changes in elemental and isotopic composition, and in the structure, morphology, and distribution of minerals. These changes constitute biosignatures that can potentially be interpreted to answer the question "is there, was there, life on Mars?". These biosignatures may be interpretable even if the form of life differs from that familiar to us on Earth.

Schieber (2001) noted that subsurface depositional environments are likely to provide more frequent and diverse opportunities for preservation of biosignatures than the shallow-water carbonate structures (e.g. stromatolites) that have been the mainstay of life detection in the early Earth rock record (Knoll, 1989; Schopf and Klein, 1992; Schopf, 1993). Subsurface habitats characterized by redox disequilibria and protection from UV radiation may be important in the search for evidence for microbial life on Mars. Such sites are probably also the only ones with long term access to water.

We consider it probable that if life forms evolved on Mars, biochemical functions would require at least some water. Recent studies indicate that water occurs in the modern Martian subsurface and that discharge of some type of fluid has occurred, at least episodically, at the surface of Mars. We contend that it is possible to develop a detailed understanding of the Martian hydrosphere and history of fluid discharge through integration of hydrological and geomorphological modeling, experimental studies, and field data for analog systems. Likely sites for development of ancient and recent Martian biomes (defined here as a chemically and biologically distinctive habitat) may be identified by targeting environments that provide energy, water, and protection from UV radiation. Based on study of patterns and locations of fluid discharge on Mars, we will select a tractable number of appropriate Earth analog sites for detailed geomicrobiological and chemical analyses. Our goal is to determine how to detect evidence for past or current life on Mars through analysis of these analog systems. Our work will involve studies of microbial communities and their biosignatures at these field sites and in the laboratory. We will adapt existing robotic devices to permit sampling and in situ analyses of otherwise inaccessible exteme environments. This will allow us to develop appropriate protocols for remote geomicrobiological investigations. The proposed research will enable design of missions to achieve maximum probability of detecting evidence for Martian ecosystems, either through in situ measurements on Mars or in samples returned from Mars.

Setting the scene: Biome constraints from past studies of Mars

A key element of the proposed work is determination of how the Martian surface and near-surface conditions now, and over geologic time, might have provided opportunities for the emergence and proliferation of life. We begin this analysis by using the results of prior studies to constraint our approach and to lay the foundation for proposed investigations.

The rocks comprising the surface of Mars are low Mg-, low S-andesites and basalts (Bandfield et al., 2000; McSween et al., 1999). Based on data from the SNC meteorites, Martian rocks are reduced and contain primary ferrous iron (probably the most abundant, redox-active constituent of the Martian surface) and some reduced sulfur (*e.g.,* McSween, 1985). However, data collected by Viking and Pathfinder indicate that the Martian dust and soil are highly oxidized, iron rich, and that sulfate is a major component (Bell et al. 2000; Foley et al. 2001). These first order observations highlight important disequilibria between the oxidized Mars atmosphere and the reduced subsurface (Table 1). Such disequilibria could form the basis for a diversity of energy-generating systems utilized by Mars life forms. Terrestrial analogs for possible Mars biomes based on disequilibria between iron (and sulfur) compounds are targeted here.

Table I: Pathfinder data (wt %) for soils and rocks from Foley et al. (2001). Please consult the original reference for details.

SOILS	Na2O	MgO	Al2O3	SiO2	P2O5	SO3	Cl	K2O	CaO	TiO	Cr2O3	MnO	Fe2O3
A-2	4.1	9.7	9.8	40.0	0.8	5.9	0.7	0.5	5.9	0.8	0.3	0.5	21.0
A-4	4.2	9.0	9.9	40.1	1.0	6.8	0.8	0.5	5.5	1.2	0.4	0.4	20.2

ROCKS	Na2O	MgO	Al2O3	SiO2	P2O5	SO3	Cl	K2O	CaO	TiO	Cr2O3	MnO	FeO
A-3	4.7	2.4	12.1	53.2	0.6	2.0	0.5	1.1	5.6	0.7	0.1	0.3	16.6
A-7	6.1	5.9	10.6	46.4	0.5	4.4	0.8	0.7	6.4	0.8	0.1	0.4	16.9

In addition to possible chemical energy sources, it is important to consider the physical constraints (radiation, fluid distribution, temperature) that may have limited (spatially and temporally) the development of life on Mars. For example, protection from UV radiation may be an important consideration. The extent to which atmospheric processes early in Mars history could have limited radiation at the planet's surface is unclear (see below). However, UV protection afforded by rocks and the subsurface may have been important in localizing life over much of Mars' history.

A key question is the persistence of liquid water on the Martian surface over time. Given the current P-T conditions, liquid water is not stable at the surface of Mars today. There is evidence for abundant fluids early in Martian history. The flat northern hemisphere (Smith et al., 1998; Smith et al., 1999) has been argued to represent evidence for a past standing body of water (Baker et al., 1992; Head et al., 1999). The development of the ancient volcano-tectonic provinces such as Tharsis is thought to play a critical role in hydrothermal circulation (e.g., Wilson and Head, 2000) and global groundwater flow patterns (e.g., Phillips et al., 2001). There is some evidence of recent surface volcanism (Garvin et al., 2000; Hartmann et al., 1999), but these features are not associated with many possible recent water-related features. Relict geothermal systems indicate abundant

hydrothermal activity early in Mars history (Farmer, 1996), and thus have been targets in the search for life on Mars (Farmer and Des Marais, 1999; Walter and Des Marais, 1993). There are also indications of possible sedimentary systems. For example, thermal emission spectrometer studies revealed a large, layered deposit of coarse-grained hematite at Sinus Meridiani (Christensen et al., 2000) that may have been deposited in water.

Surface temperatures are especially important components of analysis of potential Mars habitats and the form and distribution of water over Mars history. The cooling of the planetary interior with time and the variation of solar insolation during obliquity cycles (or tilt of the spin axis) are important contributors to Mars' subsurface and surface thermal evolution, respectively. Although a warm, wet early Mars has been proposed, it is possible that the Martian surface was not much above freezing for extended periods (Squyres and Kasting, 1994). Warm near-surface temperatures in relatively recent times may be related to obliquity cycles (Ward, 1974) that periodically increase and decrease the amount of solar insolation at the Martian surface. The obliquity of Mars is currently ~25°. When obliquities exceed ~45° the amount of insolation is such that even at current levels of internal heat loss the polar caps would melt (Fanale, 1992). Thus, Mars could periodically develop liquid water close to the surface.

The long-term loss of heat generated during Mars accretion, core formation, and the decay of radiogenic elements in the interior dictates the surface heat flow (Schubert et al., 1992; Solomon and Head, 1990). The presence of old (> 4 Ga) magnetic anomalies (e.g, Acuna et al., 1999; Connerney et al., 1999) suggests that a core dynamo was active early in Martian history. The lack of younger magnetic anomalies may reflect a rapid cooling of the core, or a change in style of mantle convection from the mobile to stagnant lid regime (e.g., Nimmo and Stevenson, 2000). Ancient surface heat flow estimates from MGS gravity and topography indicate that the northern lowlands (Smith et al., 1998) was a locus of high early heat loss (Zuber et al., 2000). This spatially non-uniform surface heat flow could be consistent with low-mode convection (Zhong and Zuber, 2000) or plate tectonics (Sleep, 1994*)*. A convective model has been proposed to explain the early focused high heat flow in the northern lowlands indicated by gravity and topography data (Zuber et al., 2000). A later-stage (<4 Ga) reactivation of the Martian dynamo also has been advocated (Schubert, 2000), which implies significant cooling of the interior, but spread over a broader period of Martian history. Constraints implied by current models for the core dynamo generation cannot easily explain possible recent evidence for liquid water at the Martian surface (Malin and Edgett, 2000) but may provide useful insight into plausible ancient hydrological processes.

Surface temperatures depend in part on the properties of the Martian atmosphere. Degassing of the interior during cooling leads to the release of volatiles that likely resulted in a thicker early atmosphere and liberated water on the surface, thus producing conditions potentially favorable for the development of life. Mechanisms of atmospheric loss (Owen, 1992) and the fate of what appears to have been abundant surface water (Carr, 1996) are not completely understood.

The surface topography of Mars provides abundant evidence for fluid flow, but quantitative interpretation of the nature of the events that occurred is not yet possible. Martian geomorphological features such as sinuous channels that have eroded back into volcanic strata appear to represent 'sapping channels' that, on Earth, are known to form

as the result of long term, localized groundwater discharge. If such a process yielded the channels on Mars (cover image, courtesy of NASA), the steep channel terminations should provide relatively protected habitats with sustained fluid flow. Thus, the origins of these channels represent important targets for geomicrobiological studies. We propose to explore processes that generate channels with features similar to the Martian channels and develop an understanding of the size, frequency, and timing of fluid discharge events. This will require evaluation of factors that control fluid flow in the Martain subsurface and that localize regions of surface discharge. Thus, the first major goal of our work is to answer the question:

"Where are the sites most likely to yield evidence for past or current life on Mars?"

Biome development on Mars: constraints from Earth

Although we cannot assume that, if life evolved on Mars, it took a form analogous to life on Earth, we can learn a great deal about how to search for life from studies of Earth biogeochemical systems. Even if the design principles for Martian life differ significantly from those we are familiar with, it is reasonable to assume that their metabolism leaves an imprint on the environment. In particular, many energy metabolisms that rely upon catalysis of otherwise sluggish or kinetically prohibited reactions generate products that differ from those in a metabolism-free system. For example, the presence of abundant, nanometer-sized sulfide minerals may be a biosignature because sulfate reduction is extremely slow at temperatures below 150 °C (see review in Druschel et al. 2002) and temperatures > 150 °C will lead to rapid sulfide mineral grain growth (Huang et al. 2002).

In the work proposed, we will focus on basalt and basaltic andesite-hosted oligotrophic environments that we believe are the best possible terrestrial analogs for life-sustaining environments on Mars. The sites chosen for initial work are groundwater springs in basaltic andesites and a subsurface, volcanic-hosted system where acidophilic (acid-loving) chemoautotrophic microorganisms (CO_2-fixing organisms that utilize inorganic energy sources) are sustained primarily by pyrite dissolution. Through analysis of these communities we will answer the question:

"where do microbial populations occur in basaltic and basaltic andesite terrains and what factors limit their distribution?"

We will combine spatially- and temporally-resolved information about microbial community structure, metabolic characteristics with details of mineral structure and mineral surface topography, elemental composition, and isotopic composition in order to define rigorous biosignatures for each environment. Through collaborations between microbiologists, mineralogists/ mineral physicists, and geochemists, we will answer the question:

"how can we establish the existence, or preexistence, of microbial communities through analysis of the physical and chemical characteristics of rocks and minerals?"

TEAM EXPERTISE, EXISTING LINKS, AND PROJECT MANAGEMENT

The following sections describe a proposed series of investigations, each of which will draw upon the expertise of a significant fraction of the researchers that will comprise our NAI team. Before describing these studies, we briefly introduce our investigators. More details are provided in the vitae listed in the later part of this proposal. Each of the individuals has a strong, independent research program that is directly relevant to the proposed research and some are collaborating with other members of the NAI group.

(1) Dr. Jill Banfield, University of California Berkeley

Dr. Banfield is a geomicrobiologist with a background in mineralogy, and interests in geochemistry of surface processes, microbial ecology, and microbial evolution. A major current activity in her research group is genomics-based analysis of extremely acidophilic archaea and bacteria and development of community genomics methods (Tyson et al. 2002, 2003a,b; Banfield et al. 2003; Hugenholtz et al. 2002a,b). The genomic data will be used to create gene expression arrays to monitor microbial activity in acidic biogeochemical systems. The other major focus in her group is study of the size-dependent structure, properties, and reactivity of nanoparticle products of chemical weathering and microbial metabolism. Dr. Banfield has prior experience working at both the Abert Lake and Richmond Mine field sites.

NAI focus: Geomicrobiology of Fe in acidic systems studied via a combination of mineralogical, geochemical, and molecular biological approaches (SSU rDNA analyses, genomics-enabled, microarray-based analyses. Iron-oxide/hydroxide biomineral formation and aging at neutral pH. Response of mineral surfaces to biologically-produced compounds.

Dr. Banfield has current collaborations with Drs. Emerson, Roden, and Luther (supported by a seed grant from NASA) and Dr. Dietrich.

(2) Dr. Janice Bishop, SETI Institute

Dr. Bishop is a planetary spectroscopist with expertise in mineral physics and chemical alteration on Mars. She has many years of experience measuring the spectroscopic properties of aqueous minerals (Bishop and Murad, 1996; Bishop et al., 1994, 1999, 2002b,c; Murad and Bishop, 2000). She also has experience characterizing and collecting geologic samples in the field for lab studies (Bishop et al., 1998; Bishop and Murad, 2002; Bishop et al., 2002a). Much of Dr. Bishop's work is applied to understanding the geochemical environment on Mars through characterization of the minerals observed on the surface. She is a Co-I on the CRISM hyperspectral (0.4 - 4.0 μm) instrument on the 2005 Mars Reconnaissance Orbiter. She has many years of experience measuring the spectroscopic properties of fine-grained Mars analogue materials and comparing them with spectra of Mars (Bishop et al., 1993, 1995, 1998, 2002d).

NAI focus: The spectral properties of weathered rocks in terrestrial analog sites to provide reference data for analysis of mineral alteration zones associated with sites on Mars targeted for geomicrobiological investigations. Mars surface chemistry and mineralogy.

(3) Dr. Kristie A. Boering, University of California, Berkeley

Dr. Boering is a chemist and earth scientist with expertise in experimental and observational atmospheric chemistry and its coupling to climate on earth and other planets on time scales from months to billions of years. Her research involves laboratory experiments on novel photochemical isotope effects and the generation and properties of aerosols, observations of radiatively and chemically important trace gas species from the ground to the middle stratosphere on earth, and numerical modeling to integrate from the molecular to the global scale.

NAI focus: Experimental simulations of atmospheric chemistry in Mars-like atmospheres over the course of Martian history in order to constrain climate and habitability uncertainties, including the stability of liquid water on the Martian surface and whether or not a UV shield in the form of an aerosol layer could have formed under various atmospheric composition scenarios. Experimental simulations of potential "abiotic" carbon isotope signatures generated in the Martian atmosphere which might otherwise be interpreted as a biomarker. Providing an atmospheric perspective to fellow NAI BioMARS members working on surface and subsurface processes and predicting potential habitats for life.

(4) Dr. Donald J. DePaolo, University of California, Berkeley

Dr. DePaolo is a geochemist with expertise in the application of isotopic techniques to problems in geochemistry and geophysics. He has worked on Ca isotope fractionation in food chains, Sr isotope geochemistry of the oceans, and is currently involved on studies of Fe isotope fractionation in the weathering cycle.

NAI Focus: Measurement of the isotopic characteristics of Martian meteorites to provide baselines for biosignature studies. Isotopic characterization of minerals formed in biogeochemical processes.

(5) Dr. William Dietrich, University of California, Berkeley

Dr. Dietrich is a geomorphologist interested in channel formation, soil production, hydrologic processes, and the geomorphic transport laws responsible for landscape evolution. Recently he has collaborated in studies directed at understanding the processes controlling the recent gullies on Mars.

NAI focus: Channel formation and evolution processes on Mars and terrestrial analogs in order to estimate duration and magnitude of water-driven channel processes.

Dr. Dietrich currently collaborates with Dr. Banfield as part of the NSF sponsored National Center for Earth-surface Dynamics

(6) Dr. David Emerson, American Type Culture Collection

Dr. Emerson is a microbiologist with specific expertise in culturing and isolation of novel organisms that oxidize iron in near neutral pH solutions. His research interests focus around Fe-based lithotrophy in low temperature environments (e.g., soils) and in hydrothermal vent systems.

NAI focus: Analysis of bacterial communities associated with aqueous and solid Fe(II) phase oxidation through enrichment culturing and culture-independent molecular (SSU rRNA-based) detection.

Dr. Emerson currently collaborates with Drs. Roden, Luther, and Banfield, and the NAI group at the Carnegie Institute, Washington DC.

(7) Dr. George Luther – University of Delaware

Dr. Luther is an inorganic chemist with expertise in geochemistry. His research includes the application of microsensor measurements of aqueous solutions (including those from extreme environments) to understanding biogeochemical systems.

NAI focus: Fe biotic and abiotic redox reactions, including mechanisms of electron transfer. Characterization of the patterns of dissolved Fe speciation in redox-stratified Fe cycling environments via voltammetric techniques applied at macro (cm-m scale) and micro (µm-mm scale) spatial scales. Electrode technology will be coupled with robotic systems through interaction with Dr. Yim. His group will make real time chemical speciation analyses of Fe and S in microbial culture experiments and work with other team members to demonstrate what chemical species microbes are using for growth.

Dr. Luther has current collaborations with Profs. Banfield, Emerson, Roden and other NAI teams.

(8) Dr. Michael Manga, University of California Berkeley

Dr. Manga's work in hydrogeology has focused on using springs to study subsurface hydrologic and geologic processes in volcanic arcs. New ongoing work is addressing the problem of how hydrologic systems interact with earthquakes, in particular how permeability and fluid pressure changes in response to earthquakes (a combination of field studies, theoretical work, and numerical studies) and how earthquakes respond to changes in fluid pressure (based on data analysis). Manga's research group is also involved in a numerical and experimental study of the global evolution of the Martian mantle, focusing presently on the thermochemical dynamics of early Mars.

NAI focus: Study of hydrosphere evolution on Mars through field, lab, and theoretical studies.

(9) Dr. Eric Roden – University of Alabama

Dr. Roden is an environmental microbiologist with interests in biogeochemical cycling in sedimentary environments, with a specific focus on the redox cycling of iron and the physiological ecology of Fe-reducing and Fe-oxidizing bacteria.

NAI focus: Biogeochemical studies of the structure and function of microbial Fe cycling communities using traditional and microscale chemical profiling techniques, process-level studies of microbial metabolism and mineral transformation, and molecular biological detection of functional bacterial groups.

Dr. Roden currently collaborates with Drs. Luther and Emerson on studies of microscale Fe redox cycling in layered, redox-stratified microbial communities and with Dr. Banfield on growth of Fe-oxidizing bacteria on silicate mineral substrates.

(10) Dr. Mark Yim, Palo Alto Research Center

Mark Yim heads a team at the Palo Alto Research Center called the Smart Electro-Mechanical Systems Area. Researchers in this group have degrees in a variety of fields including Computer Science, Mechanical Engineering, Electrical Engineering Product Design and Fine Art. The group focuses on the coupling of computation with electro-mechanical systems to exploit the rapid increases in computational ability to enable radically new capabilities in electro-mechanical systems.

The team has built modular, reconfigurable robot systems that have demonstrated a variety of firsts including, self-reconfiguring for locomotion with two topologically different gaits (rolling loop to snake-like), snake-like concertina gaits through unstructured holes (exploring gopher holes), a robot that can climb: stairs, fences, poles, over loose rubble, through 4" pipes etc. While in general these systems promise to be versatile (through the ability to chance configurations), robust (through redundancy) and low cost (through batch fabrication from many repeated modules), two near term application of these systems include search and rescue and planetary exploration.

NAI focus: Adaption of modular, reconfigurable robots for geomicrobiological investigations.

(11) Mr. Kevin Cuff, Lawrence Hall of Science, University of California, Berkeley

Kevin Cuff is currently the director of the Student Radon Research Project, an NSF sponsored instructional materials development at the University of California's Lawrence Hall of Science (LHS). Mr. Cuff is a geologist by training, with graduate experience in gas emission research on active volcanoes, who has been developing inquiry-based instructional materials and programs at LHS for the past 13 years. He is the author of

several extremely popular books that contain earth science-related inquiry-based lessons for secondary school students, and has recently developed a project-based curriculum module that engages students in authentic earth science research activities.

NAI focus: Mr. Cuff will contribute to the education and public outreach effort.

(12) Dr. Herbert Thier

Herbert D. Thier is currently an Academic Administrator Emeritus at the Lawrence Hall of Science, University of California, Berkeley. He is Founding Director of the Science Education for Public Understanding Program [SEPUP] and a number of other grants at the University. Since 1963 he has been leading Instructional Materials Development and Teacher Enhancement projects in science at the Lawrence Hall of Science. He received (with M. Linn), the JRST *Research in Science Teaching Award*, of the National Association for Research in Science Teaching in1975. Thier received the Distinguished Service to Science Education Award, of the National Science Teachers Association in 1994 and the Distinguished Service to Science Education Award, of the Connecticut Science Supervisors Association in1996.

NAI focus: Dr. Thier will be responsible for the education and public outreach component. He will coordinate interactions between the EPO team and the scientific investigators.

Project Management

As PI, Banfield will coordinate all activities within the NAI. She will be assisted in this effort by Susan Sullivan, a program assistant (temporary hire) known to all team members through her contributions to preparation of this proposal. Banfield will oversee the geomicrobiological studies (Thrusts 2 and 3) and coordinate the field-based reasearch with Dietrich, the lead investigator for the Mars history and physical constraints group (Thrust 1, see below).

Roden, Emerson, Luther, Banfield will be responsible for sampling and geochemical characterization of the specific environments targeted. Yim will interact with Luther and Banfield to supplement the field effort via robot-enabled sampling of otherwise inaccessible regions. The Roden, Emerson, and Banfield laboratories will be responsible for most of the microbiological investigations, in collaboration with Luther, who will provide fine-scale geochemical measurements. Emerson and Roden contribute expertise in microbial physiology and isolation of new organisms, Roden provides microbial system modeling skills, Roden, Emerson, and Banfield routinely use molecular biological methods, and the Banfield group contributes expertise in mineralogy and genomics. Complementing these programs are Luther, Boering, and DePaolo, who bring to the NAI expertise in solution, gas, solid characterization and isotopic analyses. Bishop contributes knowledge of Mars exploration and remote- and laboratory-based mineral spectroscopic analysis, Banfield, who has expertise in mineralogical characterization via electron microscopic and other methods, and Dietrich and Manga, who provide expertise

in field, laboratory, and modeling-based studies of hydrological and geomorphological processes.

All CoIs will contribute to a 'BIOMARS' project web site (already established to share field site information and photographs for proposal development). Sharing of this expertise through exchange visits, shared field work, and regular meetings, will greatly strengthen investigations. Streaming video conferencing capabilities have been established in the EPS department at UC Berkeley; this technology will be used for regular internet-based meetings.

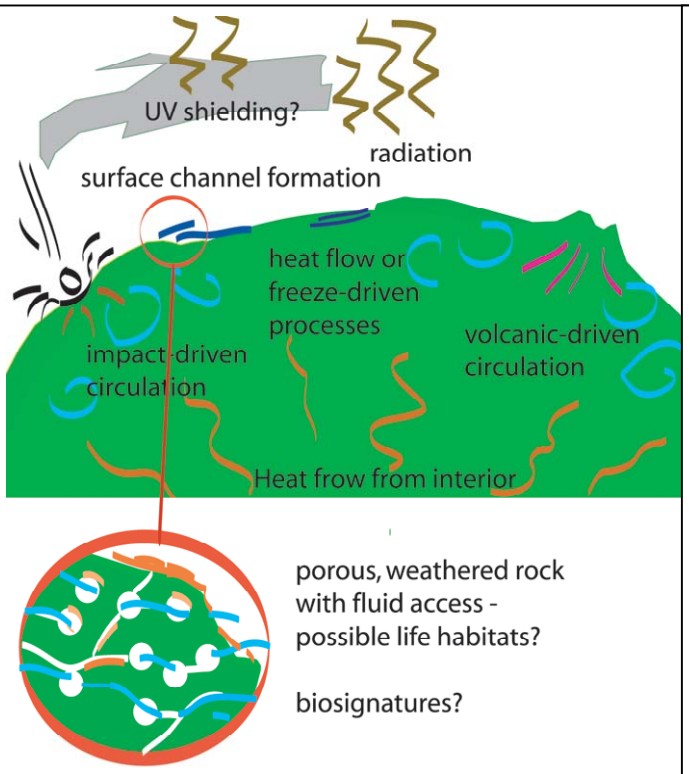
Relationship between the NAI and CIPS, and specific UC Berkeley Commitments

UC Berkeley has launched a major effort to pursue research and education in planetary science, planetary environments, and astrobiology. Two years ago, UCB created an organized research unit called the Center for Integrative Planetary Sciences (CIPS), which is directed by Dr. Geoff Marcy. CIPS has hired several new faculty (the PI and one CoI for this proposal) and instituted several educational initiatives to support a new planetary/astrobiology program. A new undergraduate curriculum has been established in planetary science under the auspices of two departments, "Astronomy" and "Earth and Planetary Science". These new undergraduate programs integrate courses in chemistry, geophysics, astronomy, biology, and atmospheric science into a coherent curriculum. The CIPS faculty will provide complementary leadership in astrobiology research and teaching, thus greatly strengthening the visibility and impact of the proposed NAI.

As described in the attached letter from Dr. Marcy, CIPS will commit a significant fraction of its resources to the NAI, should it be funded. Specifically, they propose to **provide $50,000 per year** to establish a competitively awarded **"UC Berkeley Postdoctoral Fellowship in Astrobiology and Planetary Science"** (see letter from Dr. Marcy).

As part of the proposed activities for the NAI, Banfield and colleages will develop a new undergraduate general science course that will cover planet formation, origin of life, organism-environment interactions and the search for life in the universe. This course will be coordinated via the CIPS infrastructure. **Administrative support** for course management and coordination between the NAI and CIPS (separate from the NAI administrative support requested in the budget) and **computer support** will be provided by CIPS (see letter from Dr. Marcy). For additional details, please see the section on institutional commitment.

PROPOSED WORK AT A GLANCE



THRUST 1:

ATMOSPHERIC PROCESSES
PI: Boering

MINERALOGY
PIs: Bishop, Banfield

SURFACE PROCESSES
PIs: Dietrich, Manga, Bishop

HYDROLOGIC PROCESES
PIs: Manga, Dietrich

THRUST 2:

GEOMICROBIOLOGY
PIs: Emerson, Roden, Banfield, DePaolo, Luther

THRUST 4:

ROBOTIC GEOMICROBIOLOGY
PIs: Luther, Yim, Banfield

Section 3.1 **THRUST 1**: Locations most likely to be optimal for life on Mars

Section 3.1.1 Hydrology – form, timing, volume of fluid flow near the Martian surface
Section 3.1.2 Mars surface processes: sites of sustained fluid flow on Mars?
Section 3.1.3 Atmospheric processes: the form and effectiveness of a UV shield
Section 3.1.4 Mineralogy and physical properties of solids in Martian biomes

Section 3.2 **THRUST 2**: Life associated with Mars rocks – habitats and biosignatures

Section 3.2.1 Microbial populations in chemoautotrophically-based ecosystems
Section 3.2.2 Physiology, possible role of biomineralization in energy generation
Section 3.2.3 Mineral Biosignatures associated with microbial Fe cycling
Section 3.2.4 Isotopic biosignatures
Section 3.2.5 Mineral surfaces as biological indicators
Section 3.2.6 Exploring life's extremes in an acidic Fe-S system

Section 3.3 **THRUST 3**: Novel in situ measurements, robotic geomicrobiology

Section 3.3.1 Development of sensors for in situ biogeochemical measurements
Section 3.3.2 Robotic-based geomicrobiology

3) PROPOSED WORK

Thrusts 1 and 2 (see diagram on previous page) will tackle identification of the most hospitable sites for life on Mars, analysis of the types of communities that may colonize them, and how these communities may be detected. Thrust 3 complements Thrust 2 by adding new geochemical and sampling approaches. Four approaches will be used in this work: Mars image analysis, study of terrestrial analog field studies, laboratory simulations and experiments, and numerical modeling.

The common element for the work in the three thrust areas is the set of field sites to be studied. These will provide constraints for hydrologic, geomorphologic, and geomicrobiological models and serve as sources of microorganisms for experiments and sites for biosignature analysis.

Field sites

Initially, mineralogical, geochemical, geomicrobiological, hydrologic, and geomorphological field work will be conducted at three field sites in the western US. Alternative sites may be selected as work evolves over the five-year grant period.

The first site locations are in the Miocene andesitic and basaltic volcanics and tuffs that occur near Abert Lake, Oregon. Abert Lake is a closed-basin saline, alkaline lake that occurs in graben. The lake is bounded by the spectacular ~ 2000' high basaltic andesite and basaltic Abert Rim (Fig. 1). The rim is capped by lavas considered to be approximate equivalents of the Columbia River Basalts of northern Oregon. The lake is a

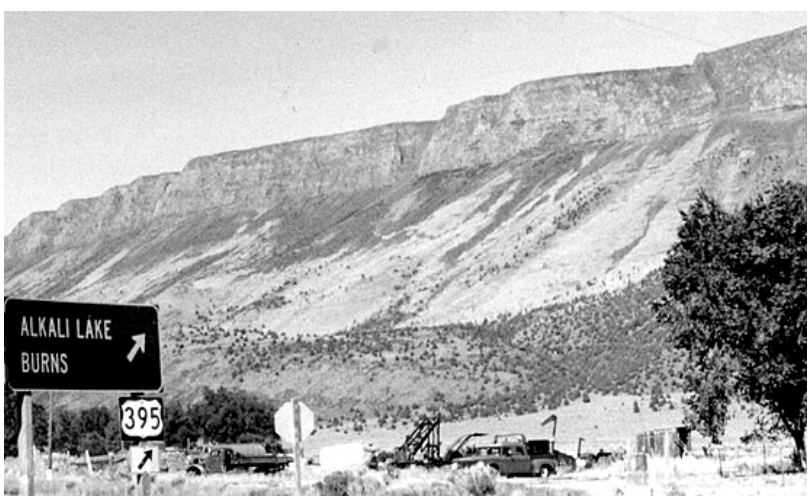


Figure 1: Photograph looking north-east toward the Abert Rim scarp. The site is relatively dry and cold, with relatively light vegetaion cover. The numerous springs associated with basaltic andesite rocks are targest Mars biome analogs.

remnant of the Pleistocence pluvial Lake Chewaucan (Phillips and Van Denburgh, 1971). The hydrology, geochemistry, history of the lake were documented by Phillips and Van Denburgh (1971), Van Denburgh (1975), and Dieke Jones (1980). The mineralogy of the volcanics, weathering products, and nature of diagenetic reactions in the lake sediments were reported by Banfield et al. (1991a,b). The area receives 12-14" rain per year, with large daily, monthly, and annual ranges in temperature (Allison 1982). The surface water runoff is limited (thus groundwater discharge features should be evident) and vegetation cover is relatively light. The geology of the region was described by Walker (1963) and

Jones and Weir (1983) and the geodynamic evolution is reviewed by Humphreys et al. (2000).

An important reason for selection of the Abert region for study is that the erosional features associated with the escarpment (Fig.2–panel B) resemble features interpreted as fluid-formed channels on Mars (Fig. 2–panel A). In addition we have identified several sites of possible seepage-driven erosion (possible analogs for the large, ancient seepage channels on Mars). Furthermore, the Abert Lake region, and surrounding areas, contains numerous active springs with a wide range of discharge temperature. In some cases, the spring waters smell of hydrogen sulfide and show clear evidence of mineral precipitation and biological activity. Recent hydrologic studies designed to explore the geothermal potential of the area have provided evidence for late Quaternary hot spring activity (e.g., Jellinek et al., 1996). Thus, the area has the potential to yield sites for studies of current geomicrobiological processes as well as places for analysis of biosignatures of extinct systems.

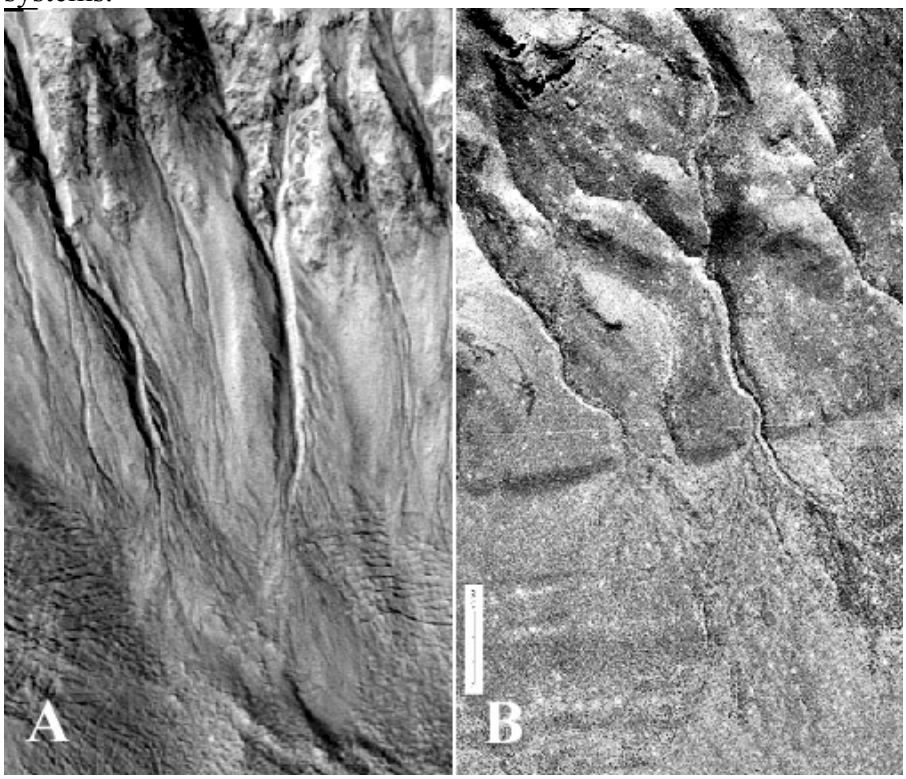


Figure 2

Aerial photos:

(A) Channels on Mars (see below) and (B) channels associated with the Abert Rim. Many potential analog sites were identified along the rim, and to the west of Abert Lake.

The second site for our work is an area adjacent to the Snake River, Idaho. The Snake River Plain extends for ~500km across southern Idaho. Compressional stress associated with mountain-building processes in the Mesozoic (>65 Ma) may have contributed to downwarping of the region. Subsequent crustal extension that created the Basin and Range province caused widespread normal faulting, so the Snake River Plain is also a large graben structure. During the last 15 Myr, numerous basalt flows have partially flooded the Snake River Plain (Malde and Powers, 1962; Armstrong et al., 1975). These basalts are, in general, more permeable than the underlying basement rocks, and so they provide conduits for water that drains into the Plain from the surrounding uplands or that falls as precipitation on the Plain itself. Some flows are more conductive than others, and where these units intersect the present-day canyon (>100 m deep) of the

Snake, springs are abundant (Meinzer, 1927). Estimates of the combined discharge of the springs in the Twin Falls and Bliss area are >150 cubic meters/sec (Stearns, 1936). The water temperature is ~16 ^{o}C, which indicates that the groundwater flow is discharging geothermal heat. Indeed, Brott et al. (1981) suggest that spring discharge removes most of the geothermal heat flow from the Snake River plain.

The most important feature of the SnakeRiver area is that discharge of large volumes of groundwater possibly caused formation of channels that resemble the sinuous channels on Mars (see cover photo: credit NASA/JPL/MSSS]. The Snake River was inundated by the Bonneville floods, and there are scabland channels along the perimeter of the canyon. The sites we have selected, however, were not created by the Bonneville releases, as they are located in a wide and deep section of the canyon where the floods did not significantly overtop the Snake Canyon walls (according to data in O'Connor, 1993;and confirmed via personal communication, 2003). The access, scale, bedrock composition and high outflow discharge rate of these features may them ideal for analog studies. Like the Abert area, the rocks are basaltic and the vegetation not extensive.

The third site for our work is a subsurface metal sulfide deposit that is undergoing active weathering to generate large volumes of acid solution. The volcanic rock-hosted deposit is pyrite (FeS_2)-dominated (>95%). At this time, we do not know that large pyrite accumulations occur, or occurred, on Mars. However, metal sulfide deposits may have developed in association with relict hydrothermal systems (and been subsequently removed by weathering). In fact, the red-orange Martian dust has compositional features not unlike neutralized acid rock drainage (high concentrations of ferric iron and sulfate; also see Figure 9, below), and there have been some suggestions that the soils are somewhat acidic.

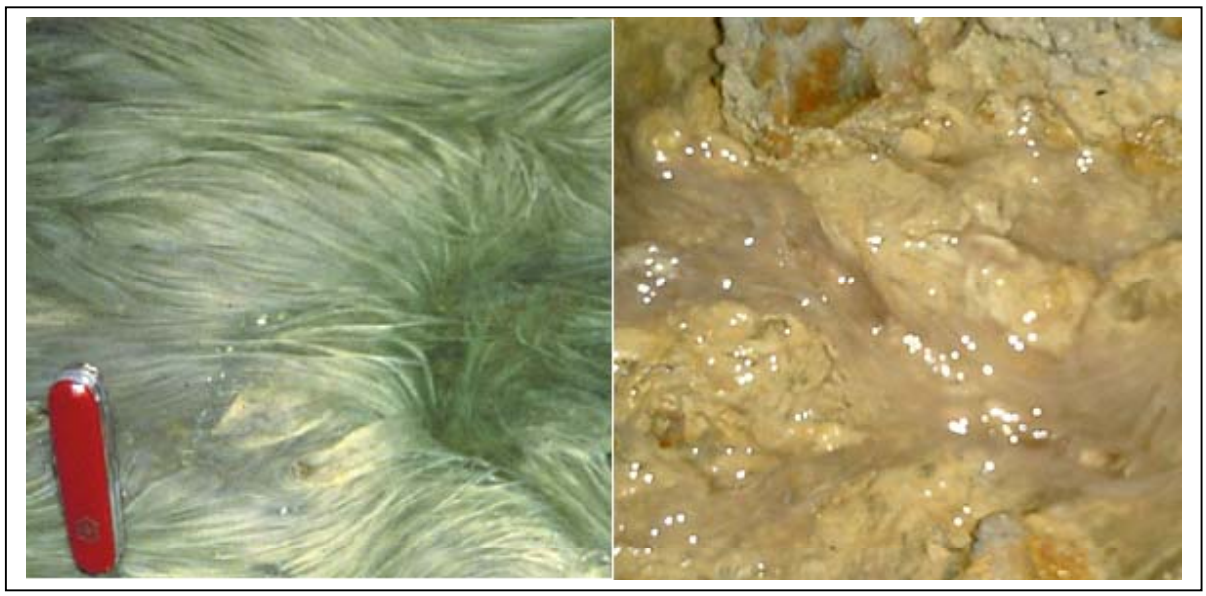


Figure 3:Biofilms in pH 0.7 solution in the Richmond Mine. At this site, our goals are to determine the limits for life in chemoautotrophic Fe-S systems, and what sets them.

Our third field site, located in the Richmond Mine at Iron Mt., CA, is of special interest because it hosts a very active chemoautotrophically-based subsurface biosphere (see Fig.

3) that is sustained by iron and sulfur oxidation. Temperatures within the deposit generally exceed 40 °C due to exothermic oxidative dissolution of pyrite. Pyrite dissolution process yields very acidic (pH 0.5–1), metal-rich (near molar concentrations of $FeSO_4$), toxic metal-rich (few to tens of milimolar Zn and Cu) solutions that pose a variety of challenges to the extremophile microbial communities that colonize the system.

THRUST 1: WHAT ARE THE OPTIMAL LOCATIONS FOR LIFE ON MARS?

An overriding goal of our work is to integrate information on geodynamics and heat flow, heat-driven crustal fluid flow, fluid-driven surface topographical evolution, fluid- and atmosphere-driven mineral weathering, and geological, biological, and radiation-driven atmospheric chemical process that dictate fluid chemistry and radiation exposure at the planetary surface so as to provide the best possible constraints for understanding the potential for biosphere development over the history of Mars. This work will be carried out by the "planetary" subgroup team (Manga, Dietrich, Bishop, Boering) and will be coordinated by Dietrich ("planetary group" lead investigator").

3.1 Mars hydrology: where was the water, why, how much, and when?

3.1.1 Mars hydrogeological investigations

Subsurface water dynamics play a key role in establishing and maintaining habitable environments. We thus propose to develop and extend a set of models in order to provide constraints on the location, discharge rate and temperature of groundwater that emerges at the surface, and hence evaluate the settings (in space and time) favorable for microbial life. Our focus will be on understanding the hydrogeologic environment in which seeps and small springs occur because they can be a source of near-surface moisture for extended periods of time. Seeps and springs are also features for which there are terrestrial analogs in which we can both study the microbial communities and test hydrologic models. A good understanding of sites with modest groundwater discharge, however, also requires an understanding of the properties and evolution of the entire hydrologic system on Mars.

The behavior of water in the subsurface cannot be studied in isolation from other regions and processes on Mars. The flow of water in the subsurface depends on the hydraulic head gradients driving flow, vertical and horizontal temperature gradients within the groundwater system, and hydraulic properties (e.g., permeability, porosity, compressibility) of the host rocks. Hydraulic head gradients will change in response to the geodynamic and exogenic processes that affect surface deformation. Temperature gradients change in time as the planet cools and vary in space due to magmatic activity. The permeability of groundwater systems depends in part on the chemistry of groundwater, which in turn depends on the composition and pressure of the atmosphere. Consequently, the geodynamic history of a planet, the style and rate volcanic and magmatic activity, and the evolution of the atmosphere and hydrologic cycle, will all influence dynamics of groundwater systems.

Subsurface hydrogeologic processes can be conveniently separated into local, regional, and global scales. At the local scale, there are three model problems of interest (see Figure 4).

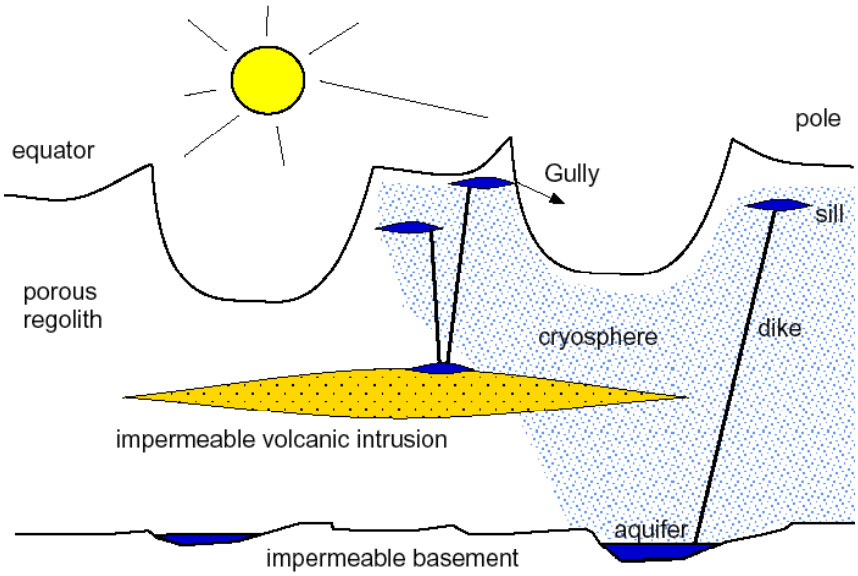


Figure 4: Illustration (redrafted from Gaidos, 2001) of "local" processes that influence the location and magnitude of groundwater discharge.

First, do intrusions provide a source of heat that may allow expulsion or drainage of groundwater (e.g., Squyres et al. 1987; Gulick, 1998; McKenzie and Nimmo, 1999; Mellon and Phillips, 2001; Harrison and Grimm, 2002) and potentially provide the heat and redox gradients required for microbial life? Second, does the formation of impact craters produce sufficiently large hydraulic gradients and heating of the subsurface to permit surface discharge (Tanaka et al., 1998)? It has been argued that individual impacts not only affect the groundwater system, but were large enough to inject water into the atmosphere, induce rain, and hence cause runoff and erosion (Segura et al., 2002). Third, as the Martian heat flow decreases and the cryosphere thickens, how does fluid pressure evolve and what are the implications for groundwater discharge (Gaidos, 2001)? In all three problems, we would like to predict groundwater temperature, flow rates, and the time scales over which the hydrologic system evolves. Dr. Manga and students have studied spring-systems in a variety of settings on Earth, including thermal springs along active faults, and basalt-hosted cold and thermal springs in the Oregon and California Cascades. These studies have focused on using measurements made at springs to characterize subsurface hydrological properties (Manga, 1996, 1999), quantify surface water-groundwater interactions (Manga, 1997), and study subsurface geologic processes (Manga, 1998; James et al., 1999, 2000; Saar and Manga, 2003). Models developed for these (and ongoing) studies of springs can be extended to Martian settings noting that a key difference is the presence of a cryosphere, which acts as a dynamic confining unit (Carr, 1979).

At the regional scale, the formation of features such as Tharsis may provide controls on the location and orientation of valley networks (e.g., Phillips et al., 2001) through changes in the crustal stress, flow geometry, and hydraulic gradients (e.g., Wilson and Head, 2002). It is at the global scale (see Figure 5) that we can best develop insight into the evolution of the hydrological cycle and water balance (Clifford and

Parker, 2001). Thus, both regional and global scale hydrogeologic systems provide the framework within which we must understand and model local hydrologic processes.

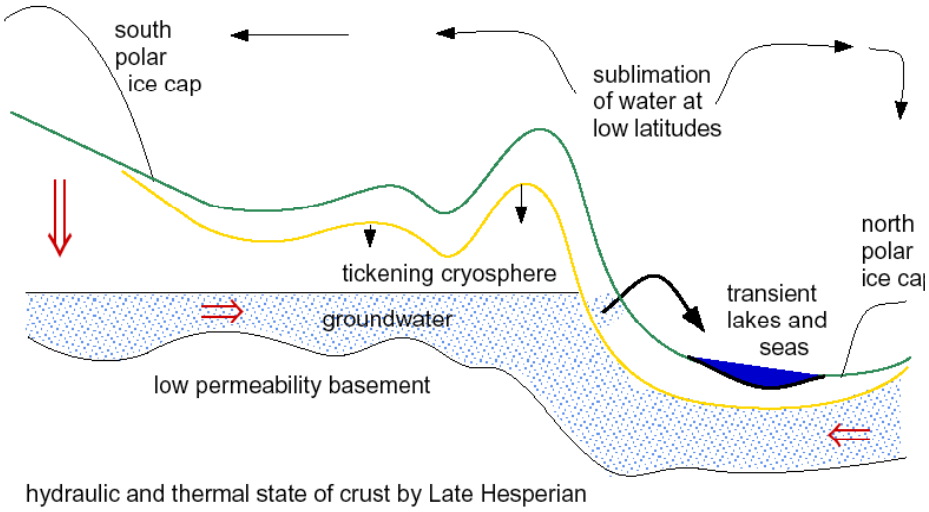


Figure 5: Illustration (redrafted from Clifford and Parker, 2001) of one model for the global hydrologic system during the Late Hesperian

The development of regional and global hydrogeologic models requires understanding the evolution of heat flow (which affects the thickness of the cryosphere) and stresses in the lithosphere. Our research group at Berkeley (Professors Mark Richards and Michael Manga, postdoc Mark Jellinek, and graduate students Helge Gonnermann, Mark Wenzel and Dave Stegman) is also involved in developing numerical and laboratory models of the global and regional (Tharsis) geodynamic evolution of Mars. The results of this work, coupled with those from previous studies, provide the needed constraints on heat flow (e.g., Schubert and Spohn, 1990; Reese et al., 1998; Zuber et al., 2000), stresses (Tanaka et al., 1991), and rate of volcanism (Hauck and Phillips, 2002). The latter is a source of water, CO_2 (which influences climate and chemical weathering), and other volatiles.

The models to be developed by the Manga group as part of the NAI collaboration will extend previous models used to study Martian hydrogeological processes (e.g., Carr, 1979; Forsyth and Blackwelder, 1998; Goldspiel and Squyres, 2000; Clifford and Parker, 2001; Gaidos, 2001; Anderson et al., 2002) in several respects.

- First, we propose to include poroelastic dynamics in order to describe fluid-solid coupling. For example, changes in hydrogeologic properties such as storage and permeability will arise from the volume change of water as it freezes and melts. That is, not only does fluid pressure change due to freezing (Goldspiel and Squyres, 2000), but as shown by Gaidos (2001) properties of the porous material itself will change. The large discharges represented in the outburst floods imply large changes in pore pressure, which in turn will influence subsurface properties, and should be important in understanding the development of chaotic terrain (e.g., Cabrol et al., 1997). The theory of *linear* poroelasticity is straightforward to include in hydrogeologic models (e.g., Wang, 2000). *Nonlinear* processes (such as consolidation and liquefaction) are still best characterized empirically; nevertheless, while they cannot be included as rigorously in models, their effects can be parameterized (e.g., Manga et al., 2003).

- We will model the evolution of porosity and permeability through weathering reactions following ideas and models developed for terrestrial analogs (e.g., Bolton et al., 1999; Fontaine et al., 2001; Lowell and Yao, 2002 for some recent studies that illustrate a range of approaches). Weathering reactions will be influenced by the partial pressure of CO_2 (e.g., Elwood and Madden, 2002), providing a connection between the evolution of the atmosphere and subsurface properties.
- We will develop models to relate the geodynamic evolution of Mars to its hydrogeologic properties following ideas developed by Rojstaczer (2002). In more detail, the amount of water and its rate of flow within the subsurface are controlled by the porosity and permeability of the subsurface. The standard procedure for Martian models is to use values similar to those on Earth, though scaled to account for the lower Martian gravity (e.g., Clifford and Parker, 2001). However, permeability and porosity on Earth are dynamic properties that reflect a balance between the rate at which the subsurface must cycle water, the hydraulic gradients caused by topography that drive this flow, the rate at which porosity and permeability are reduced by chemical and physical processes, and the regional stresses that can create new porosity and permeability. Typical upper-crustal terrestrial permeabilities of 10^{-14} m^2 (Ingebritsen and Manning, 1999) have been calculated by Rojstaczer (2002) through such a balance. The permeability in the lower crust of the Earth is constrained by the rate at which fluids are expelled by metamorphic reactions (Manning and Ingebritsen, 1999), which on Mars are limited by cooling rate and would be much lower than on Earth. Given the very different hydrologic cycle and geodynamic evolution (heat flow and stresses) of the Earth and Mars, there is no *a priori* reason to assume that Martian permeability and porosity distributions need resemble the Earth's.

As made clear in the review by Clifford and Parker (2001), care must be taken to account for the transfer of water between the atmosphere, the cryosphere, the subsurface (both saturated and unsaturated zone), and bodies of surface water.

Our proposed attempt to develop better models for the evolution of hydrogeological dynamics on Mars may appear poorly constrained. Nevertheless, there are several observations that provide bounds (in space and time) on acceptable models, and we anticipate that ongoing and future studies (including those described in this proposal) will add further constraints. Most important are estimates of the time (e.g., Burr et al., 2002), location and elevation (Carr, 2002), geologic setting (Gilmore and Phillips, 2002), and magnitude of groundwater discharge (Gullick, 2001) in groundwater seepage channels. In fact, because of the importance of understanding properties of surface discharge (rates, temperature, time scales) the surficial component of this proposal focuses on understanding the mechanics of channel formation in groundwater-dominated systems. The large outflow channels, however, also provide important constraints on groundwater systems (e.g., Carr, 1996). Additional constraints are provided by impact crater morphology (Carr et al., 1977; Stewart et al., 2001), evidence (or lack of) of hydrologic activity in craters (Cabrol et al., 2001; Russel and Head, 2002), density of the upper crust (Nimmo, 2002), the presence and age of hydrothermal alteration (Newsom et al., 2001;

Treiman et al., 2002), and the strength of faults which provides insight into fluid pressures (Barnett and Nimmo, 2002),

One of the better ways to constrain our models will be to apply them to more easily studied analog settings on Earth, including the proposed field sites in eastern Oregon and Idaho. In our past work, see Figure 6, we have shown that we can successfully model spring systems and obtain new insight intro subsurface hydrogeologic processes.

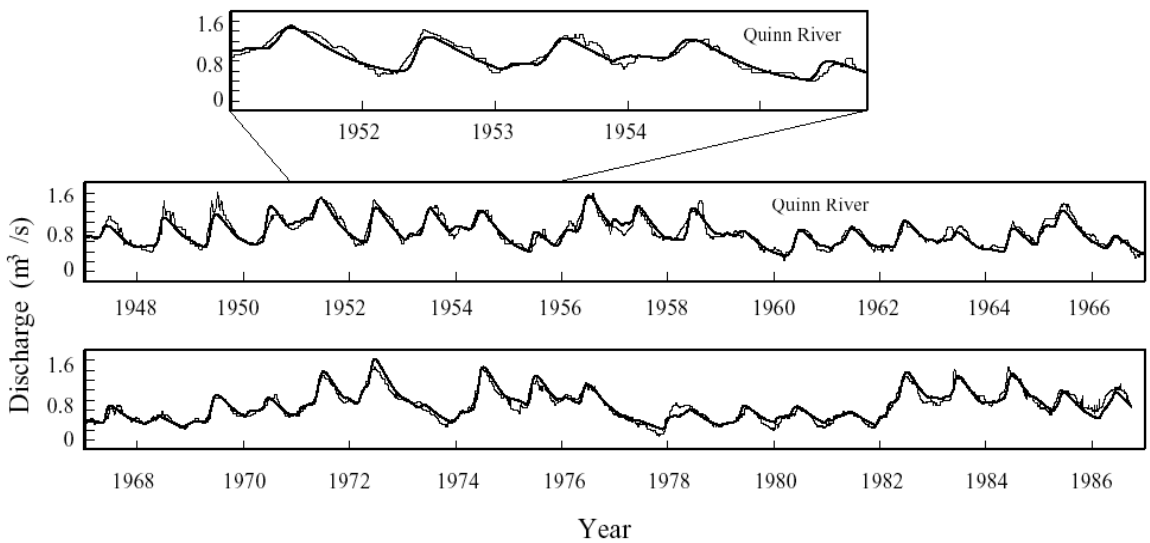


Figure 6: Predicted (bold curve) and measured (thin curve) discharge at Quinn River Spring in Oregon. The prediction is based on a calibrated groundwater flow model and allows us to determine subsurface hydrogeologic properties such as permeability, aquifer thickness, heat flow (from Manga, 2001).

Terrestrial studies are essential because they allow us to test some aspects of the models that we will apply to Mars, such as the evolution of porosity, permeability, flow rates, and water temperatures (e.g., the study springs in permafrost of Andersen et al., 2002). While we have a limited range of atmospheric conditions on Earth, we can find spring systems in areas with wide ranges of groundwater recharge rates, discharge temperatures, and ages of the host aquifers. Co-I Manga has worked extensively on the spring and groundwater systems in the Oregon Cascades and eastern Oregon where the host rocks are dominated by basaltic rocks, as they are on Mars. As a starting point for our work, we will investigate fluid flow associated with basaltic and basaltic andesite spring systems near Abert Lake, in south central Oregon and high discharge springs adjacent to the Snake River, Oregon. However, as we learn more about the hydrology and surfical processes at these sites, we may redirect our work to other locations. In Oregon, we have access to spring systems in which recharge rates vary from < 1 cm/year to > 1 m/year, discharge temperatures range from about 1°C to boiling. The spatial scale of the groundwater systems ranges from 100s of meters to 100 km. Another location in which models can be tested is Hawaii, where saline water (Martian groundwater may have a high salinity) interacts with basaltic rocks. As in Oregon, we can study groundwater discharge at springs that range from small to large, and cold to hot. Springs provide insight into the averaged or integrated behavior of groundwater systems. Our models will either predict or require knowledge of the spatial distribution of porosity,

permeability, temperature and flow rate; data made available from the Hawaii Scientific Drilling Project can be compared with models.

3.1.2 Mars surface processes: where are sites of sustained fluid flow on Mars?

One goal for our team is to evaluate the geomorphological characteristics of channels on the Martian surface to provide information about the history of fluid discharge.

The valley networks of Mars provide compelling evidence that at times in Mars' history surface water carved deep canyons and formed well-developed branching drainages (e.g. Cabrol and Grin, 2001). The origin of the surface water, the mechanisms of channel formation, and the estimates of magnitude, duration and frequency of surface water flow are debated, however (e.g. Craddock and Howard, in press; Carr and Malin, 2000; Grant and Parker, 2002, Segura et al., 2002). The small gullies recently made visible by the Mars Orbiter Camera (MOC) (Malin and Edgett, 2000a) have been interpreted as possible evidence of relatively recent water releases from the near surface, although alternative models have been proposed (e.g. Musselwhite et al., 2001). Layered deposits visible on crater rims suggest that standing bodies of water may have existed on Mars in ancient times (e.g., Malin and Edgett, 2000b; Grant and Parker, 2002). These and other observations constrain the atmospheric and hydrologic modeling proposed here as part of the effort to reconstruct the conditions under which life may have originated and persevered on Mars. One goal of the surface processes component of the proposed research is to extract more quantitative information about rates of discharge and time scales of flow in ancient and recent channel systems in order to provide more precise constraints on atmospheric and hydrologic models.

This proposal focuses on life in hydrothermal spring environments because springs provide a sustained flow of liquid water, heat, a supply of nutrients, and shielding from radiation. We therefore propose to focus on surficial features on Mars that appear to have been eroded by flows originating in the subsurface: valley networks, some of which are thought to have been formed by sustained or repeated surface flow from groundwater systems in the ancient past; and the recent gullies, which may have been formed by episodic releases of water from the shallow subsurface. Understanding the characteristics of the flows that formed these features will place important constraints on the possible origin and sustained presence of life on Mars.

Several debates have emerged regarding these two types of channel features. With regard to the valley networks, three primary issues can be identified (Carr and Malin, 2000): 1) the source of the water (groundwater outflow versus overland flow due to precipitation), 2) the mechanism of channel growth (headward advance of sapping fronts versus simultaneous incision along the entire channel), and 3) the relative duration and magnitude of the surface flow events (short-lived, episodic releases versus sustained surface runoff). The strong evidence of repeated release and reworking of the surface, at least in the Noachian (e.g. Grant and Parker, 2002; Craddock and Howard, in press), raises the challenging issue of how the groundwater system is recharged. With regard to the recent gullies, several formation mechanisms have been proposed, although most evidence suggests that water played a role (e.g. Stewart and Nimmo, 2002). The gulles are cut into scree slopes, which may themselves be an indication of widespread, water-

related activity. Scree slope inclinations on Mars are consistently lower than the angle of repose, prompting Perron et al. (submitted) to propose that freeze-thaw activity may be reshaping the slopes. Hence, near-surface hydrologic activity may be more sustained and more extensive than previously considered.

The Dietrich group, with input from the Manga group, will conduct a detailed study of each of the two types of channel features visible on the Martian surface. Each of these two foci will include quantification of geographic and geometric properties of the Martian features using Mars Global Surveyor (MGS) data, and experimental simulations and terrestrial field analog studies to identify quantitative, mechanistic relationships between channel form, flow characteristics, and substrate (bedrock) properties.

The most interesting valley networks on Mars from an astrobiological standpoint are the kilometer-wide, amphitheater-headed channels that have been interpreted as groundwater sapping features (e.g., Baker et al., 1990). These channels, such as Nirgal Valles (Fig. 7A) do not appear to be active at present, but may have been an ideal place for the development of life early in Martian history, as they appear to have been formed by sustained or repeated flow of water from a subsurface source over a long period of time. Headward advance of a "sapping" channel occurs when enhanced weathering and erosion at a seepage site leads to undermining and collapse of the headwall and sidewalls around the seepage site. Features of the Martian channels that suggest groundwater sapping include amphitheater-shaped headwalls, hanging tributary valleys, long main valleys with short tributaries, little change in cross-sectional dimensions with downstream distance, irregular tributary junction angles, and low drainage density (Laity and Malin, 1985; Howard, 1988; Higgins, 1984). In some cases these channels are discontinuous, suggesting subsurface collapse (Carr and Malin, 2000). There is debate, however, as to whether the channels were created by progressive headward advance of seepage fronts. Carr and Malin (2000) suggest that incision by overland flow released from a stationary spring head could have produced the observed morphology. These authors note that the most recent channels visible in the canyons are much smaller than the canyon dimensions, and that the canyons are sinuous in a way thought to be inconsistent with groundwater sapping processes.

Using MOC images and MOLA topographic data, we will quantify the detailed morphology and geographic distribution of these features in order to extract more information about the magnitude of flows responsible for their formation (see Grant and Parker, 2002, for an example). We will then conduct laboratory experiments and field studies of analogous terrestrial features to answer three key questions: (1) how much water is needed to cut the canyons on Mars over such great lengths (hundreds of kilometers); (2) was this incision vertical (by surface flows) or lateral (by groundwater sapping); and (3) over what time scale did the erosion occur? As suggested in earlier analyses by Howard (1988, and modeled by Goldspiel and Squyres, 2000), the volumes of groundwater flow can be estimated through an erosion theory. In both studies, however, it has been assumed that the eroding material is cohesionless. This simplifies the problem, but is probably inappropriate for Mars. Here we propose to develop a mechanistic understanding of erosion mechanisms and water flow needed to cause channel head advance by groundwater sapping of cohesive materials. We will conduct extensive laboratory experiments, building on the pioneering work of Howard and McLane (1988) and Kochel et al. (1985), to first document the differences in morphology

and erosion mechanics of seepage erosion versus vertical channel incision from a spring source. Then we will build a sufficiently large-scale tank to enable us to measure groundwater head gradients, flow rates and erosion of a seepage head formed in weakly cohesive material. We have found through extensive experiments on bedrock channel incision that we can use a measure of tensile strength to scale rock resistance appropriately (Sklar and Dietrich, 2001).

The Box Canyon, Idaho study area (Fig. 7B) is an ideal analog site to study groundwater sapping. The channel is morphologically similar to those on Mars in many respects, including its sinuous channel path. In the vicinity are numerous other actively forming sapping channels with springs at their heads (Stearns, 1936). We will obtain detailed topographic data (using LIDAR) and combine this with measured outflow to model the groundwater flow field to the seepage heads. Since steady erosion of bedrock walls cannot be observed over short time scales, we will document the long-term rate of seepage head advance using cosmogenic radionuclide exposure-age dating of exposed surfaces and quantify the pattern of fracture development and block failure at the seepage face. The wide range of channel head advances in various canyons in the area will then provide multiple solutions to constrain the development of a groundwater-driven erosion theory. These channels are located along a reach of the Snake River Canyon where the Bonneville floods did not significantly overtop the canyon walls, but backwater sediments should have been deposited in them and we will look for such deposits to add in dating rates of erosion. Our study of groundwater sapping channels will seek quantitative, mechanistic relationships between channel form; flow characteristics such as discharge, head gradient, and longevity; and substrate properties such as erodibility and conductivity.

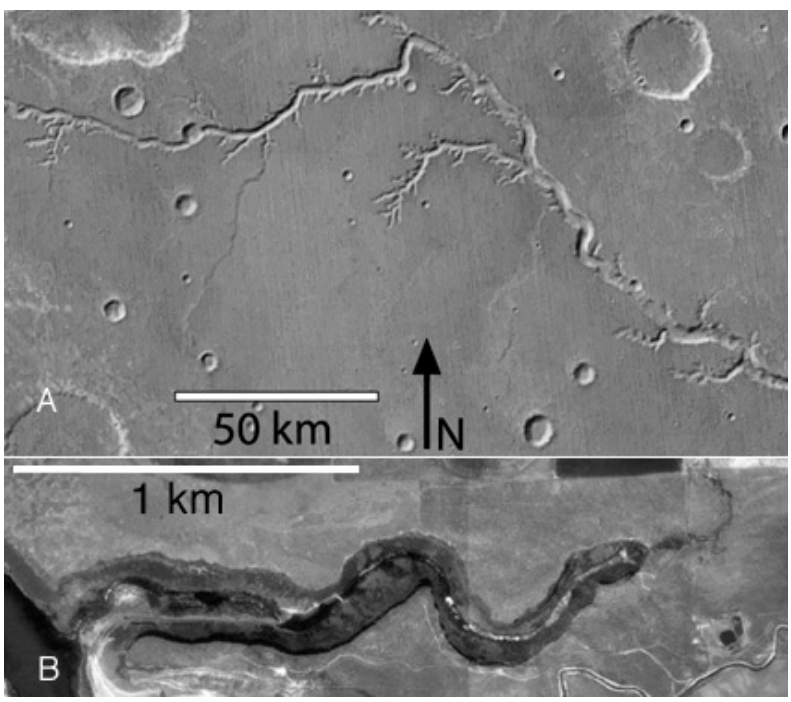


Figure 7

A: Channels of Nirgal Valles (Mars) that are interpreted to have formed by sustained groundwater discharge

B: Channel of Box Canyon, Snake River, Idaho

Our study of sapping channels (which may have fostered the development of life deep in the Martian past) will be complemented by an ongoing study of channel features

that formed in the recent past (10s of Myr) and may still be active. Kilometer-long channels formed on debris slopes, dubbed "gullies" by Malin and Edgett (2000a), are evidence of recent geomorphic activity and are the most probable location for the sustained presence of life on Mars. They appear to have been formed by short-lived mass flows containing a pore fluid, most likely water (Stewart and Nimmo, 2002). These flows appear to have originated at depths of ~100m, which suggests that there may be near-surface (but shielded) reservoirs of liquid water fed by groundwater and/or atmospheric deposition. Their origin at distinct rock layers or alcoves (Gilmore and Phillips, 2002) also implies groundwater release. The observation that some gullies have experienced multiple flow events (Malin and Edgett, 2001) suggests that there may be recharge of the fluid reservoirs, a requirement for the sustained presence of life.

The goal of our ongoing study of gully formation is to develop a mechanistic theory for channel incision by mass flows on scree slopes. We have already constructed a database of MOC and MOLA data for a 30º x 30º region on Mars that will allow us to measure the topographic characteristics and channel geometries of gullies. We have also begun preliminary experimental work to link model channel geometry to mass flow properties, including fluid content. By combining this understanding with estimates of the solid volume evacuated by a gully, we intend to infer the volume of fluid released from a near-surface reservoir. We propose to extend our analysis of MOC images and MOLA data to include a global survey of gullies. Using the procedure described above, we would estimate the time-averaged discharge of groundwater wherever gullies are present. This would provide important constraints on models of current hydrologic conditions in the Martian subsurface.

We also propose to conduct fieldwork at the BioMARS analog site at Abert Lake, Oregon, to test the relationships that come out of our laboratory experiments. In the Abert Lake site there are small channel networks that appear similar in form and scale to the recent Mars gullies. We will conduct field studies to determine the origin of these channels, distinguishing between mass flow channel incision and fluvial incision (see Stock and Dietrich, in press), and quantifying the amount of water needed to cause the erosion.

3.1.3a Atmospheric processes: the form and effectiveness of a UV shield

Atmospheric chemistry has profound implications for the climate and habitability of Mars throughout its history. The presence and stability of greenhouse gases and aerosols, for example, will regulate climate or force climate change. Photochemical processes in the atmosphere may also produce UV radiation shields (e.g., ozone on Earth), which would influence both radiative transfer (and therefore climate) and the stability of organic compounds in the lower atmosphere and at the surface. Thus, analysis of the Martian atmosphere is vital to understanding of the opportunities and challenges for early life on Mars, as well as the importance of habitat features that provide radiation protection.

Critical links between atmospheric chemistry, climate, habitability, and indicators of habitation in terrestrial-like atmospheres are currently being studied using models of early Earth (e.g., prebiotic and prephotosynthetic), current Earth, and other planetary atmospheres [e.g., Zahnle, 1986; Kasting, 1992; Pavlov et al., 2000]. A large number of parameters needed for these models, however, are untested experimentally. The co-I for

this proposal with expertise in atmospheric science, Dr. Boering, will investigate factors influencing atmospheric composition and chemistry relevant to Martian atmospheres at various times in its history through laboratory experiments. The experiments will provide new insights into the interdependence of photochemical lifetimes of greenhouse gases, the potential for the formation of photochemical hazes and whether these hazes may have warmed or cooled the atmosphere and surface and could have served as a UV shield.

3.1.2b Photochemical formation rates and optical properties of haze particles in simulated Martian atmospheres

For the early Earth, and, by analogy, early Mars, photochemical models suggest that photolysis of CH_4, perhaps produced biotically by methanogens, in an atmosphere of CO_2, N_2, and water vapor could produce a photochemical haze [Pavlov et al., 2000, 2001a]. Depending on the particles' chemical composition and size distribution, the haze could act to significantly warm the atmosphere and surface [Sagan and Chyba, 1997] or cool it [McKay et al., 1991; Pavlov et al., 2001a]. It could also provide an early UV-shield, which would increase the stability of organic molecules at the surface and the photochemical lifetimes of other UV-labile greenhouse gases, such as NH_3 [Sagan and Chyba, 1997]. Current photochemical models are sensitive to the atmospheric CH_4/CO_2 ratio and predict that CH_4 begins to polymerize when this ratio exceeds unity [Pavlov et al., 2000; Zahnle 1986]. These models, however, include a number of approximations, some of them quite arbitrary, for the rates of particle formation, and no laboratory data exist to test their predictions.

While experimental work has been done in the laboratory using shock discharges to simulate the chemistry of hydrocarbon hazes thought to occur on Titan's N_2 atmosphere [e.g., Ehrenfreund, et al, 1995; Coll et al., 1999], no experiments have been done investigating the photochemical polymerization of CH_4 in an atmosphere containing CO_2, N_2, H_2O, the likely composition of the early Martian atmosphere. We propose to measure particle formation rates and optical properties in model gas mixtures exposed to UV radiation using a new technique (Adamkovics and Boering, submitted) developed and already operational in our laboratory (Figure 8).

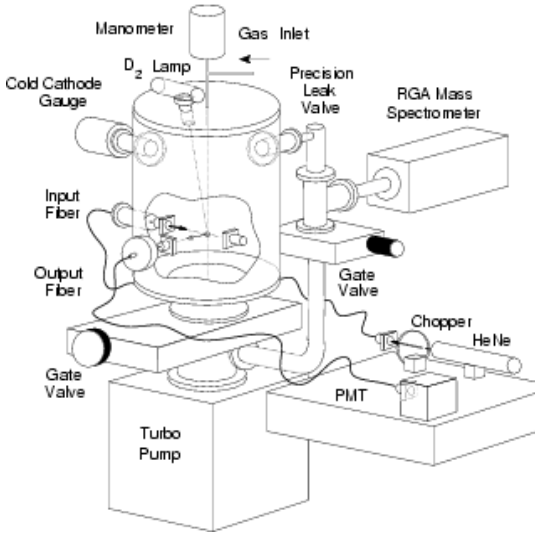


Figure 8: Experimental setup for measuring photochemical formation rates and optical properties of organic aerosols by UV irradiation of gaseous precursors simulating various possible compositions of Martian atmospheres. Complex gas-phase species formed are measured with an online mass spectrometer while aerosol formation and optical properties are measured by light scattering as a function of detection angle. Isotopic analyses of gas-phase species and particulates can also be made on collected samples offline, as can scanning electron microscopy analyses for the morphology and size distribution of the particulates.

Gas phase species will be measured in real time with an online mass spectrometer, while particle formation will be simultaneously monitored by optical scattering. By measuring the angular dependence of particle scattering, the particle size distribution and complex index of refraction will be determined "*in situ*" (i.e., while still suspended in the gas phase as opposed to measurement after deposition to a thin film). The *in situ* optical properties will be compared with collection of the particulates followed by offline field emission scanning electron microscopy (FESEM) to examine particle size and morphology. We will complement these *in situ* and SEM studies with offline chemical and isotopic analyses of the particulate material by collection of the aerosol and subsequent analysis using Gas Chromatograph-Mass Spectrometry (GC/MS) and Gas Chromatograph-Isotope Ratio Mass Spectrometry (GC/IRMS).

This new suite of laboratory measurements will significantly enhance our understanding of the mechanism(s) and kinetics of haze formation in a Martian-like atmosphere and will guide a more accurate representation of these processes in models, which, in turn, will allow more reliable predictions of Martian climate and the UV flux to the Martian surface to be made.

3.1.4 Mineralogy and physical properties of solids in Martian biomes

The analysis of the nature of fluid flow associated with channels in the Martian surface relies upon understanding of the properties of the materials through which the fluids are flowing. Similarly, geomicrobiological analyses depend upon an understanding of the mineral chemistry and reactivity, as these factors can control the options for metabolic energy generation and the physical characteristics of habitats.

Knowledge of the nature of Mars surface materials relies upon compositional data collected from a few sites on Mars and spectral information obtained by orbiting instrumentation. Interpretation of the minerals present depends upon the availability of appropriate reference spectra. Thus, spectra from Mars analog sites will be important in the iterative process of determining the nature of potential habitats associated with Mars surface channels.

The co-I with expertise in remote characterization of the mineralogy of planetary surface materials, Dr. Bishop, will provide constraints on the mineralogy and physical properties of materials in channels on the Martian surface. The spectral measurements will be performed using a FieldSpec Pro FR purchased for this project from Analytical Spectral Devices (ASD) that covers the range 0.35 to 2.5 μm and has a spectral resolution of 2-3 nm.

During year one spectral measurements will be made using the ASD visible/NIR instrument at SETI/NASA-ARC of mineral standards in the lab with a solar simulated NIST traceable irradiance source, and outdoors using this lamp and solar radiation in separate tests. Using the lamp and a fixed sample/standard distance will produce spectra that are readily reproducible, while using direct solar radiation outdoors will introduce variation in the source that will lead to atmospheric lines and increased noise. Measuring samples outdoors with the lamp can help constrain these differences. As some field measurements at variable distances will be desired, it will be necessary to gain an understanding of how the field conditions affect/mask the spectral properties of the

rocks/minerals. These tests will ensure that the users are familiar and experienced with the instrument and the spectral character of the minerals of interest in order to maximize field time and facilitate selection of rocks to be collected for the study.

Spectra of minerals, soils and rocks will need to be measured in a dehydrated environment in order to obtain spectra that can be readily compared with spectra on the surface of Mars. A number of clay minerals, iron oxides/oxyhydroxides, some carbonates, some sulfates, and several Mars analog soils have already been measured under dehydrated conditions (e.g. Bishop and Pieters, 1995; Bishop and Murad, 1996, 2002; Bishop et al., 1995, 1996, 1998a,b, 1999, 2001, 2002a,b,c,d,e) and are available in Co-I Bishop's library for this project. Co-I Bishop and her student/lab technician will convert these data from the lab spectral resolution to the spectral resolution of spectrometers measuring data on the surface and in orbit around Mars. During years one to two Co-I Bishop will work with PI Banfield and the team to decide which mineral/soil spectra in Co-I Bishop's library are relevant to the project. Mineral standards will include a variety of clay minerals, opal and silica polymorphs, carbonates, sulfates, and iron oxides/oxyhydroxides.

During years two and four Co-I Bishop and her student/lab technician will visit field sites with the co-Is in order to take spectral measurements of rocks and terrain, help document the field sites, and collect samples for detailed lab studies. The spectrometer will be taken to the Aber Lake and the Snake River sites. The Snake Rivers site is of particular interest because we expect big outcrops of alteration minerals (iron oxides, clays, sulfates, carbonates, etc) due to the high groundwater discharge. For the acid Richmond Mine, rocks will be brought back to the lab for spectroscopic characterization.

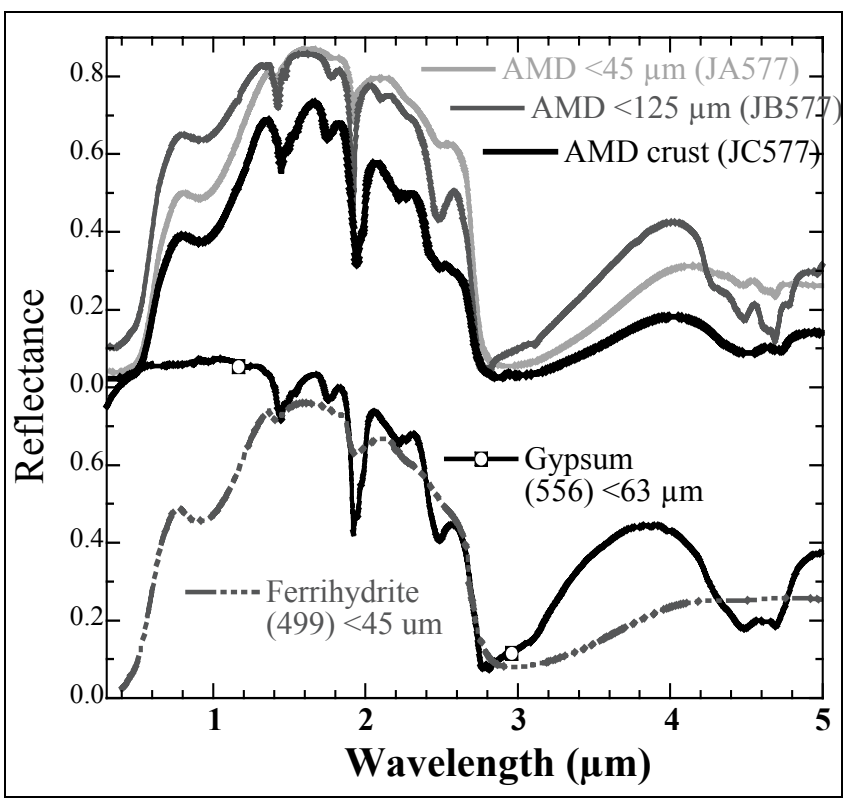


Figure 9: Visible/NIR reflectance spectra of neutralized acid mine drainage (AMD) material from iron mountain measured under dehydrated conditions. Similar reflectance spectra are shown of two main components of this sample, ferrihydrite and gypsum, for comparison. These spectra cover the spectral range of Omega (Mars Express) and represent a dry Mars-like sample state.

During years two to five portions of the rocks collected in the field will be crushed and dry sieved into size separates for detailed analysis. Because spectral properties of minerals change with the grain size, it is important to measure both coarse and fine size fractions. The size fractions will include coarse material containing rock chips and fragments 125-250 µm in size, material less than 125 µm, and material less than 45 µm. If the rocks contain altered rinds or are otherwise only partially weathered, then the alteration products are normally most abundant in the <45 µm fraction. The mineralogy of these samples will be determined by X-ray diffraction and transmission electron microscopy. The Bishop group will identify minerals at the field sites for which spectra are not currently available, obtain reference minerals, and their spectra. In all samples, mineral particle size will be determined confirmed scanning electron microscopy and, in the case of ultra-small particles, by peak broadening analysis of X-ray diffraction spectra. Compositional data will be obtained via X-ray fluorescence and mass spectroscopic measurements. Reflectance spectra of the size separates of these samples will be measured at the NASA-supported RELAB facility at Brown University.

Bishop and her group will read these data into ENVI and process them for several sets of Mars spectral data. Emphasis will be placed on convolving the data to the Omega spectrometer (0.4 - 5.0 µm wavelength range, 2003 Mars Express mission) and CRISM spectrometer (0.4 - 4.0 µm wavelength range, 2005 Mars Reconnaissance Orbiter mission) because these instruments will be measuring data where a number of aqueous minerals important to our project can be detected (e.g. through bands due to OH, water, carbonate and sulfate). As shown in Figure 9 spectra of the AMD material contains several bands due to gypsum and ferrihydrite that could be detected in the Omega spectra of Mars. CRISM will be measuring data both in a low spectral resolution global survey mode as well as a high spectral resolution mode for selected spots on the surface. Co-I Bishop is a CRISM team member and will be working with both the Omega and Crism datasets; she will therefore be informed about changes in the specific spectral channels measured by both instruments. Spectral analyses will be performed in order to determine limitations on abundance for the minerals of interest to our project for the various instruments and measurement modes. During years three to five Co-I Bishop and her group will perform similar work on the spectral data of samples measured in this project.

3.2 THRUST 2: LIFE ON MARS: HABITATS AND BIOSIGNATURES

Introduction

The overriding questions to be addressed in the geomicrobiological studies relate to the nature of microbial populations that can colonize relatively hostile, rock-dominated habitats somewhat analogous to those expected on Mars, the factors that limit colonization, and the form of the record of the existence of these communities. Work in this area will be the focus of the "geomicro" subgroup (Emerson, Roden, Luther, Banfield) and will be coordinated by Banfield ("geomicro team" lead investigator).

Figure 10 illustrates the general approach we take to field and experimental geomicrobiolgoical studies. Because of the extensive information already available on microbial populations and biogeochemical processes occurring at the Richmond Mine (Edwards et al. (1999; Bond et al., 2000b; Bond et al., 2000a; Edwards et al., 2000; Baker

et al., 2003, Druschel, Baker et al. in review; Baker et al., submitted), the focus of work in this acid habitat will be on microbial adaption and the processes and factors that limit microbial colonization. In contrast, work at the Abert and Snake River sites will focus on poorly understood connections between silicate mineral weathering, iron cycling, microbial community structure, and biosignature formation.

Solids are likely to yield the best durable biosignatures in the Martian samples. Solid products of metabolism are most likely to be preserved in the circum-netural pH, basalti rock-dominated systems. Particular attention will be paid to relationships between mineralization, cell distribution, and cell preservation in the solid materials will be characterized. Emphasis will be placed on the structure, chemistry, and morphology of solids formed by inorganic and biological processes.

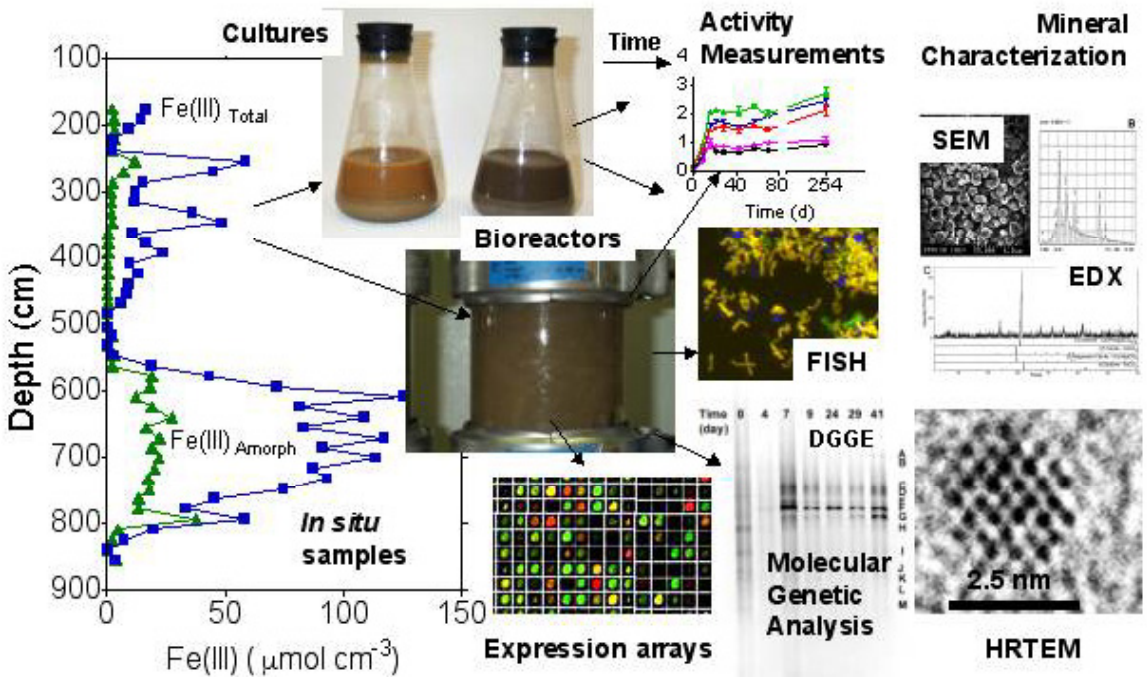


Figure 10: Schematic diagram illustrating our approach: Methods include scanning (SEM) and transmission electron microscopy (HTEEM), energy-dispersive (compositional) analysis (EDX) and fluorescence in situ hybridization (FISH).

Recent considerations of potential sources of energy for subsurface life on Mars have focused on the availability of both energy (H_2, CO) and oxidant (O_2) in the Martian atmosphere (Weiss et al., 2000). However, the existence of lithotrophic microbial communities on Earth that are driven by energy from geologic processes (e.g. H_2 generation coupled to tectonic activity or mineral weathering; Stevens and McKinley, 1995; Chapelle et al., 2002) suggest the possibility that life on Mars could be supported by subsurface as well as atmospheric sources of energy. Reduced and oxidized Fe mineral phases represent prime candidates for redox-active compounds capable of supporting subsurface life on Mars and other extraterrestrial environments (Jakosky and Shock, 1998; Santelli et al. 2001, Irwin and Schulze-Makuch, 2001; Schulze-Makuch, 2002).

When solutions flow through rocks, minerals dissolve. In basaltic rocks, weathering of abundant ferromagnesian silicates (e.g., olivine, pyroxene, amphibole) and sulfides (e.g., pyrite) begins with oxidation of ferrous iron bearing minerals. These reactions consume oxidants (e.g., nitrate or dissolved oxygen. Subsequent interaction between these oxidant-depleted fluids and minerals yields ferrous iron rich solutions that can migrate through the subsurface. Even in weathered basaltic rocks from Abert Rim that are exposed to Earth's atmosphere, evidence of significant iron mobility (and subsequent redeposition) has been documented (Banfield et al. 1991). Redox gradients form in subsurface regions where reduced, iron-bearing fluids mix with more oxidized fluids, or where the fluids have increased access to the atmosphere.

Ezymatic oxidation of iron by microorganisms at near neutral pH is often precluded by extremely rapid inorganic oxidation kinetics. However, reaction kinetics are slow in redox gradients where the oxygen concentration is low, and in very acid solutions (see below for discussion of acidic solutions). The two target environments for microbial iron oxidation in near-neutral pH systems are (i) in proximity to surfaces of Fe-rich minerals that are undergoing the early stages of dissolution in groundwater solutions and (ii) in gradient zones where reduced and oxidized fluids mix and are cycled.

The existence of "iron bacteria" able to oxidize ferrous iron in circum-neutral pH solutions has been known for over a century. However, comparatively little effort has been directed towards their investigation. In the last few years, however, there have been a number of important new discoveries in this area. For example, although the ability of iron oxidizing bacteria to conserve energy from iron oxidation remained uncertain for many decades, it is now well established that at least a subset of species (including members of the well known *Gallionella* genus) are able to fix CO_2 using energy derived from iron oxidation (i.e., they are autotrophs, thus can underpin a subsurface biosphere). Additionally, a greater diversity and more widespread distribution of iron oxidizing organisms in near-surface environments has been demonstrated, both microaerobically (Emerson and Moyer, 1997; Emerson, 2000; Sobolev and Roden, 2001) and anaerobically through coupling of Fe-oxidation to nitrate-reduction (Straub, et al 2001; Hauck, et al 2001). To date, only a handful of key papers have appeared because the number of researchers working on iron-oxidizing microorganisms has been small and cultivation and characterization of these organisms is difficult. Profs. Emerson and Roden, leaders in this effort, bring key expertise in this area to the NAI team. Given its potential importance on Mars (and on Earth), the biological and geochemical importance of chemoautotrophic iron-oxidizing prokaryotes in basalt-dominated systems is an important focus of this proposal. This metabolic group may be especially relevant to early life on Mars if the ability to utilize ferrous iron as an electron donor was an early evolutionary development, as some analyses suggest.

Simultaneous with the recognition of the wider range of habitats for iron-oxidizing neutrophiles (microorganisms living in near-neutral pH solutions) has been acceptance of the importance of deep subsurface microbial communities. Much of the analysis on deep subsurface communities has focused on hydrogen and methane-based metabolisms (e.g., Stevens and McKinley, 1995, Chapelle et al., 2002). We will not attempt to duplicate this effort. Rather, we will focus on nearer-surface systems where iron oxidation is coupled to oxygen or nitrate reduction.

Microoganisms may be able to utilize ferrous iron released by dissolution of iron-bearing minerals. The redox potential of ferrous iron phases (including structural ferrous iron in primary or secondary minerals) can be substantially lower than that for dissolved ferrous iron. Thus, ferrous iron bearing minerals may provide a unique basis for energy metabolism. Springs with visible, orange, iron oxide accumulations are not uncommon. These may be the excellent analogs for Martian biomes, such as those that may have been associated with the Sinus Meridiani deposit. We speculate that a very significant range of iron oxidizing organisms will be discovered in appropriate rock-dominated microaerophilic environments (e.g., at the interface between solutions and ferrous iron-rich minerals).

Ferric iron mineral products represent a potential electron acceptor for anaerobic metabolism of organic carbon or hydrogen. Together, iron-oxidizing and iron-reducing life forms may regulate a coupled iron and carbon cycle.

We propose a detailed investigation of the biogeochemistry and microbial community structure of subsurface and near-surface environments at the Abert Lake and Snake River study sites, which preliminary analyses suggest may provide plausible analogs to potential Fe-based life systems in shallow subsurface environments on Mars (for example, where water generated by geothermal heating of subsurface ice deposits; Carr, 1996) may permit development of microbial communities based on Fe(III) oxide reduction, Fe(II) oxidation, or coupled Fe reduction and oxidation (Figure 11). The motive for these studies stems from our assertion that careful documentation of the biogeochemical characteristics and microbiological composition of terrestrial Fe-based life systems is an appropriate first step toward development of the knowledge base and analytical techniques that will eventually be required to detect signatures of existing or past life on Mars and other extraterrestrial bodies.

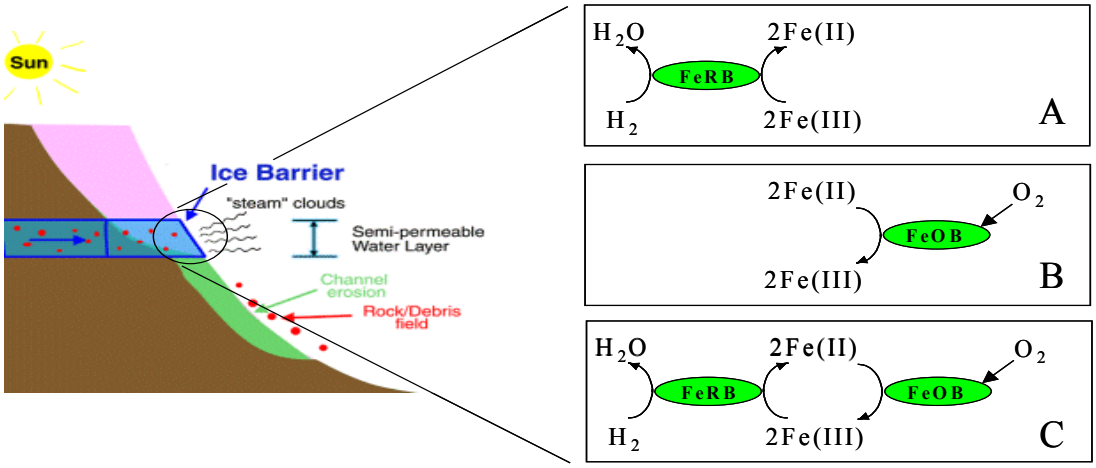


Figure 11. Potential setting for subsurface Fe-based microbial life on Mars driven by (A) Fe(III) oxide reduction (geothermal H_2 source), (B) Fe(II) (e.g. andesite) oxidation, or (C) coupled Fe(III) reduction and Fe(II) oxidation. FeRB = Fe(III)-reducing bacteria; FeOB = Fe(II)-oxidizing bacteria.

We hypothesize that basaltic rocks will host significant populations of organisms that are sustained by Fe cycling, especially by iron oxidation. We will characterize the structure and activity of microbial populations to test this idea. Mineralogical

biosignatures at the field sites will proceed along three interrelated lines (1) detailed studies of in situ geochemical distributions correlated with information about microbial community structure; (2) process-level studies of mineral transformations coupled to Fe metabolism by pure or mixed cultures retrieved from field samples; and (3) controlled studies of microbial Fe redox metabolism in bioreactors seeded with organisms from the field site.

3.2.1 Microbial populations in chemoautotrophically-based ecosystems at field sites

In order to determine the size of microbial populations sustained by iron cycling in near-surface environments, soils, weathered rocks, solutions, and associated microorganisms will be collected from sites of groundwater discharge at the Abert Lake and Snake River spring sites. Similarly, organisms to be used in the study of the factors that limit growth of microbial populations sustained by metal sulfide mineral dissolution will be collected from the Richmond Mine site. Robotic sample collection from otherwise inaccessible regions of the Richmond Mine is described in Section 3.3.2

The environmental chemistry will be determined at the time of sample collection of samples from all three sites. This work will benefit from real time microanalysis (hundreds to sub 100 μm-scale) of O_2, pH, Eh, T, ferrous iron and sulfur compound concentrations. Chemical analyses will include determination of oxygen and pH using standard electrochemical microsensors, soluble Fe(II) and Fe(III) gradients using voltammetric microsensors, at microscale (≤ 100 μm) resolution. Novel aspects of the in situ geochemical measurements are discussed in section 3.3.1. At the Abert and Snake River sites, analyses will utilize well-established 'peeper' technology, where multiple membrane-enclosed diffusion chambers separate microbes and pore water and allow measurement of important pore water chemistry in the absence of microbes. Solid phases will be identified by direct electron microscopic and x-ray diffraction characterization. In some cases, refrigerated microtome frozen subcores will be microtome sectioned. Where appropriate, selective chemical extraction-based analysis of the solid-phase materials will be conducted.

How large a subsurface microbial population might be sustained by dissolution of ferrous iron-rich silicates comprising basalts and basaltic andesites on Earth, thus possibly associated with weathering of basaltic rocks near the Martian subsurface? What is the size of populations sustained by coupled iron oxidation and reduction or other processes occurring in the basaltic rocks? What sorts of microorganisms occur, and what factors control their distribution?

In order to answer these questions, microbial populations at the Abert and Snake River sites will be evaluated via a combination of culture-based methods (Emerson and Roden). Firstly, the microbial populations sizes will be determined by direct total cell counts and quantification of extracted lipids, as well as by Most Probable Number (MPN) assays for aerobic and anaerobic Fe-oxidizers and for Fe-reducers, S-reducers and heterotrophs. In each case the base medium will be based on the chemistry of the field site to the best of our ability to determine what that is. Attempts for both enrichments and MPNs will also be done using solid mineral phases (Emerson laboratory). In all cases, a subset of the highest dilution MPN tubes that show growth will be used for continued

enrichment and isolation of the physiologically most relevant microbes (see Section 3.2.2).

The microbial populations in basaltic rock-dominated habitats will be documented via molecular phylogenetic analysis (primarily SSU rRNA gene sequencing work by Emerson, Roden, and Banfield laboratories). Using the sequence data, we will design fluorescence in situ hybridization (FISH) probes to quantify population abundances at the species and higher levels. This work will follow methods reported by Edwards et al. (2000) and Bond et al. (2000), and Bond and Banfield (2001). Real time PCR (qPCR) quantitation of specific populations of the Fe-oxidizing microbial community will be carried out in the Emerson laboratory using Taq-man primer-probe combinations. The probes may be the same used as for FISH analysis of neutrophilic iron-oxidizing bacteria (FeOB). In addition, we will also use terminal restriction fragment length polymorphism (tRFLP) as a tool to assees overall community diversity at the field sites. These latter techniques will done using established procedures for qPCR (Suzuki, et al 2000) and tRFLP (Liu, et al, 1997) Data from different sites and conditions will be compared in order to understand the factors limiting microbial populations and how population structural changes relate to geochemical changes.

Biomineral products in basalt and basaltic andesite habitats will be characterized in detail using optical and electron microscopic methods, X-ray diffraction, electron microprobe-based compositional analyses, and isotopic analyses (isotopic work is described in section 3.2.4).

3.2.2 Physiology, and possible role of biomineralization in energy generation

To complement the field-based studies of bacterial communities and associated biosignatures, the physiology of isolates will be studied in the laboratory. Particular attention will be given to identification of neutrophilic Fe-oxidizers and establishing the ecological roles (e.g., in carbon and nitrogen fixation) of different phylotypes. Culture-based studies to identify iron-oxidizing organisms will be critical if we are to determine their abundance and impacts on their geochemical environments.

Neutrophilic Fe-oxidizers from the field sites will be cultivated using bioreactors and other growth methods (see above) useful for carrying out basic physiology and ecological studies. At present our research group has 9 strains of lithotrophic, neutrophilic FeOB in pure culture isolated from a wide range of environments that can be comparative studies (see Table 2 in the Results of Previous Support section, below). In addition, we will isolate new FeOB and iron-reducing bacteria (FeRB) from the field sites and from the bioreactors. The isolates will be extensively characterized, including physiology studies aimed at determining optimal growth conditions and substrates. The forms of C- and N-metabolism will also be determined using standard isotope methods, ^{14}C-uptake and $^{12}C/^{13}C$ ratios for C and ^{15}N-uptake for N. The phylogeny of the isolates will be determined uisng 16S rDNA analyses, and their genotypes will be evaluated used –rep-PCR (De Bruijn 1993, Versalovic et al. 1998), and their phenotypes using MALDI-TOF-MS. These latter results will be compared with existing data for other species from other sites. This work will be conducted primarily in the Emerson laboratory. The cultivation of iron-cycling microorganisms from relevant environments is essential for experiments to investigate the impact of active cells on mineral surface evolution and for

experiments designed to measure isotope fractionations. In essence, these pure culture studies will 'ground-truth' our in-situ studies.

Pure cultures will be analyzed with microelectrodes to determine, in detail, the physico-chemical parameters for growth. These cultures will be the used in bioreactor studies designed to explore microbial communities and biosignature development.

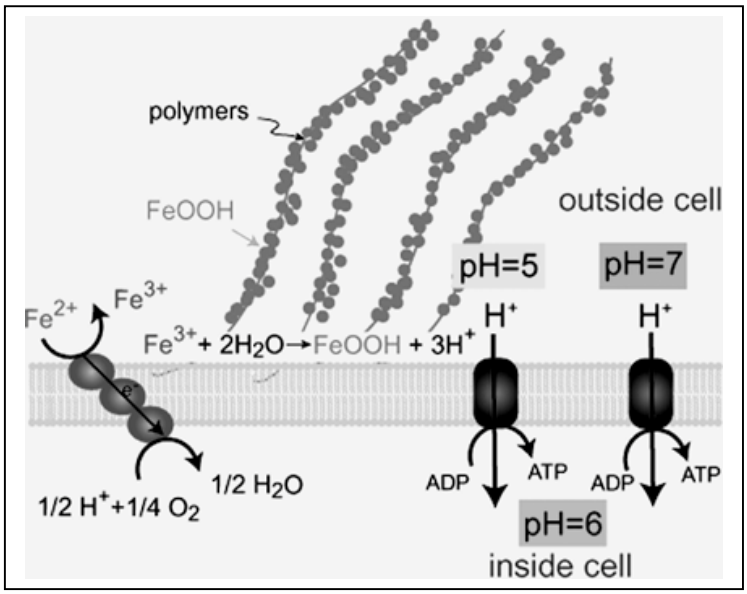


Figure 12:

Diagram indicating possible connections between proton generation, pH gradients, proton motive force, and energy generation. Increased energy generation is possible if proton supply, rather than Fe^{2+} supply, is rate limiting. Diagram: Chan and Banfield.

A hypothesis developed by the Emerson and Banfield groups posits a special and unusual connection between mineral precipitation and energy generation by iron-oxidizing neutrophilic microorganisms. Metabolisms based on iron oxidation in neutral and alkaline environments colonized by iron-oxidizing bacteria are thermodynamically possible, but the energy yield is only moderate. Protons must be pumped against a pH gradient (a challenge not faced by acidophilic iron-oxidizing prokaryotes). As noted by Emerson (2000), mineral precipitation liberates protons. If mineral precipitation occurs in proximity cells, the proton electrochemical gradient is changed, leading to an increase in the proton motive force (pmf). We postulate that the reason for extensive (and expensive!) production of extracellular polymer strands is to localize iron oxyhydroxide precipitation adjacent to the regions of the cell surface where ATP synthase complexes are concentrated. This could result in a higher energy yield if the rate of energy generation by the cell is limited by proton supply, not by supply of ferrous iron. This idea is illustrated in Figure 12.

As shown, rate of utilization of electrons from Fe^{2+} is directly linked to the rate of supply of protons (it is unknown whether proton pumping accompanies flow of electrons from Fe^{2+} to O_2; it appears that they are not in acidophiles). Either the supply of Fe^{2+} or protons could limit the rate at which energy can be generated. If the latter, energy generation can be increased if proton transport into the cell is accelerated by increase in the pmf due to proton release following iron oxyhydroxide precipitation. We will test this hypothesis by measuring microscopic pH environments in proximity to active cells, by altering the concentrations of ferrous iron and pH to establish growth limitation, and by determining if there is a correlation between sites of polymer secretion and ATP production. If requested genome sequence for an isolate (PV-1, a mesophilic iron-

oxidizing bacterium from a hydrothermal vent site (Emerson & Moyer, 2002) becomes available (DOE Joint Genome Institute microbial sequencing proposal, resubmission encouraged), the additional prediction of a correlation between the location of iron oxidizing enzymes (e.g., cytochromes, multi-Cu-oxidases) and polymers structures will be tested. If our hypothesis is correct, the implication is that intimate association of polymers and minerals should be widespread and important. This work will be conducted via collaboration between the Emerson and Banfield groups.

3.2.3 Mineral Biosignatures associated with microbial Fe-oxidation and Fe cycling

We will extend the field-based studies of iron- and sulfur-cycling microbial communities by studying the structure, dynamics and products of these systems in the laboratory. Process-level studies will be conducted with pure and/or mixed cultures obtained from the field sites. CoI Roden, working closely with Co-Is Emerson and Luther, will lead this aspect of the proposed work. The goals will be to provide information on the rates, pathways, and end-products of specific enzymatic processes, e.g. microbial reduction of Fe(III) oxides, oxidation of soluble and solid-phase Fe(II) compounds, and sulfate-reduction. In addition, bioreactors (Figure 13) will be used to investigate the ability of microorganisms obtained from the field sites to thrive and generate biogeochemical and mineralogical signatures of life under conditions that approximate those which may be present in shallow subsurface environments on Mars. The reactors (1-10 dm in length) will be packed with selected Fe-bearing mineral phases (e.g. Fe-rich basaltic glass, Fe(II) silicate minerals, in mixtures containing some cases FeS_2 (to simulate basaltic rocks), and Fe(III) oxides in a quartz matrix. Reactors will be inoculated with a consortium of organisms from field sites. The reactors will be designed to allow for control of temperature, pressure, water content, and gas composition. The water content (a crucial factor for life in the Martian subsurface) and rate of fluid flow through the reactors will be regulated by altering the degree of pore space saturation through control of hydrostatic pressure at the outflow end of the reactor (e.g. Seyfried and Rao, 1987).

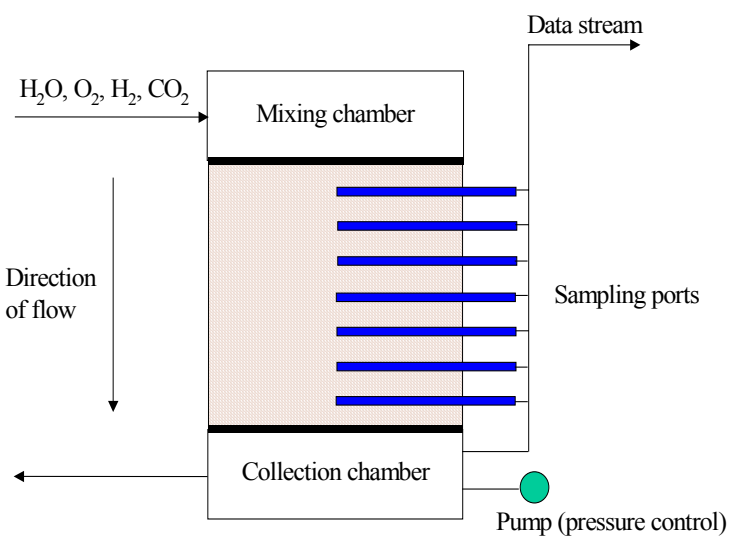


Figure 13 Diagram of bioreactors to be used for studies of Fe-based microbial metabolism under conditions approximating the Martian subsurface environments.

We anticipate that macroscale (tens of cm to mm) and microscale (mm to micron scale) **chemical gradients** associated with bacterial processes will develop within the bioreactors.

Methods described in section 3.3.1 will be critical for gradient characterization measurements, to be provided by Co-I Luther. Chemical heterogeneities should be paralleled by **variations in the microbial communities**, to be documented by Co-Is Roden and Emerson, and Fe-bearing mineral phases formed, to be documented by PI Banfield. The evolution of redox gradients, development of microbial communities, and alteration of mineralogical properties will be followed over time in order to gain insight into the potential biogeochemical structure and mineral biosignatures of Fe-based subsurface life. Several of the necessary techniques have already been applied to studies of Fe(II) oxidation and coupled Fe(III) reduction/Fe(II) oxidation by pure cultures of Fe(II) oxidizing and reducing microorganisms (Sobolev and Roden, 2002; Roden et al., 2003).

Distributions of redox-active and related chemical species that either consumed (e.g. O_2, H_2, CO_2, Fe(III), S(-II)) or produced (e.g. Fe(II), SO_4^{2-}, H^+) as a result of Fe-driven microbial metabolism will be compared to those predicted on the basis of non-reactive fluid and/or gas transport plus abiotic chemical reactions (using standard **numerical reactive-transport modeling** techniques; Steefel and MacQuarrie, 1996; Boudreau, 1997) in order to provide independent verification of the role of microbial activity in controlling redox speciation in the reactors. Co-I Roden has recently employed such techniques for analysis of biogeochemical Fe cycling in freshwater wetland sediments (Roden, 2003) and experimental microbial Fe cycling microcosms (Roden and Sobolev, 2001).

Microbial communities that develop in the bioreactors will be sampled in a limited way during operation, and more extensively upon termination, as described above for the community analysis of the field sites. **Bioreactor microbial population analysis** methods will include FISH and real time PCR quantitation of specific groups, MPN quantitation and enrichment, as well as either tRFLP or 16S rDNA clones libraries to assess overall community diversity within an individual bioreactor and for comparison to the field sites.

An important characteristic of neutrophilic microbial iron oxidation, especially when it occurs at sites of fluid mixing (e.g., groundwater entering a swamp; intersection of subsurface fluid flow paths, etc.), is the generation of polymers that become loaded with iron oxyhydroxide minerals. In modern systems, the activity of iron-oxidizing bacteria is evidenced by distinctive, highly mineralized cells and cell products. In modern samples, these are highly recognizable, especially when they are produced by *Leptothrix* and *Gallionella* spp. (Figure 14). In the last few years, micropaleontological studies have revealed compelling evidence that these same structures are preserved for millions of years as evidenced by analysis of ancient hydrothermal sites and 'red beds' where it was evident that Fe oxidation and cycling were occuring. Filamentous Fe-oxides and Fe-silicates analogous to extant Fe-oxidizing bacteria such as *Gallionella* spp. and *Leptotrhix ochracea* can be found in the fossil record at ancient hydrothermal vent sites, as well as other sites where the physico-chemical conditions appear conducive for Fe-oxide precipitation (Juniper and Fouquet, 1988; Juniper and Sarrazin, 1995; Little, et al 1999; Preat, et al 2000). A recent review of a wide variety of specimens suggested these types of biosignatures could be quite common (Hofmann and Farmer, 2000) The most dramatic of these are microfossils associated with the Pilbara Craton in Australia that are

dated to an age of 3.2 bya, and that are associated with pyritic deposits (Rasmussen, 2000).

Preliminary work in aquifer and ocean systems reveals that the **morphology** of polymer-mineral aggregates varies significantly with habitat, and that many of the resulting biomineral accumulations have features that are highly diagnostic of their biological origin. Consequently, we will examine the detailed nature of mineralized polymer products of Fe-oxidizing prokaryotes. Mineral-polymer mixtures from the field site, cultures, bioreactors, and in situ studies (see below) will be characterized by optical, scanning electron, and transmission electron microscopy. These methods provide information about the size, shape, and structure of minerals and the way they are associated with polymers. Comparison of mineralized biopolymers from different species will enable us to determine the conditions under which these potentially very important biosignatures form. In parallel, we will synthesize iron oxyhydroxides via a variety of inorganic methods and characterize these with the same techniques used to study the natural biomineral materials. We will also conduct polymer mineralization experiments, following the approach of Nesterova et al. 2002. It may be to distinguish biomineral signatures that strongly indicate the preexistence of polymers such as large prebiotic molecules or cell degradation products because preliminary evidence indicates that iron oxyhydroxides can preserve evidence of these (e.g., micron-scale mineralized polymer filaments are reported by Chan et al. in review and shown in Figure 14 as curvy filaments).

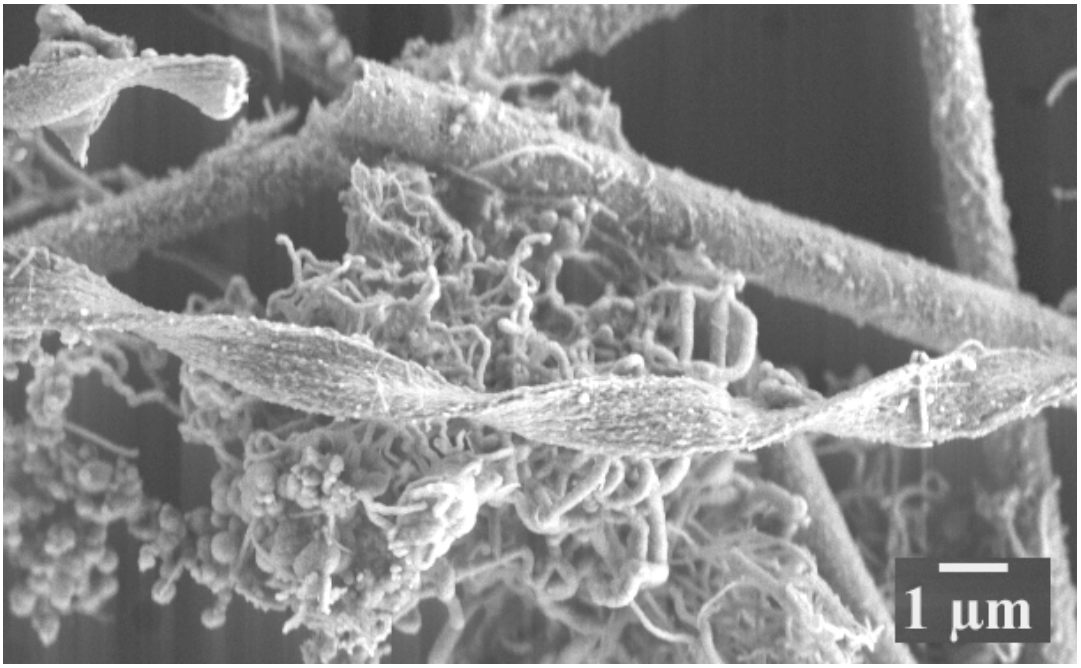


Figure 14: Scanning electron microscope image of distinctive mineralized biopolymers: sheaths, stalks, and fibrils

Some Fe-oxidizing microorganisms generate Fe-oxide products but apparently do not generate energy from this reaction (*L. discophora* and *S natans*). In other cases (ES-1

and PV-1), iron oxidation is associated with energy transduction (Emerson & Moyer, 1997 & 2002; Neubauer, et al 2002). HRTEM analysis of the polymers-mineral materials will be conducted to determine if this difference influences the type of minerals formed. This work will include physiology experiments, where cells are grown under different oxygen concentrations, Fe concentrations, temperature, and pH, as well as shaking/physical regimens, all aimed at understanding how these conditions may effect Fe-oxide mineralization by these two groups.

Microorganisms generate very distinctive mineral products. For example, nanocrystalline magnetite can be produced directly by magnetotactic bacteria (Blakemore, 1975) or indirectly through production of Fe(II) by dissimilatory Fe(III)-reducing bacteria that is later incorporated into magnetite (Lovley et al., 1987) and sulfate-reducing bacteria can generate extremely small metal sulfide particles. In principle, Fe-reducing organisms could have been responsible for generation of the carbonate-embedded magnetite nanocrystals in the Martian meteorite Allan Hills 84001 (McKay et al., 1996). Although recent detailed electron microscopic evidence suggests that the magnetite nanocrystals in the Allan Hills 84001 meteorite are not of biological origin (Barber and Scott, 2002), this by no means rules out the possibility that biosignatures of Fe-based life on Mars and in other extraterrestrial environments may eventually be identified. Rather, these recent analyses provide important information for constraining when potential mineralogical biosignatures such as magnetite do and do not provide valid evidence of biological activity. Given the vast amount of prior work on magnetite as a biosignature, will examine the basaltic rock-dominated habitats and bioreactors studied for evidence of magnetite produced biologically, focusing primarily upon its distribution and mineralogical context.

The relationship between organic compounds, especially exopolymers, and Fe biomineralization is of considerable interest. Organic molecules could be stabilizing aqueous Fe (III) complexes (see section 3.3.1). Large exopolymers are probably critical in biomineralization and biosignature preservation. Consequently, an important aspect of the culture work will be to determine the **chemistry of polymers** (especially sheaths, stalks, and fibrils) formed by neutrophilic and acidophilic Fe-oxidizing bacteria. Preliminary studies indicate that it is common for all the neutrophilic Fe-oxidizers isolated in Dr. Emerson's laboratory to form a variety of exopolymers (when Fe oxides are removed with hydroxylamine, large amounts of organic polymer remains). It is possible that some exopolymers form a matrix or scaffold upon which Fe-oxidation occurs, whereas others are passive substrates on which Fe-oxyhydroxide nanoparticles accumulate. In addition to the role polymers could play in localized pmf generation, as hypothesized above, they may also provide a means by which the cells can prevent themselves from becoming encased in an impermeable crust of Fe-oxides. Based on studies of sheath structures formed by heterotrophic Fe and Mn-oxidizing bacteria, these exopolymers may be complex acidic polysaccharides (Emerson and Ghiorse 1993).

We will determine the nature of these biopolymers because we hypothesize that their specific nature will influence the composition, structure, defect structure (crystallinity), and particle size of the associated Fe-oxide and Fe-oxyhydroxide minerals. This work, to be carried out by the Emerson group, will involve pure cultures already on hand (Table 2) and newly isolated species, as they become available. The principle composition of sugars/polysaccharides, proteins, and lipid content will be determined.

Assuming that it is principally polysacchride, then an analysis of the major carbohydrate moieties, e.g. amino sugars, uronic acids, neutral sugars, etc. will be performed. This latter work will be accomplished most readily by sending samples to the Center for Complex Carbohydrate Research at the University of Georgia, which provides a reasonable fee-based service for these types of analysis.

Relationships may exist between the nature and rate of production of the polymer matrix and the environmental conditions. This will be examined by determining the ways in which environmental conditions regulate exopolymer production in cultures. The experiments will utilize conditions similar to those described above for effects of environmental conditions polymer-biomineral formation and will be performed in parallel with those experiments in Dr. Emerson's laboratory.

In the proposed work, our team will examine the detailed compositional characteristics of polymer-mineral assemblages associated with iron-oxidizing microorganisms that colonize basalt-spring systems. We will determine the ways in which the iron oxyhydroxides differ in **element and trace element composition and morphology** from inorganic products formed due to rapid inorganic iron precipitation. Thus, we will investigate whether a biological origin can be established in the absence of preserved cell materials. This work will involve continuation of the collaborations among Emerson, Roden, Banfield.

An important question that must be dealt with when developing biosignatures for analysis of ancient samples is **'how well do they age?'**. As noted above, the biomineral products formed by iron oxidation have characteristics that are highly diagnostic of the presence of microorganisms (e.g., Fig. 14, Chan et al., in prep; Banfield et al. 2000) and polymers (Chan et al. in prep). Although some of the most obvious features survive in some geologic settings (see above), it is unclear to what extent the distinctive morphological, microstructural, and microchemical characteristics of these complex biomaterials will survive over long time periods. We anticipate that subtle features of ancient iron oxide deposits that are crucial to biosignature analysis may be lost. For example, the distinctive particle size, submicron-scale impurity content, and crystal orientation of nanoparticles may be eliminated by crystal growth and phase transformation reactions. It is important to know which of the interpretable characteristics (e.g., submicron-scale mineralized tubes formed by *Lepthothrix* sheaths and ribbon-like structures formed by *Gallionella*) may be retained. Because little is known about the extent to which the morphology and microstructure of biomineral products are preserved over geologic time, we will experimentally investigate aging of biomineral materials.

We hypothesize that the presence of polymers, and their specific composition and structure, may be important determinants of the evolution of textural characteristics of polymer-mineral composites during diagenesis. Consequently, Dr. Banfield's laboratory will study thermally-driven changes in cell-(and cell product-) mineral assemblages in order to understand biosignature preservation in ancient samples (e.g., size, microstructure, morphology, composition). The fate of microbial and simulated polymer-mineral assemblages will be evaluated in conducting coarsening experiments at between 40 and 150°C under hydrothermal conditions, in air, and under vacuum. This work will involve biomineral materials generated in the Roden and Emerson laboratories, inorganically precipitated mineral controls, and synthetic polymer-mineral aggregates. Samples will be characterized by powder X-ray diffraction, BET, and high-resolution

transmission electron microscopy (HRTEM). The kinetics of growth and transformation reactions will be monitored using peak area analysis and peak broadening analysis of X-ray diffraction data (see methods of Penn and Banfield, 1998).

Isotopic fractionation commonly accompanies biomineral formation. **Isotopic composition** of minerals formed as the result of microbial iron and sulfur cycling represents an important component of the NAI team's proposed research (below).

3.2.4 Isotopic biosignatures

i) Biological and inorganic isotope fractionations recorded in rocks

A vital aspect of this work is to establish baselines for non-biological isotope fractionation effects. This will be accomplished through the work of Dr. Boering, dealing with atmospheric isotope fractionations that may be recorded in minerals, and via measurements on Martian meteorites and materials from terrestrial analog systems via Dr. DePaolo.

Dr. DePaolo's work will focus on analysis of Fe and Ca (but also meaure S, H, O, as appropriate) isotopes, which are fractionated mainly by biological processes. His group will determine whether the magnitude and sense of the fractionations are discriminatory, thus diagnostic of biological processes.

As noted above, Fe is a redox sensitive element and may be critical to microbial metabolism on Mars. Recent work suggests that biologically produced Fe- bearing minerals may have distinctive isotopic compositions. Microbially-mediated iron reduction (Beard et al., 1999), organic chelation (Brantley et al., 2001), and anoxygenic photosynthesis (Croal et al., 2002; Johnson et al., 2002) fractionate iron isotopes ratios by 1.3 to 1.6‰, concentrating the light isotope preferentially in the dissolved state.

However, recent studies of natural and experimental systems have also demonstrated that abiotic processes can fractionate iron isotopes. Bullen et al. (2001) investigated the abiotic precipitation of ferrihydrite, a ubiquitous process in the natural environment, and showed that the ferrihydrite precipitate was enriched in the heavy isotope by about 1 to 2‰ relative to dissolved Fe^{+2}(aq) (Bullen et al., 2001). More recent results (Skulan et al., 2002) suggest that equilibrium precipitation of goethite alone does not fractionate iron significantly. Skulan et al.'s (2002) results exclude the Fe^{2+}-Fe^{3+} oxidation step, while Bullen et al.'s (2001) study includes this step. In addition, Skulan et al. (2002) suggest that kinetic isotope effects during relatively rapid precipitation of goethite can create a solid that is 1.3‰ lighter than the Fe^{3+}(aq) in solution.

The interpretation of the abiotic experiments is complicated by issues of aqueous speciation, which may also fractionate iron isotopes. Spectroscopic studies (Schauble et al., 2001) indicate that iron isotope fractionations between coexisting species in solution may be significant (δ^{56}Fe range of 7.8‰ at 25˚C between various chloride and hydroxide iron species). Experimental evidence (Johnson et al., 2002) indicates a +2.8‰ fractionation between Fe^{3+} and Fe^{2+} in solution.

Hence, there is a significant likelihood that the Fe isotopic composition of minerals could be indicative of a biological origin. This cannot at present be proven. The best way to approach this problem would be by studying natural terrestrial systems

that might be analogous to those on Mars, and establishing just how large Fe isotopic effects are in these systems.

What we know about terrestrial rocks so far is that igneous rocks do not vary significantly in their δ^{56}Fe values, with a total range between 0.1 and 0.6‰ (Beard and Johnson, 1999; Beard et al., 1999; Johnson and Beard, 1999; Johnson et al., 2003). However, some natural samples do have significant variations; these include chemical (± biological) precipitates such as marine Fe-Mn nodules and crusts (Beard and Johnson, 1999; Beard et al., 1999; ZHU et al., 2000), and soils, stream waters, and precipitates from streams (Bullen et al., 2001; Fantle and DePaolo, 2002). It is possible that all of these observed fractionations are ultimately due to microbial processes, but this cannot be proven yet because the appropriate systematic studies have not been done.

Although not necessarily essential to life on Mars, Ca may be included in biominerals. On the Earth, Ca isotopes are apparently significantly fractionated in nature almost solely by biological processes (Skulan et al., 1997). Typical mineral matter for terrestrial organisms has δ^{44}Ca values that are about 1.5‰ lighter than the Ca source (Skulan and DePaolo, 1999). Some marine organisms (certain foraminifers) can apparently produce larger fractionations, up to about 3‰ (Nagler et al., 2000). Calcium bearing minerals could therefore provide a potentially conclusive test for the existence of life on Mars. If there was never any life, there should be very little Ca isotope fractionation – the existence of life would essentially be required if significant Ca isotopic variability were observed.

The work plan for this approach will be integrated with other work on terrestrial analogue sites. Fe and Ca bearing minerals associated with biological activity (both in situ and in experimental systems) will be measured for isotopic composition to determine the natural range of values that can be expected. Co-I Roden has already participated in NASA-supported studies of Fe isotopic fractionation coupled to dissimilatory Fe(III) oxide reduction (Johnson et al., 2002). These samples will be compared to precipitates in systems where biological activity is not a factor, and analyzed in the context of experimental results from our experiments and those in the literature. In addition, we will characterize the Fe and Ca isotopic composition of carbonate and other minerals in Martian meteorites. The meteorite data may be highly interesting whether or not significant fractionations are observed. They will also be useful as baseline data for future sample returns.

ii) Isotopic fractionation in the Martian atmosphere: constraints for interpreting inorganic fractionations recorded in rocks

Recent studies on Martian meteorites have shown that isotopic signatures in oxygen and sulfur that could only have been produced by photochemical processes in the atmosphere have been preserved in the rock record [Farquahar et al., 1998, 2000a, 2000b; Bao et al., 2000]. It is possible that atmospheric photochemistry producing hydrocarbon hazes (discussed above in section 3.1.3) could also result in a carbon isotopic signature that is preserved in the Martian rock record. If the particles produced from the precursor CH_4 are isotopically light, it is also possible that a carbon signature from atmospheric processes could be misinterpreted as a biologic signature. Indeed, this atmospheric scenario has been proposed as an alternative explanation for the presence of isotopically

light kerogens (degraded organic carbon) found in the geologic record 2.8 Gy ago on Earth [Pavlov et al., 2001b]. For example, the current interpretation for Earth's rocks is that the very light kerogens are created by an additional fractionation of the carbon isotopes due to the rise of methanotrophic organisms that consumed the methane produced by methanogens when O_2 levels rose high enough in Earth's atmosphere to support methanotrophs [Hayes, 1994]. Yet, this interpretation would have to be investigated further if the carbon isotopic composition of a photochemical haze produced from the photolysis of CH_4 in an N_2, CO_2, and H_2O (i.e., early Earth-like) atmosphere is isotopically light. Using the experimental technique outlined above in section 3.1.2b, we can test the hypothesis of Pavlov et al [2001b] that photochemistry alone could impart an isotopic signature in carbon in the martian rock record that could potentially be mistaken for a signature of metabolism. Particles formed in our reaction chamber with a simulated martian atmosphere will be collected and analyzed offline for the bulk and compound-specific $\delta^{13}C$ values. These new measurements will provide a baseline for the contribution of abiotic atmospheric processes to the isotopic composition of carbon in martian rocks and meteorites.

3.2.5 Mineral surfaces as biological indicators

Although its clear that heterotrophic microorganisms can profoundly affect both the rates of silicate mineral weathering by producing acids and complexing ligands (Barker et al. 1997), little attention has been focused on the possibly distinctive effects of microbial activity on surface topographic evolution.

Microbial activity in proximity to mineral surfaces may lead to changes in surface structure and composition (see Banfield et al. 2001 for review). For example, recently Welch et al. showed that surface etching of apatite (the primary mineral source of phosphorus to the biosphere) is dramatically different in the presence of organic ligands compared to inorganic ions (Welch et al. 2002). Similarly, Teng and Dove (1997) showed that surface step geometries on calcite are highly dependent on surface-binding ligands. We will investigate the ways in which microorganisms impact their surroundings primarily though studies of chemoautotrophic prokaryotes growing in proximity to metal sulfide surfaces and microbial communities living in the porous surfaces of phosphate and silicate minerals in basaltic andestite rocks from the Abert Lake region. We will compare surfaces dissolving in the absence of cells and after exposure to microbial communities in laboratory experiments (section 3.2.3). Surface characterization will be by field-emission scanning electron microscopy and via surface compositional analysis (e.g., x-ray photoelectron spectroscopy). Particular attention will be paid to phosphate mineral surfaces, as these have been shown to focus microbial activity in weathered rock (Taunton et al. 2000). An example result may be the finding that insoluble elemental sulfur accumulates on dissolving metal sulfide surfaces but is absent on metal sulfide surfaces in biological experiments containing S-oxidizing prokaryotes.

Our investigations of mineral surface features potentially diagnostic of microbial activity also will involve situ studies of biosignature formation at the field sites. It may be difficult to convincingly establish that features of field-collected samples can uniquely be interpreted as biosignatures because the initial state of these materials is unknown.

Consequently, in addition to laboratory experiments, we will conduct **in situ studies** to investigate biosignature formation at the Abert and Snake River sites. In these studies, samples of well-characterized minerals will be emplaced at the field sites for periods ranging from a few months to up to 4 years. Samples will be retrieved and characterized in terms of microbial surface colonization, morphology change, and compositional change. In the first experiments, carefully prepared, precharacterized (dimensions, surface features, weight) slices of Fe-rich volcanic glasses, olivine, and pyroxene will be placed into microaerophilic sites in the field. Analogous slices of metal sulfide minerals (e.g., marcasite, pyrite, arsenopyrite) will be placed at another site (these experiments are similar to those reported previously by the Banfield group in the Richmond Mine, see Edwards et al.1998). It is anticipated that the minerals will dissolve at different (but predictable, and measureable) rates, releasing ferrous iron (and sulfide ions) into the environment. Cell numbers will be evaluated using methods noted above; FISH analyses and qPCR methods will be used to evaluate population structure. Mineralogical methods (see above) will be used to document changes in surface structure and to identify mineral precipitates. Isotopic features (e.g., Fe, H, and O) will be determined, as described below. Addition of a fresh source of energy (e.g., the reactive sulfide mineral in ground water) may locally (and conveniently) stimulate metabolic activity and mineral dissolution and precipitation phenomena of interest.

In one experiment, the Banfield group will emplace a kilogram of fine-grained, sterilized pyrite into a confined region at the Abert site. This creates the potential for considerable local acidification. This experiment is designed to explore how rapidly microbial colonization of distinctive habitats occurs. Are there a few cells of acidophiles in basaltic rocks (e.g., 'everything is everywhere'?) available to colonize the acid habitat, or are microbes rapidly dispersed from remote acidic sites? This experiment will run for five years.

3.2.6 Exploring life's extremes in an acidic Fe-S system

Our third field site is a very acidic system associated with a volcanic-hosted metal-sulfide deposit. In combination with work on neutrophiles described above, research at this site will extend out scope to cover much of the pH range for biological iron oxidation (from ~ possibly < 0 to 9 (environments with pH ~ -3.5 were described in currently inaccessible regions of the Richmond Mine by Nordstrom et al. 2000).

The Richmond Mine sustains a remarkably productive subsurface biosphere (Fig. 3), and provides an analog for possible localized microbial habitats associated with volcanic-hosted iron sulfide accumulations on Mars. Acid solutions form when oxidative dissolution converts pyrite to aqueous ferrous iron and sulfuric acid. Microbes play a key role in environmental regulation because they catalyze the slow (inorganic) reoxidation of ferrous iron, producing soluble Fe(III) which is the primary sulfide oxidant at low pH. Microbial catalysis of iron oxidation can dramatically increase rates of metal sulfide oxidation, allowing microbial communities to regulate the pH at values optimal for their survival.

The extremely acidophilic microbial communities that populate this site have been extensively studied by the Banfield group. Prior research has revealed the structure of microbial communities (at the level of species, a defined by SSU rDNA analysis) and

explored correlations between species makeup and geochemical conditions. The work to date has been limited to pH > 0.5 habitats and has not evaluated the limits of life for these organisms or those in other mine habitats, or the metabolic processes that are important in setting these limits.

The Richmond Mine acid mine drainage system at Iron Mt., CA, offers a unique opportunity to explore the answers to these questions because (i) it contains regions more extreme than virtually anywhere else (estimated to have temperatures > 65 °C in combination with extreme acid) and (ii) it is one of the first systems for which extensive community genomic data are available (~ 20 mb at the time of submission of this proposal, ~140 million mb anticipated). These data are the product of separate DOE and NSF-funded genomic sequencing and analysis efforts to evaluate lateral gene transfer (DOE) and ecological response to normal system perturbations (NSF).

In the proposed work, samples will be robotically collected from the most extreme (and currently unstudied) environments within the Richmond Mine system. This has recently become possible because extensive tunnel reconstruction (part of the ongoing remediation effort) has provided access to entry points to old stopes (caverns created by mining) and tunnels. We have EPA and mine owner approval to deploy robots into regions that cannot safely be accessed by human investigators. Preliminary to this proposal, we tested a prototype robot in the environment (see section 3.3.2). This test provided important information about modifications needed in order to sample in the extreme environments of the tunnel system. The proposed robot modification work is described in section 3.3.2. One of the two objectives for the robotic sampling is to obtain samples of organisms adapted to the most extreme conditions possible for characterization and to be used as innoculum for experiments to investigate extreme adaption. The other objective is to develop geomicrobiological-robotics protocols for remote sampling.

The robotics project will represent a collaboration between the Yim (Robotics), Luther, and Banfield groups. The Luther group's special contribution will be in in situ analysis of sulfur intermediates and measurement of soluble Fe(II) and Fe(III). A particular goal of this project is to implement these electrodes on the robot so that relevant data can be collected at the sampling location.

Organisms will be isolated in the Banfield laboratory. Isolation procedures for a subset of organisms have been used successfully by this group (however, this has not been a priority). The physiology of existing uncharacterized and new isolates will be analyzed.

In the proposed laboratory experiments to explore extreme environmental adaption (relative to the normal habitat for these organisms), we will use both batch cultures and specially designed bioreactors that replicate the natural environment. Use of bioreactors will probably be critical to permit co-cultivation of microbial populations. A diagram of the proposed bioreactor is shown in Figure 15. Important features are darkness (to simulate the subsurface), a long reactive path length with adjustable gradient (flow rate), and controlled temperature, pH, humidity, and gas mixture. The system will include computer-controlled sensors to permit continuous monitoring of most physical and chemical parameters (ionic strength monitoring, temperature, pH) and chemical and biological 'scrubbing' prior to recirculation. The system is designed to be compact and modular, making many duplicate systems practical. Microbial populations size and

makeup will be monitored by FISH, direct cell counts, and (if necessary) quantificaiton of total lipids. Sampling will target planktonic, biofilm, and sediment-attached cells.

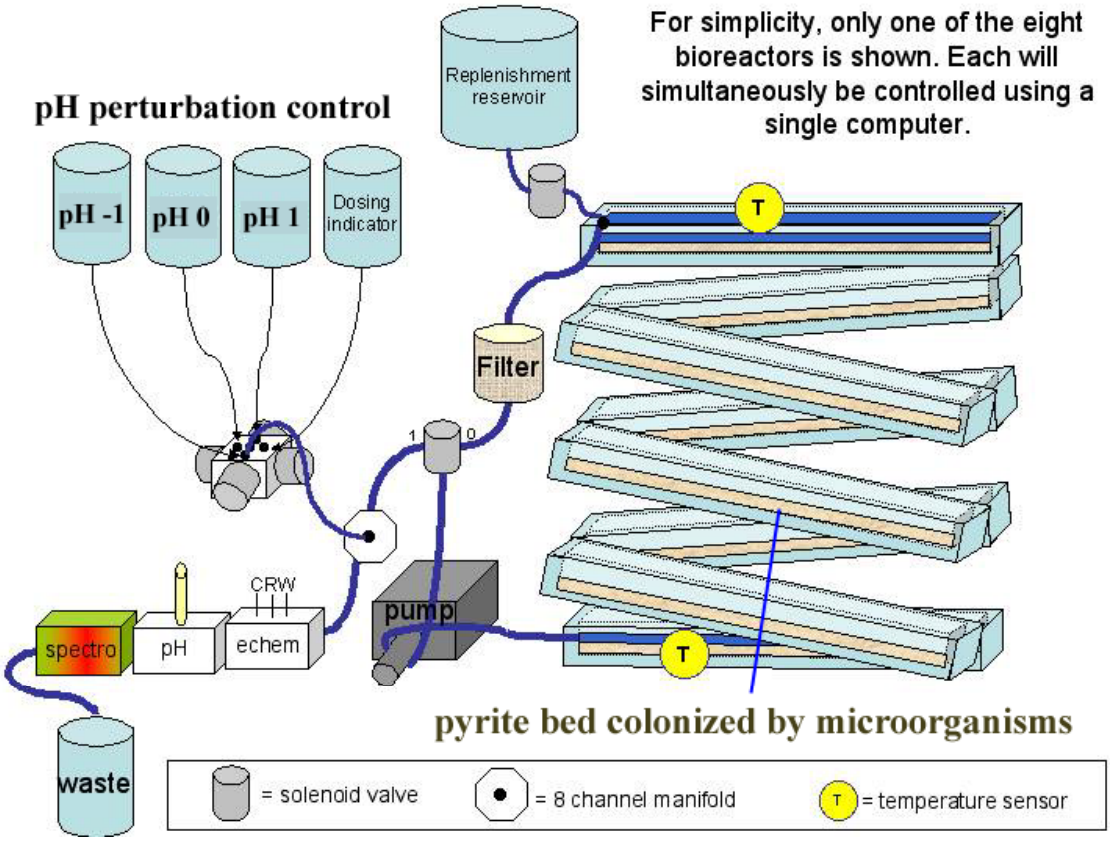


Figure 15: Proposed variable gradient bioreactors. The bioreactor will be enclosed (dark), temperature, fluid flow, humidity (gas mixture) and pH be controlled.

The Banfield group will evaluate the response, at the level of gene expression, of microorganisms colonizing pH 0.5-1.0 habitats to environmental challenges. Our objective will be to determine how organisms respond, and the sequence of events that marks failure to adapt (i.e., what determines the limits of adaption). Extremes to be investigated include cold, low water abundance, high ionic strength, and extremely low pH. At this time, we know that some microorganisms can grow at pH values as low as ~ 0 and temperatures in the range of 30-50 °C. Our goal will be to determine the limits of pH and temperature tolerance.

Information about gene expression (pathway- and organism-specific activity) of members of the populations over time (and in response to changed environmental conditions) will be obtained by using gene expression arrays (e.g., Wu et al. 2001). These are currently being designed for above mentioned parallel projects in the Banfield laboratory. The arrays are possible because genomic data for a pH 0.7 community will be available. Current estimates of the array size for this project is up to ~10,000 proteins, this corresponding to all genes for 6 species (species defined at the 16S level) and evidence for relatively little genome variability within species (Tyson et al. 2003).

Although the extreme acid study sites have high chemoautotrophic microbial activity, it may be difficult to detect most categories of biosignatures in them. Typically, the entire sulfide mineral assemblage is dissolved and very little secondary mineral precipitation occurs (unless some pH neutralization occurs). Furthermore, the long-term durability of organic products (e.g., lipids) at low pH is limited. Potentially promising biosignatures for this system are primarily in the form of etching patterns on sulfide mineral surfaces. These are potentially difficult to interpret (Edwards et al. 2000b). However, at least some (as yet unidentified) microorganisms do etch themselves into surfaces, creating extremely deep etch pits distinct from those formed inorganically. Attention will be given to characterizing these microbe-mineral interfaces (primarily via differential interference contrast and optical microscopy, SEM and FISH with existing or new probes). However, the primary questions to be pursued in this proposed NAI effort relate to microbial adaption and tolerance under relevant environmental extremes.

3.3 THRUST 3: NOVEL IN SITU MEASUREMENTS AND ROBOT-BASED GEOMICROBIOLOGY

Introduction

A key aspect of the proposed work is integration of Mars remote sensing, field-, and laboratory-based studies. **In situ measurements** and **sampling** will be important aspects of this work.

- In both the field and laboratory studies, **high-resolution geochemical measurements** of ion concentrations, redox state, fluxes, and temperature at the macro (cm-m scale) and micro (µm-mm scale) spatial scales will provide crucial information about metabolic options, active microbial metabolic processes, constraints for microbial cultivation-based studies, and biosignature analyses.

- As the ultimate question (about life on Mars) will probably be answered largely by remote investigation (e.g., involving robots), we feel that it is important to develop the **methods and approaches needed for robotic-based studies** in parallel with development of strategies for the scientific investigations.

It is <u>not</u> our goal to stimulate a major robot development program under this NAI. Rather, our intention is to work closely with scientists who are actively involved in robot development in order to explore the challenges and opportunities. If the NAI is awarded, other interations with NASA robotics scientists (e.g., Dr. Silvano Colombano, NASA Ames, who has been to the Richmond Mine site with the Banfield group) will be sought (to be funded by other avenues) in order to broaden this effort.

3.3.1 Development of sensors for in situ biogeochemical measurements– simultaneous detection of multiple chemical species that are biologically relevant

Until Dr. Luther's group developed a solid-state gold-amalgam (Au/Hg) voltammetric microelectrode for sediment work (Brendel and Luther, 1995), it was not

possible to measure non-gaseous biologically important species in freshwater and seawater solutions with microelectrodes for two reasons. First, the membrane-covered electrodes are not permeable to ions (other than the proton) and second, the potentiostats for the application of potential were relegated to only one fixed potential where more than one species may be electroactive. These two features did not allow for the determination of the important redox metals Fe(II) and Mn(II). Using the solid-state gold/amalgam (Au/Hg) microelectrode, Dr. Luther's group is able to measure dissolved O_2, H_2S, I^-, Fe(II), Mn(II), polysulfides and FeS_{aq} (Luther et al, 1998; Rozan et al, 2000; Theberge and Luther, 1997) because each redox species, if present, produces a current that can be detected in one potential scan [this is analogous to varying wavelength and monitoring absorbance in UV-VIS spectroscopy]. A key advance from our present work is the finding that FeS_{aq} was present in Contrary Creek, VA waters rather than Fe^{2+} alone (see results of previous work). This means that microbes are using a different source of reduced Fe than expected.

Also germane to this proposal, the electrode measures soluble Fe(III)species (see Huettel et al, 1998; Taillefert et al, 2000, 2002), which are metastable to precipitation only in the presence of organic ligands and are molecular clusters of unknown composition. These clusters are transportable, used by microbes (Dollhopf et al, 2000) and are intermediates in the formation of iron-oxide nanocrystals, as found by Banfield's group (e.g., Banfield et al. 2000).

Dr. Luther's group uses a portable electrochemical system designed with an instrumental company (Analytical Instrument Systems, Inc.) to monitor *in situ* redox species from environments ranging from microbial mats to sediments (Luther et al, 1999; 2001) to hydrothermal vents (Luther et al, 2001, 2002; Nuzzio et al, 2002). The calibration / validation of the electrodes and initial salt marsh results were described in Brendel and Luther (1995). Several papers, as noted above, have been published describing field research with the solid-state microelectrode and an *in situ* intercomparison of the O_2 data from the solid state electrode with the traditional Clark O_2 microelectrodes. The comparison yielded excellent agreement (Luther et al, 1999). Also, other trace metals (Cu, Pb, Cd, Ni, Zn) can be measured (Sundby et al, 1999). Most importantly, the electrodes have shown that chemical speciation drives hydrothermal vent ecology (Luther et al, 2001) and that molecular biology corresponds with *in situ* redox measurements (Glazer et al, 2002). Both Fe and S speciation can be measured and these redox species are a significant chemical source for microbes in areas ranging from ground waters, seeps, acid mine drainage sites (AMD), etc**. Thus, the electrode can be used to prospect for specific life forms.** The Luther group proposes this use in this proposal, and to couple this technology with the robotics equipment of Dr. Yim to analyze chemistry in extreme environments.

In addition to *in situ* measurements in the field, Au/Hg electrodes allow the possibility of studying primary and secondary diagenetic and microbial reactions in lab studies in real time. Recently, Dr. Luther's group (Dollhopf et al, 2000) has used the microelectrodes to follow Fe(III) and Mn(IV) reduction in cultures of *Shewanella puterfaciens*, strain MR-4. These solid-state electrodes are robust and will be used in culture experiments with other team members. With Dr. Roden's group (Roden et al, 2002; see results of previous support), we have already demonstrated the electrode's ability to investigate Fe dynamics in a gel experiment, which contained Fe oxidizing

microbes above Fe reducing microbes. Thus, we propose to use our electrodes in the proposed bioreactor system for real time measurement of redox chemical species (see section 3.2.3). The electrode system will permit us to obtain more accurate kinetic data on microbial processes and assess which redox chemical species are used by microbes. Traditional sampling and analytical methods can create artifacts and lead to incorrect interpretation of chemical and biological processes (Luther et al, 2001, 2002).

3.3.2 Robotic-based geomicrobiology: sampling inaccessible regions of the Richmond Mine

The challenges of sample collection and site characterization in some underground regions of the Richmond Mine have some parallels to sample retrieval and analysis on Mars: the samples occur in places where it is currently impossible for people to go; the habitats are extreme, simultaneous imaging, environmental characterization, and sample selection are required. Robots have the potential to solve these problems.

However there are significant differences as well. Subsurface locomotion requires mobility that is different than the Martian terrain explored in the past. Not only are there mobility constraints in climbing over obstacles (e.g., rocks), but size constraints requiring small profiles and as 'sediment' (pyrite accumulations in the tunnels) conditions that maybe submerged by acid solutions. In the context of the Richmond Mine system, the environment has regions that have extreme pH and temperature. As noted above, work to date has been concentrated on the 'moderate and moderately extreme' sites (pH 0.5 – 2) but more acidic environments occur in pools in caverns in other parts of the mine system. At this time, we have no idea of the types of microorganisms that will be encountered at temperatures above 50 °C or pH values of close to, and below 0. Our first goal will be firstly to determine if such organisms exist.

Since the requirements for specific in situ geomicrobiology and biogeochemical experiments in these areas involve mission specifications that are significantly different than those used in current robotic exploratory missions, these experiments will also lead to determining and validating requirements for possible future robotic mars missions.

We have conducted a preliminary test of a modular robot, known as a 'PolyBot', in the tunnel system and determined the types of modifications necessary to allow it to function in the mine environments. We propose to carry out these modifications, then develop methods to permit sample collection and necessary *in situ* measurements. Robotics work will be carried out by the Yim group, who will work with the Banfield group to develop sampling protocols and test the system.

PolyBot is a modular reconfigurable robot (MRR) that is compact, light, durable, and versatile (Figure 16). It is made up of repeated modules that may be arranged into different configurations depending on the task. The system promises to be extremely versatile (from reconfigurability), robust (from redundancy) and low cost (from economies of scale from repeated units). The Yim group (Yim 1993; Yim et al. 2003) has shown that MRRs possess the basic relevant abilities that make it potentially invaluable in extraterrestrial planetary exploration: it can move over a variety of terrains, including rough gravel, through a gopher hole using a snake-like concertina gait and through a man made obstacle course (made by US marines). NASA Ames recognized the promise of such as system and adopted an early prototype design of PolyBot in their

system called "snakebot" to study the usefulness for use in planetary exploration. This system received a large amount of international press.

The snake-like concertina gait demonstration was the first exploration of its kind inside an unstructured natural tunnel by a snake-like robot, and may be particularly useful for more highly constrained areas in the Richmond mine system. Even more apropos, early demonstrations of the system were shown exploring the surfzone (under water tested at a local beach), which included a simple liquid sampling tool on it as initial analysis for the Richmond mine exploration. Because it has been designed as a generic tool, PolyBot can also manipulate complicated and heavy objects, as required for sampling and delivery and operation of other *in situ* test equipment (see section 3.3.1). In addition, PolyBot has demonstrated its versatility over a variety of challenges, including riding a tricycle, climbing fences, stairs, poles, manipulating boxes, and self-reconfiguration; some in tethered and some in untethered forms (Yim et al. 2000, Yim et al. 2002). The ability to reconfigure its modules is potentially very useful in Mars exploration: small robots can rapidly explore some areas and join up in order to traverse difficult areas. Reconfigurable versions may be alternately able to form the shape of a rolling hoop, walking spider or slithering serpent, as needed, allowing them to access a greater diversity of places. The modular form ensures redundancy, a useful strategy to deal with component failure.

Figure 16: Photograph of a modular, reconfigurable robot similar to that used in a preliminary test in the Richmond Mine. The robot will be modified to improve durability in acid solutions, and interfaced with tools to permit *in situ* chemical measurements and collection of samples from regions where people can not go.

The Yim group proposes to further harden the robots for the acid environments, adapt them to be able to take and return samples and measurements, tune the mobility for the Richmond Mine, specifically to optimize performance in fine pyrite mud and acid pools, and to test and develop interface and methods for humans to be able to control and semi-teleoperate robots for our tasks (subsurface navigation, sample and return measurement).

The robotics work will begin by testing robots in accessible sites within the mine. The two primary objectives of the first phase of research are to optimize sample

collection and in situ analysis methods and to evaluate robot vs. human sampling bias. In the later case, sampling will be conducted in the same area by human and robot and results (biases) examined. Results will be used to fine-tune robotic sampling methods. Once the robot performance is fine tuned, the second phase of sample collection in inaccessible regions will begin. We plan approximately two on site trips per year for this work.

4.0 INITIAL RESULTS: GEOMICROBIOLOGY IN Fe-S SYSTEMS

Approximately 1.5 years ago, a team of investigators (Banfield, PI; Roden, Emerson, Luther, and Sturchio, Co-Is) received two years of seed funding from NASA (~$50,000/ yr/investigator) to conduct preliminary research that represents the foundation for the geomicrobiology part of this proposal (Sturchio, now chair of his Department at the University of Illinois at Chicago, decided not to participate in this full proposal). A summary of collaborative research accomplishments associated with the seed funding is provided below.

i) Can Fe-silicate dissolution sustain Fe-oxidizing bacterial populations?

Experimental work was conducted in the Banfield group, in collaboration with the Emerson and Roden groups, to determine if Fe-oxidizing bacteria can derive metabolic energy by oxidizing Fe(II) released during dissolution of ferrous-iron-rich silicate minerals (e.g., fayalite (Fe_2SiO_4), pyroxene (e.g., $CaFeSi_2O_6$, biotite ~ (K $(Mg,Fe)_3Si_3AlO_{10}(OH)_2$). The initial experiments by the Banfield group used the moderate acidophile *Acidithiobacillus ferrooxidans* (previously known as *T. ferrooxidans)*, which was successfully grown on fayalite (Santelli et al.2001 and Welch et al. 2002). Although this choice of system required use of fairly acidic solutions, it was useful because *A. ferrooxidans* is unable to grow on trace organic components, which are difficult to completely eliminate from experiments. By coupling kinetic analysis of the rates of release of ferrous iron via silicate mineral dissolution as a function of temperature and pH with analysis of the rate of utilization of ferrous iron by bacteria, it was possible to predict that basalt weathering reactions at ocean temperatures should sustain populations of > 10^5 cells/cm^3 (Welch et al., in prep). In research in collaboration involving the Banfield, Emerson, and Roden groups, experiments using isolates of iron-oxidizing bacteria with Fe-bearing silicate minerals as the source of dissolve ferrous iron were undertaken (ongoing work). The goal is to further test the predictions of iron-oxidizing bacteria population sizes sustainable by dissolution of Fe(II)-bearing minerals in basaltic rocks.

ii) Studies of microbial Fe(II) oxidation and Fe redox cycling in circumneutral environments

Research on neutrophilic iron-oxidizing bacteria, and the potential for microbial Fe redox cycling, was conducted at several different field sites. All of these sites have conspicuous flocculated Fe-oxides associated with characteristic structural remains of neutrophilic Fe-oxidizers, principally *Lepthothrix ochracea* and *Gallionella ferruginea*. The Emerson and

Luther groups have studied a surface stream (Contrary Creek) in Virginia which has a pH that ranges from ca. 5.5 to 6.5, and Fe(II) concentrations in the range of 50 to 100 µM. Emerson's group has made monthly measurements of Fe(II), pH, temperature, O_2, and conductivity at several sites in the stream. They also made visual and microscopic observations of the microbial community in the more acidic region of the stream, which indicated that the iron oxides have large numbers (~10^7 to 10^8 mL^{-1}) of tightly associated bacterial cells. Furthermore, they demonstrated proof of concept that tRFLP can be utilized to both document community diversity and track changes in dominant microbial groups in the community.

Roden's group has conducted a preliminary evaluation of the potential interaction between iron-oxidizing bacteria and dissimilatory Fe(III)-reducing bacteria at a groundwater Fe seep located near the campus of The University of Alabama in Tuscaloosa, AL. MPN analyses indicated the presence of 10^3 mL^{-1} and 10^4 mL^{-1} of culturable iron-oxidizing bacteria and dissimilatory Fe(III)-reducing bacteria, respectively, in the seep materials. These findings suggest that both Fe-oxidizing and Fe-reducing bacteria are actively involved in Fe redox cycling in the seep materials. The potential for Fe(III) reduction activity was clearly demonstrated by experiments in which materials were incubated under anaerobic conditions and changes in Fe(II) concentration were monitored over time (Fig. 17, upper panel). Fe(III) oxide reduction in electron-donor amended materials (Fig. 17, lower panel) resulted in the formation of distinctive, black-colored Fe(II)-Fe(III) hydroxide mineral phases. Molecular biological (16S rDNA extraction, PCR amplification, and analysis by construction of clone libraries and/or denaturing gradient gel electrophoresis) and fluorescence in situ hybridization (FISH) studies of the Fe cycling bacterial communities (using 16S rRNA probes available for known iron-reducing bacteria together with newly-developed 16S rRNA probes for circumneutral iron-oxidizing bacteria) in the seep materials are underway, and collaboration with G. Luther on microscale analysis of Fe redox distributions in the seep materials are planned for the near future.

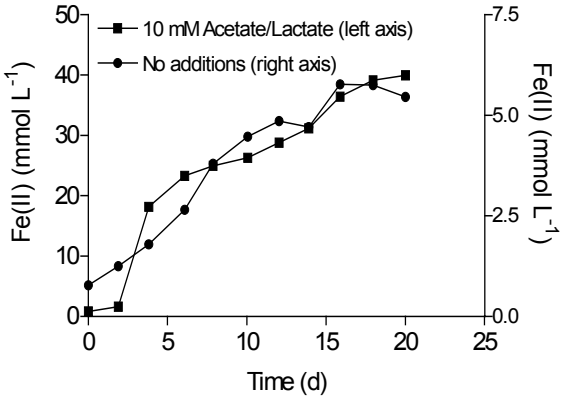


Fig. 17 Fe(III) reduction (upper panel) and reduced Fe mineral production (lower panel) in anaerobically- incubated Fe seep materials with (left) and without (right) electron donor amendment.

The Banfield group has studied biomineralization in the pH 7.2-8.6 carbonate-hosted subsurface tunnels associated with the Tennyson ore deposit in Wisconsin. Approximately 20 cm thick Fe-oxide biomineral accumulations have formed since the mine system was flooded ~ 32 years ago. We have extensively characterized the mineralogy of these materials, and described novel, elongate mineralized cell products (a central few nm wide fiber of akaganeite surrounded by flocculated ferrihydrite (Chan et al. in prep.). We have constructed clone libraries and FISH studies to explore the phylogenetic diversity of the community, made in situ measurements (using 'peepers') of solution chemistry, and developed an understanding of how fluid flow, geochemistry and microbial activity and interconnected in this system to generate highly diagnostic mineral biosignatures (Chan et al. in prep #2). Mixed cultures of iron-oxidizing bacteria have been established for further phylogenetic analysis, and for characterization via collaboration with the Emerson group.

Research involving the Banfield, Sturchio, and Roden groups was carried out at Soda Spring in western Oregon. Chemical data was collected by the Sturchio group for spring waters in which abundant Fe-oxide biomineral accumulations were forming. Results show a gradual decrease in Fe concentration from the vent to Pool 4, whereas all other measured constituents are essentially constant (including Mn). There is a large decrease of all constituents in Pool 6 due to dilution of inflowing stream water. The chemistry of this spring water is consistent with acid attack of intermediate volcanic rock by groundwater that has absorbed magmatic CO_2, with concomitant neutralization of the acidity through hydrolysis of minerals. The high Si (near saturation with amorphous silica) is typical of acid waters that have reacted with glassy volcanics. The low SO_4 and Cl- indicate that the magmatic gases absorbed by the water were already cooled (SO_2 and HCl absorbed at higher temperature). The SO_4^{2-} concentration is similar to that expected from an air-saturated water in which sulfate is generated by consumption of dissolved atmospheric oxygen with sulfide (dissolved or in the form of sulfide minerals). No nitrate (< 0.1 mg/L) was detected by ion chromatography. Stable isotopes (H, O, C) and dissolved gas measurements are currently being conducted. In parallel, the Banfield group has characterized the biomineral products of Fe(II) oxidation and are currently engaged in isolating FeOB from the spring materials. Most biominerals are in the form of ferrihydrite aggregations and some mineralized stalk structures. FISH data on these biomineral samples indicate abundant β-Proteobacteria. Roden's group has demonstrated the presence of cultivatable Fe(III)-reducing bacteria in a majority (5 of 8) spring samples.

iii) Development and application of methods for in situ analysis of microbial Fe cycling communities

An important aspect of both field and laboratory (experimental) based studies of bacterial Fe redox metabolism involves development of field sampling methods that allow *in situ* measurement of important physicochemical parameters across redox boundaries. These data are necessary for understanding of the range of environments suitable for Fe-oxidizing and Fe-reducing microbial communities, as well as for refinement of culture-based strategies that are essential for the metabolic characterization

of the relevant organisms. Initial analyses of in situ Fe and O_2 dynamics have been completed using the microanalysis tools developed by Dr. Luther's group.

Field sampling using *in situ* voltammetry was performed at the Contrary Creek site in Virginia. This work has provided a unique data set on the chemical speciation of iron and sulfur that could not have been obtained by other techniques. The data clearly illustrate the importance of *in situ* analysis for identification of the major redox active forms that exist in natural waters. The system was initially thought to be Fe-dominated, based on colorimetric measurements soluble Fe(II) and Fe(III). However, the *in situ* data showed that molecular iron-sulfide clusters, FeS_{aq}, were dominant over dissolved free Fe^{2+}. Figure 18 shows the results of a profile from the Contrary Creek area taken on November 8, 2002. Successive cyclic voltammetry (CV) scans were performed in situ using a glass Au-amalgam microelectrode scanned between a potential of –0.05 and –1.8 vs. Ag/AgCl reference at a scan rate of 2000 mV/sec. Using this method, it has been determined in laboratory experiments with creek water and in other field work that potentials for Fe^{3+} will be at about –0.25 V, O_2 at –0.4 V, H_2S/ HS^- at –0.6 V, $FeS_{(aq)}$ at –1.2 V, H_2O_2 at –1.4, and Fe^{2+} at –1.6 V.

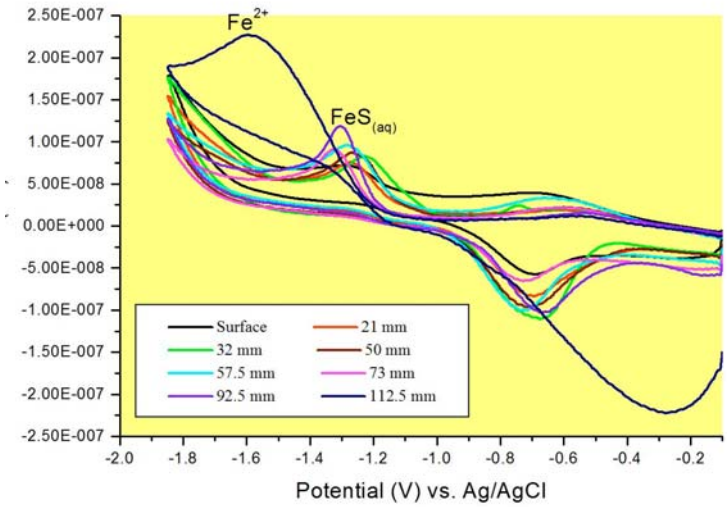


Fig. 18. Cyclic voltammetry scans from the Contrary Creek March area taken on November 8, 2002. Scans were performed in situ at different depths using a glass Au-amalgam microelectrode between a potential of –0.05 and –1.8 vs. Ag/AgCl reference at a scan rate of 2000 mV/sec.

The predominant features of most scans along this profile (and 2 others) taken at lateral positions 5 cm in either direction, showed a forward peak at approximately –1.2 V with the associated return peak at about –0.7 V. Iron [total Fe^{2+}] measured by the ferrozine method for these samples was 171 μM. The in situ CV scans (Fig. 18) show that the majority of the iron (and all the sulfide) is in the form of $FeS_{(aq)}$ clusters. Note that the $FeS_{(aq)}$ peaks around –1.2 V (vs. Ag/AgCl) shift up to 100 mV through the profile. This is possibly due to changes in the molecular composition of the cluster (e.g., if it is Fe_4S_2 or Fe_3S_2, etc.) or to complexation of the cluster with organic compounds – we are currently testing these possibilities in the laboratory. Only at the deepest point in the water column did the iron exist as dissolved free Fe^{2+}. Analysis of the O_2 levels show that the stream water was microaerophilic, as there were very low levels of oxygen present (less than 10 μM, but above the Ar-purged standards). These data have altered our thinking of the key redox species that are being used by microbes in this system. We

are now testing for sulfur oxidizers in addition to the Fe-oxidizers previously found in this system.

The Luther and Emerson groups have cultivated iron-oxidizing and iron-reducing bacteria the Great Marsh in Delaware. In addition, during a Chesapeake Bay cruise during the last week of July, the Luther and Emerson groups collected in situ voltammetry data and samples for cultivation of Fe(III)-reducing bacteria. The Luther group will carry out culture work on organisms isolated by Emerson and follow Fe chemistry in situ during incubations as documented below with the Roden group.

The Roden and Luther groups have investigated coupled Fe oxidation and reduction in experimental Fe(III) oxide-reducing microcosms in the presence and absence of a newly-isolated neutrophilic Fe(II)-oxidizing bacterium strain TW2 (Sobolev and Roden, 2003). Based on the ability of TW2 to cause unique alterations in patterns of Fe(III) oxide deposition in opposing gradients of Fe(II) and O_2 (Sobolev and Roden, 2001), we hypothesized that this organism might lead to enhanced coupling of Fe redox cycling at the aerobic-anaerobic interface of the microcosms. The existence of such biologically-catalyzed Fe redox cycling in structured (layered) microbial communities has important implications for patterns of biogenic mineral accumulation (Emerson, 2000), as well as Fe isotopic fractionation signatures (Johnson et al., 2002) in bacterial Fe cycling systems – e.g. systems which could conceivably exist on the surface of Mars. Results are described in a manuscript (Roden et al., 2002). In summary, the findings indicate that close juxtapositioning of FeOB and FeRB and rapid microscale Fe redox cycling occurred within the cocultures, such that the majority of the Fe was maintained in the reduced state at the aerobic-anaerobic interface despite the presence of detectable O_2 in the bulk aqueous phase. The lower concentration of dissolved Fe(II) in microcosms (as determined by voltammetric microelectrodes at the University of Delaware) inoculated with TW2 compared to those containing *S. alga* only (Fig. 19) provided direct evidence for enzymatically-enhanced Fe(II) and O_2 scavenging. More detailed studies of the distribution of FeOB and FeRB within Fe cycling aggregates, as well as more rigorous quantification of dissolved and solid-phase Fe(II) and Fe(III) pools

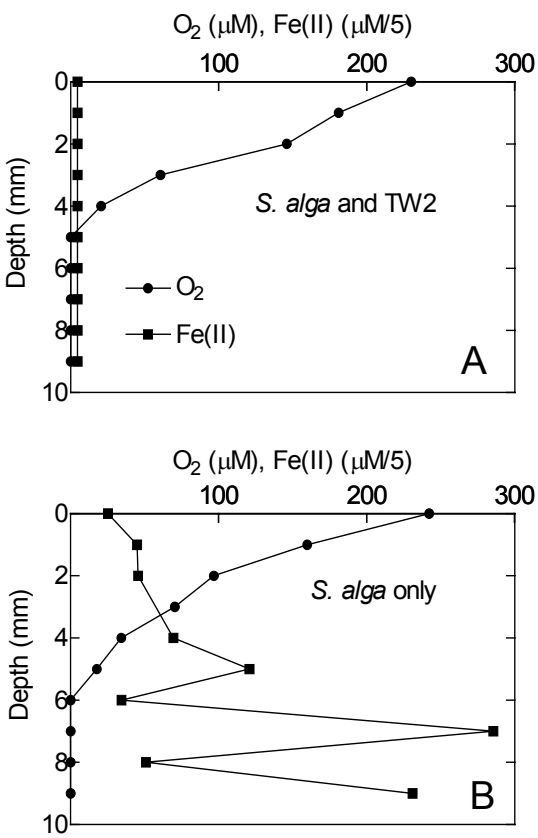


Fig. 19. Voltammetric microelectrode profiles of dissolved O_2 and Fe(II) in Fe cycling microcosms containing both *S. alga* and TW2 (A) or *S. alga* only (B). Data are averages of triplicate profiles. From Roden et al. (2003)

at the redox interface, are planned to verify our conceptual model (Roden et al., 2003) for the behavior of our coculture systems.

In situ voltammetry investigations of the chemistry of mats from salt marshes and a hot spring in Yellowstone showed Fe and O_2 profile data similar to the lab experiments described above.

Strain	Source	Obligate FeOB	Oxide type	Lab
ES-1	Groundwater,	Yes	Particulate	Emerson
ES-2	Groundwater	Yes	Particulate	Emerson
PV-1	Marine hydrothermal vent	Yes	Filamentous	Emerson
JV-1	Marine hydrothermal vent	Yes	Particulate	Emerson
CCJ	Rhizosphere	Yes	Particulate	Emerson
BrT	Rhizosphere	Yes	Particulate	Emerson
LD-1	Rhizosphere	Yes	Particulate	Emerson
Br-1	Rhizosphere	Yes	Particulate	Emerson
TW-2	Wetland	Mixotrophic	Particulate	Roden

Table 2: Strains of neutrophilic Fe-oxidizing bacteria (FeOB) isolated by the Emerson and Roden labs, and available for aspects of the proposed research.

iv) Influence of neutrophilic Fe-oxidation rates.

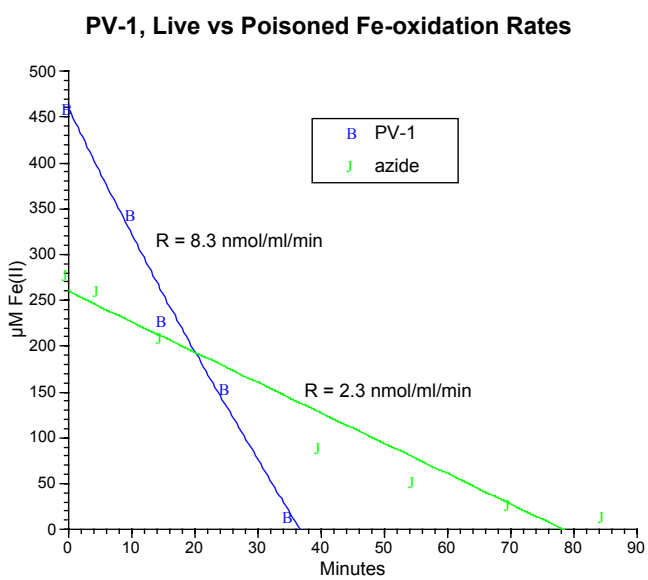


Fig. 20. Graph showing rates of Fe-oxidation for live PV-1 versus PV-1 poisoned with azide. The live rate was determined, then azide was added to the BR and the poisoned rate determined.

A focus of the Emerson lab during the last 18 months has been aimed at understanding the percentage of Fe-oxidation that is directly mediated by microaerophilic FeOB, and how much Fe(II) they need to oxidize to meet their energy requirements. We have used bioreactors that can control pH, temperature, O_2 supply, and Fe(II) supply to study these questions. A simple experiment, illustrated in Fig 20, demonstrates that the marine strain PV-1 may directly mediate nearly 80% of the Fe-oxidation in this system. A more detailed analysis of a freshwater strain, BrT, indicated that it could account

for up to 60% of the Fe-oxidation in the system that was directly mediated by the cells and that the growth yield was 0.70 g of CH_2O (a proxy for cell C) per mol of Fe(II) oxidized. Furthermore, there was evidence that while live BrT accelerated rates of biotic oxidation versus abiotic oxidation, the presence of the cells and exopolymers could also suppress the abiotic oxidation of Fe(II) (Neubauer, et al, 2002). The strains of iron-oxidizing bacteria isolated by the Emerson and Roden labs and available for proposed research are listed in Table 2.

v) Connections between geochemistry and metabolism in acid systems

We have investigated the oxidation of tetrathionate ($S_4O_6^{2-}$), which along with thiosulfate ($S_2O_3^{2-}$), is an intermediate in the oxidation of pyrite in the natural environment and acid mine drainage systems. Hydroxyl radical (OH^*) forms on pyrite surfaces and enhances the oxidation kinetics of these intermediate sulfur species. We have also investigated the oxidation rates for tetrathionate in the presence of Fenton's reagent (Fe(II) and H_2O_2 which forms hydroxyl radicals). The reaction leads to sulfate and is too fast to be directly measured using UV-Vis spectrophotometry. Cyclic voltammetry experiments in acidic solutions indicate that the reaction of $S_4O_6^{2-}$ with OH^* goes through an unknown intermediate, tentatively assigned as $S_3O_4^{n-}$, which is stable in an inert atmosphere but not in air. An outer-sphere electron transfer mechanism for the reaction of $S_4O_6^{2-}$ with OH^* to form $S_3O_4^{n-}$ is proposed. Competitive reaction kinetics within the context of the Haber-Weiss mechanism suggests that the rate constant for the oxidation of polythionate with OH^* is diffusion controlled and $\sim 10^8$ M^{-1} sec^{-1}. This work represents a collaboration between the Luther and Banfield groups (Druschel et al., in review).

vi) Fe stable isotopic signatures of biogenic magnetite and Fe(II)-carbonate minerals

Biogenic magnetite and Fe(II)-carbonate minerals were generated in Roden's laboratory using the well-characterized iron-reduciing bacteria *Shewanella putrefaciens* and *Geobacter metallireducens*. The minerals were collected and dried under anaerobic conditions and shipped to the University of Wisconsin for analysis of Fe isotopic (^{54}Fe, ^{56}Fe) composition by multi-collector ICP-MS. These studies provide the first data on the isotopic composition of Fe(II)-bearing minerals generated during dissimilatory bacterial Fe(III) oxide reduction, and are included in a manuscript submitted for publication which describes the current state of knowledge concerning constraints on Fe isotope fractionation during biogeochemical Fe cycling (Johnson et al., 2002). The results indicate that magnetite will have $^{56}Fe/^{54}Fe$ ratios that are approximately equal to the Fe(II) in solution as the isotopic equilibrium is approached, whereas the $^{56}Fe/^{54}Fe$ ratios for Fe(II) will be 1-2 ‰ higher than Fe carbonates, where the largest fractionations are measured in Fe carbonates that contain limited Ca substitution. The relative order of $\delta^{56}Fe$ values for these biogenic minerals is therefore Ca-bearing Fe carbonate < siderite < magnetite, which is similar to that observed in natural samples such as banded iron formations, as well as that predicted from Fe isotope fractionations calculated from spectroscopic data. The results of these and ongoing collaborative studies open the way

for use of Fe stable isotope analyses for assessment of the participation of biological activity in Fe redox cycling in natural sedimentary environments.

5.0 "BIO M A R S" AND THE ASTROBIOLOGY ROADMAP

Mars represents one of the few places where, in the near future, it may be possible to answer the fundamental questions that underpin the study of the origins, evolution, distribution, and future of life in the universe. Our work is primarily designed to tackle the question: '**Does life exist elsewhere in the universe**?" (Astrobiology roadmap (AR), fundamental question 2, goal 2) by targeting the habitat types on Mars with the highest probability of sustaining life and developing biosignatures for detection of past and current life (AR goal 7). The findings (life detection in combination with understanding of early Mars evolution) will impact thinking about the related question 'How does life begin and evolve? (AR fundamental question 1, goal 3). Should it happen that evidence for life is found on Mars, understanding of where it evolved and how life changed in response to changes in the abundance of water and the atmosphere will provide information about the drivers and consequences of dramatic climate change on the millions of years timescale. This understanding can be used to project the outcomes of environmental change on Earth. Thus, the proposed studies will seek information that, in the long term, may address the question: '**What is the future of life on Earth and beyond?**" (AR goal 3).

Although unintentional, Mars exploration may result in **colonization of Mars by Earth-derived microbial life.** The organisms most likely to survive are those that colonize terrestrial Mars-like habitats similar to those studied in our work. Thus, the proposed NAI is directly relevant to the question of the **potential of microbial life to adapt and evolve in environments beyond their planet of origin** (Astrobiology roadmap goal 6). Our work also may have direct relevance in the distant future if there is an attempt to deliberately introduce life to Mars.

6.0 SUMMARY

Three major interconnected research thrusts have been defined. Firstly, we will identify the places on Mars with the highest probability of protection from extreme radiation, bioavailable energy, and long-term access to water. The requirement for UV protection early in Mars history will be deduced from atmosphereic studies. This search will be accomplished by a novel, comprehensive approach in which we will deduce current and past conditions at the Martian surface by coupling studies of core behavior and planetary evolution to models of hydrosphere development and fluid discharge at the surface. Thus, we will generate targets for geomicrobiological studies. Our second research thrust is to determine the limits for life of in Mars-like Earth environments identified in the first research thrust (AR, goal 5), identify the energy options most likely to generate biosignatures, and identify the form of biosignatures expected as the result of utilization of this energy (AR goal 7). Our work will position NASA to optimize the chances of detecting Martian life, if it exists, or once existed.

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Plan for Strengthening the Astrobiology Community

Institutional Commitment

UC Berkeley has launched a major effort to pursue research and education in planetary science, planetary environments, and astrobiology. Two years ago they created an organized research unit called the Center for Integrative Planetary Sciences (CIPS). CIPS is directed by Prof. Geoff Marcy. CIPS has hired several new faculty (the PI and one of the CoIs for this proposal) and instituted several educational initiatives to support a new planetary/astrobiology program.

A new undergraduate curriculum has been established in planetary science under the auspices of the ``Astronomy" and ``Earth and Planetary Science" Departments. These new undergraduate programs integrate courses in chemistry, geophysics, astronomy, biology, and meteorology into a coherent curriculum. They will form a solid basis for the undergraduate and graduate programs for the NAI.

As part of the proposed activities for the NAI, PI Banfield and colleagues will develop a new course that will cover planet formation, origin of life, organism-environment interactions and the search for life in the universe. This course will be coordinated via the CIPS infrastructure. **Administrative support** for course management and coordination between the NAI and CIPS (separate from the NAI administrative support requested in the budget) and **computer support** will be provided by CIPS (see attached letter from Prof. Marcy).

There are many sources of support available to fund undergraduate research. Our education and outreach efforts will build upon several ongoing programs at UC Berkeley. SUPERB (Summer Undergraduate Program in Engineering Research at Berkeley) offers outstanding underrepresented engineering students the opportunity to gain research experience by participating in research projects with engineering faculty and graduate students. SUPERB is targeted to students of color, first-generation college students, educationally disadvantaged students, or students from historical minority institutions. The Berkeley Edge is a newly established recruitment, retention and advancement program for traditionally underrepresented minority graduate students in science, mathematics and engineering (jointly by the NSF and UCB). The Berkeley Edge goal is to identify, recruit, retain, and assist in advancing talented minority students to the professoriate.

There are several graduate programs available for training of graduate students working in astrobiology. These include opportunities associated with CIPS, Earth and Planetary Science, Environmental Science, Policy and Management, and the Microbiology Program.

As described in the attached letter from Prof. Marcy, CIPS will commit a significant fraction of its resources to the NAI, should it be funded. Specifically, they propose to **provide $50,000 per year** to establish a competitively awarded, advertised **"UC Berkeley Postdoctoral Fellowship in Astrobiology and Planetary Science".**

The Miller Fellowship Program provides funding for Postdoctoral Fellows. Recently, EPS has received approximately one per year. There is currently one Miller Fellow in the Geomicrobiology Program.

Under CIPS, we have already hired three new tenure-track faculty members, namely Drs. Eugene Chiang, Michael Manga, and Jill Banfield. Their research spans areas of planet formation, the chemical composition of planets, and the biogeochemistry of the atmosphere, oceans and mineralogy and surfaces, especially with regard to conditions suitable for biology. We plan additional faculty hires in the next year. CIPS has also hired a new Senior Fellow, Greg Delory, who specializes in the surface morphology and water on Mars.

Support for the proposed research will also be available through the following programs:

1.) The university provides up to two graduate fellowships to prospective graduate students each year. First year graduate students admitted to Earth and Planetary Science or Ecosystem Science are typically covered for at least one semester with fellowships.

2.) The department provides approximately ten Graduate Student Instructor (GSI) positions for graduate students per year.

3.) Department provided $1 million dollars for laboratory facilities and renovations to establish the geomicrobiology program.

4.) The campus has committed $300,000 in matching funds for new TEM and XRD instrumentation (MRI proposal in review).

5.) The department supports the following labs through a commitment of space (1,300 square feet) and staff (one full time staff member): Thin Section Lab, Electron Microprobe Lab, XRD lab and XRF Lab. Equipment in these labs is valued at $2,000,000.

6.) PI Banfield and Co-I Dietrich share an internet-based teleconference system that can be used by the NAI team.

7.) The campus supports the following technical staff: Tim Teague, Analytical Specialist (EMP, XRD, etc); Dave Smith, Machinist.

Banfield Group

Space: Rooms, 274, 452, 453 McCone and 108, 113, 120, 128 Hilgard totaling approximately 3500 square feet of lab space.

UC Paid Staff: $20,000/year in staff support via the AES program.

Equipment Value: $900,000

Dietrich Group

Space: Rooms. 269, 273, 313, 398 McCone totaling approximately 1,000 square feet of lab space.

Equipment Value: $500,000

DePaolo Group

Space: Rooms 5, 465, 467, 469 McCone Hall, totaling approximately 2,000 square feet of lab space.

UC Paid Staff: $21,000 /year in staff support.

Equipment Value: $1.2 million

Manga Group

Space: Rooms 6, 7, and 175 McCone Hall, totaling approximately 2700 square feet of computer and experimental lab space.

Equipment Value: $150,000 value

Boering Group

Space: Rooms DG24-DG26, CG2-CG4 Giauque Lab, Hildebrand Hall, totaling approximately 1,000 square feet of lab space.

UC Paid Staff: $5,000 /year in staff support.

Equipment Value: $800,000

Other Institutions

In addition to the commitment of the University of California, Berkeley, each of the proposing institutions will make available to this effort a variety of facilities and equipment to support NASA's Astrobiology Program. These resources will greatly benefit the implementation of the proposed research effort, the proposed training, education and outreach plan.

The University of Alabama

Dr. Eric Roden occupies an 1100 square foot laboratory in a new energy/environmental sciences building on the University of Alabama campus. Autoclaves for microbiological work are located across the hall. An auxiliary 200 square foot lab is available for reactive transport experiments with sediment cores. Dr. Roden has recently received federal funding for several research projects dealing with microbial Fe transformations in sedimentary environments. The equipment and other infrastructure accumulated through these awards will be fully available for the proposed NAI project. Prof. Roden's laboratory space and associated equipment has a dollar value of at least $0.25 million. Prof. Roden also has direct access to a central Freshwater Analytical Chemistry laboratory, and a Geological Science laboratory, both of which provide a variety of equipment and resources relevant to the NAI project (Volume II, Facilities and Equipment). An Electron Microscopy/Confocal Microscopy Facility housed in the Department of Biological Sciences is also available for research on the project. Each of the above are multi-million dollar facilities supported by both external grants and University funds.

The University of Alabama provides a variety of support mechanisms for research and graduate training in aquatic-environmental sciences which are relevant to the proposed NAI project. The Department of Biological Sciences includes a section in Ecology and Systematics (E&S) which has approximately 15 faculty with research interests in ecology and environmental science. Recently, a University-wide Center for Freshwater Studies (CFS) was initiated at UA. The CFS embraces a broad range of faculty and students with expertise in biological, geochemical, hydrological, and policy/management aspects of freshwater affairs. Several faculty involved in the CFS, together with counterparts at the University of Mexico, were awarded funding for an NSF IGERT project designed to provide interdisciplinary graduate training in ecological, geochemical and hydrological aspects of freshwater aquatic sciences. Prof. Roden, is one of ten UA faculty participants in the IGERT project. The Department of Biological Sciences is also the recipient of a university graduate program enhancement award which includes several graduate research assistantships that are available (on a competitive basis) to support ongoing faculty research lines. The varioussupport structures which these programs provide will be fully available to students and faculty collaborators on the NAI project.

The University of Delaware

Facilities necessary for conducting the proposed research are available at the University of Delaware in Lewes in either Dr. Luther's laboratories or as general equipment. Dr. Luther's laboratory (1500 sq. ft) has a trace metal clean facility (600 sq. ft class 10,000 clean room containing 4 class 100 clean benches) which is equipped with cleaning, storage and analytical facilities. In addition, a 200 square foot class 100 clean laboratory van for use on land and sea is available. UD has an equipment facility, which includes ICp-ES, Alpkem autoanalyzer, Carlo Erbe CNS analyzer.

Seven students have been supported by research assistantships, one more by a teaching assistantship. Also, three postdoctoral students have been supported by research grants and another was supported with his own NSF postdoctoral fellowship. A renovation of $400,000 for trace metal clean facilities has been performed and the estimated total cost of equipment in the laboratory is $600,000. The College of Marine Studies has a fleet of ships available for research; small boats can be rented on an hourly basis and the R/V *Cape Henlopen* is a UNOLS coastal vessel.

American Type Culture Collection

Dr. David Emerson has a research laboratory in a shared ATCC/George Mason University research facility in Manassas, VA. This laboratory has 800 ft^2, and is equipped with a COY anaerobic glove box, multiple incubators, anaerobic gassing station, a PCR machine, a research quality Olympus epiflourescence microscope coupled to a CCD camera, a chemical fume hood, and all the equipment necessary for routine microbiology, including three biological safety hoods. Dr. Emerson also oversees another 800 ft^2 of shared laboratory space for fermentation and process studies. This includes three Applikon fermetners, two 3-liter, and one 5-liter capacity for growing Fe-oxidizers under special conditions, and an HP gas chromatograph for methane and other gas measurements. In addition there is shared instrumentation including digital gel documentation systems, hybridization ovens, high temperature ovens, high speed and ultracentrifuges, spectrophotometers, a scintillation counter, a Bio-Rad Biologic HPLC system, as well as other equipment. For preservation of microorganisms and sensitive biologics, Dr. Emerson has access to a -80°C freezer and a large capacity (4000 vial) liquid nitrogen storage vessel. All freezers and mission-critical equipment are maintained on an alarm system that is monitored 24 hours. His laboratory also has several Macintosh computers (PPC 7500, G# & I Mac), and Pentium-based wintel machines. The total cost of equipment in these facilities is approximately $300,000.

This research complex also houses a state-of-the-art DNA sequencing facility. This includes three ABI 377 flourescent sequencers, two ABI 310 flourescent sequencers, three Robbins 96 well robots, Speed Vacs, and all the front end instrumentation such as preparative centrifuge, agarose gel electrophoresis systems, and acrylamide gel electrophoresis system required for high throughput phylogenetic analysis. He also has access to a network of 36 Silicon Grpahics workstations and a large disk array to support data handling and phylogenetic analysis analysis.

Dr. Emerson also has routine access to the ATCC bacteriology laboratories (he supervises two technicians there, in addition to his research lab.) These labs are well equipped for microbial characterization work; instrumentation includes a MIDI system for fatty acid methyl ester (FAME) analysis, and a riboprinter from Qualicon, as well as a Bio-log system for C-source utilization studies.

SETI Institute

Dr. Bishop's laboratory will be used for sample preparation for spectral analysis. This includes preparation of size separates of samples collected in the field through grinding and sieving. The lab will also be used for weighing, storage and labeling of samples.

Dr. Bishop's office at NASA-ARC is equipped with a computer and software sufficient for the spectral analyses to be performed here, although it is envisioned that these will need to be upgraded over the 5-year duration of the project.

Dr. Bishop also has access to the spectroscopy facilities at the NASA-supported Reflectance Experiment Laboratory (RELAB) at Brown University under the direction of Professor Carlé Pieters. The laboratory is specifically equipped for geologic remote sensing measurements. This includes a bi-directional visible to near-infrared reflectance spectrometer and a biconical Nicolet Nexus FTIR spectrometer. This is a multi-user, NASA-supported facility and a description and user's manual can be found at (http://porter.geo.brown.edu/relab/index.html).

Palo Alto Research Center

Dr. Yim has a lab with a diverse set of prototyping tools including a small 4 axis CNC machine, 100W laser cutter, fused-deposition modeling machine as well as a variety of metal and plastic working tools. These tools can be used to modify already existing modular robotics systems that will be used in this proposal as needed. These include over 200 modules from robot systems that span over 3 generations and multiple iterations of development.

PARC also has a class 100 clean room to perform surface and bulk micromachining on a variety of substrates. The facility contains deposition tools for conductors (e.g., AlCu, MoCr, ITO), semicon-ductors(e.g., poly-Si, a-Si), and insulators (e.g., SiON, SiO2, SiNx).

PARC has many electronics and mechanics prototyping equipment including a 5-axis CNC machine, fused-deposition modeling prototyper, 100W laser cutter, fully equipped and staffed machine room and electronics shop.

A technical information center staffed by trained librarians provides immediate access to technical journals and literature search services. All of these resources will be available to this project.

Parent Science Proposal Title
BIOMARS - BIOsphere of Mars: Ancient and Recent Studies

Education & Public Outreach Proposal Title
Searching for Life on Mars

Summary of Proposed Costs
Year 1: $64, 697; Year 2: $64, 769; Year 3: $64, 710; Year 4: $63, 227; Year 5: $64,477

BIOMARS E/PO Abstract
Key earth, space, and biological science themes encompassed in the BIOMARS Parent Proposal will also serve as the focus for the project's educational and public outreach development efforts. To effectively address these themes from an educational standpoint, BIOMARS' multidisciplinary team of scientists will work closely with educators from the University of California at Berkeley's Lawrence Hall of Science to develop, field-test, and implement a set of materials that employ proven effective teaching and learning strategies. Educational materials that enhance existing middle and high school science curriculum by presenting important, standards-based science and technological education content will be developed. In addition, materials will be produced that increase public awareness and understanding of relevant issues in Astrobiology in particular, and science in general. By integrating aspects of earth science and life science, developed materials will demonstrate the interdisciplinary nature of space exploration. Effective implementation of these materials will be ensured. To achieve BIOMARS educational goals, a partnership has been established between the BIOMARS team of multidisciplinary scientists and science educators from the University of California at Berkeley's Lawrence Hall of Science (LHS). LHS is the university's popular public science center, which is also world-renowned as a leader in the development of high quality, standards-based educational materials based on current research and related understandings of how students think and learn. Project educational materials will be disseminated through the use of the LHS infrastructure, and its well-established national and international network of educators. To ensure that all materials developed effectively address the project's educational goals, all materials will be pilot and field-tested for educational effectiveness prior to their release to the public.

Principal Investigator:
Dr. Jillian Banfield

E/PO Co-Investigators:
Mr. Kevin Cuff (Lead) and Dr. Herbert D. Thier

Goals and Objectives of the E/PO Effort
The key to BIOMARS educational materials becoming truly effective and implemented on a broad scale lies in their ability to become woven into the fabric of contemporary reform-minded education efforts underway throughout the nation and world. As such, our principal goal will be to produce and prepare for dissemination an innovative set of

educational materials that provide strong support to the educational goals of the *National Science Education Standards* (National Academy of Science, 1996), as well as state frameworks and district guidelines. Achieving this goal will help accomplish the related overall project goal of increasing public awareness and understanding of the value and importance of BIOMARS scientific endeavors. To achieve our goals, our main objectives will be to develop materials that:

- Closely correlate BIOMARS-related scientific investigations to inquiry-based learning of important standards-based earth, space, and life science concepts.
- Provide opportunities for students to learn important science content by asking questions, gathering and evaluating scientific evidence, and making decisions based on evidence.
- Encourage students to assess the benefits and tradeoffs associated with their investigations.
- Sustain high levels of enthusiasm and interest, by emphasizing the relationship between students' investigative learning experiences and relevant interests and issues in their own lives.

E/PO Structure and Content

The topics of possible life on Mars, life in extreme environments, and extraterrestrial exploration tend to readily capture public attention. Our group will use the broad appeal of these subjects to engage middle and high school students and general public audiences in activities that increase their understanding of BIOMARS-related content. The education team will work closely with the BIOMARS scientific team to:

1) Generate, test, and implement a project-based learning module for middle and high school science classrooms.
2) Adapt some of these materials for use with the general public.
3) Develop and implement a series of one-day educator training workshops.
4) Develop and implement a series of one-day public events, that include presentations made in a small, public planetarium.
5) Initiate the development of electronic versions of the school and public materials and personal profiles of project scientists.that can be used at home and by schools and public science centers nationwide.

1. BIOMARS Learning Module Development

The module will consist of inquiry-based investigations that provide students with opportunities to evaluate information the way scientists do. They will be project-based in nature, and as such their themes will engage students in grade appropriate, investigative activities whose focus is related to the efforts of the BIOMARS scientific team. Thus, students will work in teams analyzing evidence of past and present fluid distribution and flow on Mars, evidence related to the evolution of Mars' atmosphere and hydrosphere, and evidence of Martian surface feature development throughout its history. They will relate this evidence to the search for and understanding of possible Martian habitats, and use accumulated understandings to make decisions for example on where future sampling on Mars should occur. Overall, the module will be multidisciplinary in nature, and as

such it will enable teachers to enhance their existing space science, life science, geology, and technology classroom curricula. By integrating these subjects the module will present concepts that are closely aligned with those that comprise state and national standards documents, and also demonstrate the interdisciplinary nature of space exploration. A BIOMARS Educator's Guide will be produced for users of the module. Educational resources developed will address the following key concepts in earth, life, and space science: What are the requirements for life on earth as we know it? In what extreme environments does life exist on earth? How do organisms survive, and in fact thrive in extreme environments? What do landscapes and rocks tell us about the past? How are conditions on other planets different than earth? How do we detect life on other planets? Is there life on Mars? What would we see on Mars? How did Mars evolve differently from earth over its 4.5 billion year history? These concepts are aligned with the following Grade 6-12 Content Standards addressed in the *National Science Education Standard*s: Science as Inquiry, Earth and Space Science; Life Science, Physical Science; Unifying Concepts and Processes; Science and Technology; and History and Nature of Science.

The following is a tentative list of specific topics from which activities will be developed for inclusion in the module:

a. Investigating Redox Reactions. Students react Ferrous Chloride with Hydrogen Peroxide to investigate the production of free oxygen as a model reaction for what could take place on Mars.
b. Exploring Sulfur-based Reactions. Students initiate chemical reactions involving Ferrous Sulfate to form various precipitates.
c. Investigating Growth in Acid Environments. Students grow various organisms such as yeast and brine shrimp in varying acidic environments.
d. Life on Earth and Beyond. Students explore the issue of the possibility that biomes on Earth may be different than those found elsewhere.
e. Investigating Mars' Surface. Students examine photographs and maps of Mars to develop an understanding of how its visible features may have formed.
f. Exploring subsurface fluid flow. Students investigate fluid flow by injecting fluids of varying viscosities into gel models, and use what they learn to develop ideas about fluid circulation on Mars.
g. **Lessons from the Martian Atmosphere**. Students discuss the relationship of all education project investigations to the students' interests and responsibilities as a citizen. For example investigations regarding the evolution of life in an atmosphere without free oxygen will be related to issues related to protecting the quality of Earth's atmosphere.

2) Adaptation of Materials for Public Use.
Selected activities from the module will be adapted for use by the general public at Science Centers, other public science facilities and for individual and family use through the World Wide Web.

3) Educator Dissemination Workshops.
As a means of popularizing and disseminating the BIOMARS Educator's Guide, a BIOMARS educator workshop will be held at Lawrence Hall of Science each project

year. These workshops will be attended by middle, high school and informal educators from throughout the greater San Francisco Bay Area, who will be recruited from a pool of individuals associated with LHS teacher and informal educator professional development programs. The workshops will include the presentation of background information on the pertinent science and instructional practices associated with the module, as well as direct hands-on experience with its investigations.

4) One-day Public Events . A series of public events will be designed and held, which will be entitled: Mars Astrobiology Days. These events will involve both the project's scientific and educational teams. Presented will be interactive public activities describing the BIOMARS project and its findings, particularly in relation to the lives of the attendees. In addition attendees will have the opportunity to experience some of the activities described in 2 above. Events will take place first at LHS, and possibly later at other Science and Technology Center sites, such as the New York Hall of Science. Presentations will be videotaped for possible classroom use and/or display on a project-related Web site. In addition, programs that include photographs, diagrams, and hands-on activities that highlight research of the BIOMARS scientific team will be presented in the LHS interactive planetarium. Once fully developed and tested, BIOMARS-related planetarium presentations will be inserted into the rotation of standard LHS planetarium program offerings.

5) Scientific Team Profiles. Development of electronic profiles of members of the project's scientific team, including its lead PI, Professor Banfield, will be initiated. These profiles will present a given scientist in an informal, personal manner, by tracing their development from childhood to the present. The profiles are intended to show the public how nascent interests and influences may result in careers within particular fields of study. Development of these profiles will complement an LHS initiative that is in the early stages of development, which is intended to help increase public understanding of science by displaying such profiles in portable kiosks that reside at the Boston Museum of Science, the Minnesota Museum of Science, and LHS.

Capability And Commitment Of The Proposer And The Proposer's Team

Professor Jillian Banfield is the PI for the parent proposal and is fully committed to be a participating member of the E/PO team. Professor Banfield has had significant experience in the development of general science courses and demonstrations designed to capture the interest and to effectively teach science concepts in undergraduate courses for non-science majors (<http://www.geology.wisc.edu/%60jill/306.html eudora="autourl"> http://www.geology.wisc.edu/`jill/306.html). She has designed an on-line course "Science Behind the News" to appeal to high school and undergraduate students and K-12 teachers seeking continuing education opportunities. Development of BIOMARS educational materials will allow her to work with the other science team members and E/PO specialists from LHS to incorporate the science and technological content of her research into the space, earth and biological science curriculum of classroom teachers.

A team of educators from LHS will coordinate BIOMARS E/PO efforts under the leadership of Kevin Cuff and Dr. Herbert D. Thier. Kevin Cuff is a project director within LHS' Center for Curriculum Innovation (CCI), and the former director of LHS

geology education group. Over the past decade, Mr. Cuff has directed several CCI endeavors that have resulted in the development of project-based earth and physical science materials that have been published and have achieved widespread circulation. These have included numerous modular units and courses that convey pivotal concepts and help promote the development of essential understandings and inquiry abilities. These materials are also actively used as tools to promote innovation in the preparation of secondary science teachers and graduate preparation in science education. As a ranking member of the CCI staff, Mr. Cuff has intimate knowledge of, and ready access to its teacher professional development networks, which are used to provide ongoing support and education in the use of innovative materials and methods developed at the center. BIOMARS E/PO efforts will greatly complement those of other CCI groups who currently contribute to NASA-funded programs that support education and outreach efforts, including the Kepler Project. Working closely with Mr. Cuff will be Dr. Herbert Thier, the former Academic Administrator and the founding director of the Science Education for Public Understanding Program (SEPUP) at LHS. He has been a leader in the field of inquiry-based instructional materials development for over four decades. In addition to SEPUP, Dr. Thier has played a major role in the development of numerous other renowned programs that together have produced materials used by many millions of students, teachers and other educators nationwide and throughout the world.

Project Management Structure

The management structure for the E/PO component of the proposal will be collegial and cooperative. The strong commitment of the overall Project PI to E/PO activities will help the E/PO team to build strong cooperative relationships with the various project scientists so that their accomplishments can help inform the E/PO effort. All materials developed by the E/PO component of the project will be submitted for scientific review by project scientists. As CoIs, Mr. Cuff and Dr. Thier and will work closely on the overall design and carrying out of the E/PO effort. CoI Cuff will be responsible for day to day efforts of the E/PO project. Furthermore, relationships will be established with CCI projects such as Great Explorations in Math and Science (GEMS), Full Option System Science (FOSS), and SEPUP to ensure that BIOMARS E/PO materials and experiences provide support to widely adopted and used materials developed by these projects.

Contribution to Nationally Recognized and Endorsed Education Reform Efforts

Development of BIOMARS educational materials will result in science learning experiences that stimulate the evolution of a deeper understanding of the nature of science; particularly its reliance on inquiry, creativity, and labor performed by teams of people working toward a common end. In addition, by embedding key inquiry and science concepts in a project-based format, the BIOMARS learning module will provide an innovative set of multidisciplinary investigative experiences that foster changes in the way science is taught and learned. Use of module materials in classrooms and with the public will help develop student and public understanding of science. Furthermore, BIOMARS E/PO educational materials will be congruent with NSES recommendations, which stress the need for new emphasis on inquiry-based learning that focuses on development of deeper understandings of science embedded in the everyday world.

Broader Impact of the Implementation of E/PO Materials

Presentations and materials will be developed that contribute to an improvement in science literacy by promoting increased understandings of the nature of science in general, and that increase public awareness and understanding of BIOMARS scientific endeavors in particular. To ensure that these resources are beneficial to users from a diverse set of socio-economic backgrounds, they will incorporate methodologies such as hands-on inquiry, collaborative learning, multi-sensory observation, and interdisciplinary explorations. Given their emphasis on providing the public with access to scientists engaged in cutting-edge scientific research of great interest to the public at large, components such as the Mars Astrobiology Days, along with their associated planetarium programs will likely become a part of LHS regular public outreach programming. Through the established connections that the LHS-based E/PO team has with museums such as the New York Hall of Science, the Boston Museum of Science, and the Natural History Museum of Los Angeles County, the finished BIOMARS educational materials will be made available to these and other public science centers. The BIOMARS Educator Guide will encourage students to investigate relevant questions and issues from a project or problem based approach that helps them to develop a real and personal interest in an area of concern. The guide will constitute a new educational resource that provides opportunities for students to effectively learn and apply important inquiry skills, while also learning key standards-based earth, biological, and physical science concepts. The interdisciplinary, standards-based nature of the guide will make it easier for the investigations in it to be infused into classroom curricula, which will make the guide more desirable to teachers. Successful development of the guide will therefore lead to the creation of a highly useful, exemplary model of a multidisciplinary, project-based educational resource that can be used to integrate science, mathematics, and technology education studies. This model, along with other project-related findings and educational products will become disseminated throughout the science education community through publication within the LHS Web site and presentations at meetings and conventions. In addition, all educational products will be disseminated through the LHS established partnerships with school districts throughout the United States.

Evaluating the Effectiveness and Impact of the Proposed E/PO Efforts

In addition to piloting and field-testing the BIOMARS educational module and presentations and activities prior to public implementation, we intend to evaluate their infusion into core science programs being used in schools and science centers locally and nationwide. We will do this by tracking not only the degree to which project products are included in the offerings of LHS and its partner science center/museum public programs, but also reports from the field regarding the actual use of the materials in schools. One way that we will accomplish this is by offering potential users materials they can request on the Web. Then we will evaluate the use of the materials placed on the Web by monitoring the number of individuals who register to use them (free of charge), and also by tracking requests for further information and materials as described above. We will also survey teachers who use the materials and attendees at project Public Outreach events to get their impressions of the value of the materials and presentations and how to improve them.

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From Stars to Genes: An Integrated Study of the Prospects for Life in the Cosmos

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From Stars to Genes: an Integrated Study of the Prospects for Life in the Cosmos

Proposal to Participate in the NASA Astrobiology Institute – Cycle 3

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Executive Summary

Building on seven years of experience and accomplishments in the planning, implementation, and operation of the NASA Astrobiology Institute, UCLA lead team members propose to embark on another five-year plan of research, education, and public outreach in astrobiology that makes full use of the remarkable sets of talents and tools that have been assembled for these purposes over the past five years. The UCLA Center for Astrobiology (CAB) is one of five centers of the UCLA branch of the University of California-wide Institute of Geophysics and Planetary Physics (IGPP) and one of the eleven founding lead teams of the NASA Astrobiology Institute (NAI). Research, education, and public outreach activities of the Center are aimed at the long-term questions and goals of astrobiology, namely: How do planetary processes give rise to life? How are planets formed? Are there habitable planetary bodies outside our Solar System? When and how did life appear on Earth and, possibly, Mars? What are the biological innovations that allow life to climb to complexity on planetary timescales?

The team consists of more than 50 investigators. If this proposal is successful, UCLA will contribute ~ $1.3 million to match the $7.2 million requested of NASA. In addition to the University contribution, and in the spirit of the Cooperative Agreement, CAB participants bring to the project a wide range of skills and resources. Our plan calls for distributing these resources in ways that maximize the scientific and educational output beyond that which would be achieved by the investigators on an individual basis. We will use a method of strategic allocation of funds to promote interdisciplinary research that would be difficult to carry out without the existence of the Center. Examples of interdisciplinary research planned for the next five years include: collaborations between astronomers, atmospheric chemists, and cosmochemists that are leading to entirely new theories for the way that our Solar System evolved; the bringing together of paleontologists, atmospheric chemists, and isotope geochemists to study atmospheric evolution on ancient Earth; integration of advanced orbital and rotational dynamical calculations into studies of mass extinctions and the climate history of Mars; and collaborations between paleontologists and microbiologists with the goal of understanding the origins of eukaryotic life. In some cases the impetus for interdisciplinary work proposed here has come from merely bringing workers with disparate backgrounds together at CAB meetings. In other cases CAB funds will allow individuals to have access to facilities they might not have been aware of, much less had access to, previously. In still other cases the support of a student or a researcher will allow an investigator to participate in transdisciplinary studies that they would not otherwise engage in. In all cases, the new avenues of research will further the goals of astrobiology as a discipline and will be possible through the activities of the UCLA Center for Astrobiology in the coming years.

The title of this proposal reflects the breadth of research to be carried out if it is funded. Our investigators and collaborators have expertise in astronomy, cosmochemistry, geology, geobiology, geophysics, geochemistry, paleontology, planetary science, microbiology, organismic and molecular biology, and, of course, astrobiology. They represent UCLA's Departments of Atmospheric Sciences, Chemistry & Biochemistry, Earth & Space Sciences, Mathematics, Microbiology, Immunology & Molecular Genetics, Molecular, Cell & Developmental Biology, Organismic Biology, Ecology & Evolution, Physics & Astronomy, and the Molecular Biology Institute, as well as many other off-campus institutions.

The team assembled for this proposal includes a comparatively large (relative to our previous group) number of astronomers and cosmochemists. This reflects our goal of assuming a leadership role in helping to strengthen the astro component of astrobiology, as suggested by the National Research

Council's Committee on the Origins and Evolution of Life in their 2002 assessment of NASA's astrobiology program. While astronomers may not work directly with microbiologists, CAB astronomers will work with the cosmochemists in ways that they have not before, and the cosmochemists in turn will work with geochemists who at the same time will collaborate with microbiologists in novel ways. In this fashion, progress in a given area will be informed by work in even the most dissimilar discipline when the need arises. Such a *chain of informed inquiry* is crucial to a field that seeks to define the range of possible habitable environments in all planetary systems and to match this range of habitats with the full spectrum of possible forms of life.

The UCLA astrobiology research plan for the next five years focuses on four themes: (1) Extrasolar Planetary Systems; (2) Habitability of Planets and their Satellites; (3) Earth's Early Environment and Life; and (4) Evolution of Biological Complexity. UCLA lead team members have considerable experience in each of these areas. Their accomplishments range from the recent detection of asteroids and comets around another star to identifying the earliest evidence for life on Earth. Some members of our team have been at the forefront of technological advances in measuring isotope ratios at microscopic scales in natural materials, capabilities that are central to many of the studies planned as part of this program. Others are Principal Investigators or Co-Investigators on exciting new astronomy missions that are equally important to our proposed research program. Still others are leaders in the fields of microbiology and paleontology.

How typical is our Solar System? This is a key question that arises when one attempts to gauge the likelihood for life elsewhere in the Galaxy. UCLA lead team members are beginning to answer this question by studying both extrasolar planetary systems and the Solar System itself. The most successful method for detecting planets beyond the Solar System has been to measure the wobbles exerted by planets on their stars. The method, by its very nature, is biased towards detecting giant planets in close proximity to the star. It is generally recognized that such planetary systems – with giant planets much closer to their stars than Jupiter is to the Sun – are unlikely to harbor life. Members of the UCLA team are developing and applying methods more suitable for detecting planetary systems that more closely resemble the Solar System. The underlying assumption is that these systems are more likely to have Earth-like planets. Even where planets can not be detected, our group proposes to search for indirect evidence for planet formation. Rock and ice debris around some stars may signify the existence of comets and asteroids, the precursors to, or vestiges of, rocky planets that could resemble Earth. We will do all of this with access to the finest tools, including the Keck observatories and new platforms for observations from space (SIRTF) and in the stratosphere (SOFIA) that permit imaging with infrared wavelengths with minimal interferences from air, as well as various radio telescope facilities.

Looking over the shoulders of the astronomers are those members of our team studying meteorites and the story these rocks have to tell about the origin of the Solar System. By virtue of their involvement in the Center for Astrobiology, the cosmochemists and astronomers are working together to discover the ways in which water might be incorporated into Earth-like planets, and whether photochemistry in the disks of dust and gas surrounding young stars is instrumental in determining the nature of the organic and inorganic building blocks of planets. This synergistic activity has already resulted in proposals for new astronomical measurements that would not otherwise have been made.

While hunting for extrasolar planetary systems and comparing them to the Solar System is requisite for gauging the prospects for life elsewhere, it is not sufficient. It is also necessary to define those objects that are habitable. Our team is addressing this issue by studying the factors that might control the habitability of the icy moons of Jupiter, the time-dependent climate of Mars, potential links between the dynamics of rocky planets and their long-term habitability, the influence of orbital

dynamics on the prospects for Earth-like planets in extrasolar planetary systems, and the role that impacts play is survival and evolution of life.

Manifestations of Earth's earliest life are controversial and our understanding of the environment that nurtured early life on Earth is poor. Sophisticated isotopic analyses of Earth's oldest materials, including >4.0 billion year old zircon crystals and 3.9 to 2.5 billion year old sulfur minerals will be used by our team to determine the ages of Earth's atmosphere, hydrosphere, and geodynamo, relate these ages to the earliest signs of life, and assess how the emergence of life changed our planet. Experiments will be performed to investigate interactions between ancient microorganisms and their environs. Chemical interactions between organisms and their inorganic and organic environs will be monitored using the newly developed transition metal isotope systems. The pooled results from these disparate studies will provide new information about the antiquity of life on Earth and provide a firm basis for life-detection on other bodies in the Solar System.

The appearance of Eukaryotes in Earth history coincided with dramatic changes in the planet's climate, ocean chemistry, atmospheric chemistry, and tectonic configurations, suggesting that emergence of biological complexity may be linked to evolving climate. Understanding the early part of this progressive history may therefore reveal general principles that are applicable to the growth of complexity in any living system. UCLA lead team investigators are studying the evolution of eukaryotes using information from molecular biology and the fossil record. The goals are to better understand the order in which important universal properties of eukaryotes (nucleus, sterols, cytoskeleton, endoplasmic reticulum, organelles, multicellularity, etc.) were acquired, and to try to date these events using both the fossil record and molecular clocks. The nature of the common ancestor and the source of the prokaryotic donations to the genome(s) of the last common ancestor of all living eukaryotes are outstanding problems in evolutionary biology that will be addressed by our work. As part of this effort, CAB members will also examine the steps by which eukaryotes obtained and lost their energy-producing organelles.

The Cambrian period of Earth history was a time of rapid evolutionary innovation. The exponential advance in animal diversity and complexity near the start of the Cambrian demands understanding as it is one of the signature features in the evolution of life on our planet. Our group will examine this rapid advance by studying the morphological development of trilobites, the emergence of skeletons by searching for the responsible developmental genes, and fossils that represent stem groups of modern phyla that reveal the ways in which animal body plans developed.

The UCLA Center for Astrobiology will continue to be engaged in numerous activities that serve to strengthen the field of astrobiology in general. These activities include continued support of the UCLA Astrobiology Society, introduction of an Astrobiology General Education course, participation in the NAI Minority Institution Involvement Faculty Sabbatical Program, participation in the Minority Institution Astrobiology Collaboratory (MIAC), organizing public lectures, and convening of Rubey Colloquia, which serve as forums for rapidly developing topics of interest. The Astrobiology Society is the first student-run organization devoted to fostering the discipline of astrobiology at the university level. In the coming years the Society will be engaged in replicating their success at other institutions, especially those serving minorities in particular. UCLA was one of the first hosts in the new Faculty Sabbatical Program and this connection has resulted in a closer tie between our lead team and the MIAC.

Research and Management Plan

1 INTRODUCTION

BUILDING on seven years of experience and accomplishments in the planning, implementation, and operation of the NASA Astrobiology Institute, UCLA lead team members propose to embark on another five-year plan of research, education, and public outreach in astrobiology that makes full use of the remarkable sets of talents and tools that have been assembled for these purposes over the past five years. Research will focus on four themes: (1) Extrasolar Planetary Systems; (2) Habitability within the Solar System and Beyond; (3) Earth's Early Environment and Life; and (4) Evolution of Biological Complexity. Sections of the proposal that describe the research plans of each of these themes are cross-referenced in Table 1 with the seven goals of the NASA Astrobiology Roadmap (astrobiology.arc.nasa.gov/roadmap). The UCLA team is well placed to advance Astrobiology - the discipline - as this exciting new field increasingly contributes to the recruitment of young people into careers in science and technology and to long-term planning for NASA missions.

The UCLA Center for Astrobiology (CAB) is one of five centers of the UCLA branch of the UC-wide Institute of Geophysics and Planetary Physics (IGPP) and one of the eleven founding lead teams of the NASA Astrobiology Institute (NAI). The Center is composed of faculty, researchers, students, and staff, plus collaborators at other institutions, who have expertise in astronomy, cosmochemistry, geology, geobiology, geophysics, geochemistry, paleontology, planetary science, microbiology, organismic and molecular biology, and, of course, astrobiology. Research, education, and public outreach activities of the Center are aimed at the long-term questions and goals of astrobiology, namely: How do planetary processes give rise to life? How were planets formed? Are there habitable planetary bodies outside our Solar System? When and how did life appear on Earth and, possibly, Mars? What are the biological innovations that allow life to climb to complexity on planetary timescales?

Clearly, answers to these questions will require advances in a broad spectrum of disciplines. It will be necessary to establish a firm astrophysical context for the birth and evolution of our Solar System if we are to assess the likelihood for similar systems elsewhere. We will require a better knowledge of the diverse forms that life might take in different environments if we are to gauge the habitability of bodies in the Solar System and beyond. Conversely, searches for the bounds of habitability will be guided by the range of environments found to exist on other planets and their satellites.

In order to begin to answer these transdisciplinary questions, and explain them to others, special people and special tools are required. These are listed below. This proposed investment of time and real resources ($1.3 million) represents a substantial commitment by UCLA and the associated institutions to the success of the cooperative endeavor known worldwide as the NASA Astrobiology Institute.

Table 1. The UCLA CAN-3 proposal in the context of the 2002 Astrobiology Roadmap

Astrobiology ROADMAP Goals	**UCLA theme EXTRASOLAR PLANETARY SYSTEMS**	**UCLA theme HABITABILITY IN PLANETARY SYSTEMS**	**UCLA theme EARTH'S EARLY ENVIRONMENT AND LIFE**	**UCLA theme EVOLUTION OF BIOLOGICAL COMPLEXITY**
Goal 1 Habitable Planets	Proposal §5	Proposal § 6.2, 6.3, 6.4		
Goal 2 Life in our Solar System		Proposal § 6.2, 6.5, 6.6, 7.2		
Goal 3 Origins of Life			Proposal § 7.4, 7.5	
Goal 4 Earth's Early Biosphere and its Environs			Proposal § 7.2, 7.3, 7.4, 7.6	
Goal 5 Evolution, Environment, and Limits of Life				Proposal §8
Goal 6 Life's Future on Earth and Beyond		Proposal §6.5		
Goal 7 Signatures of Life	Proposal §5.2		Proposal §7.5	

2 UCLA LEAD TEAM INVESTIGATORS

David G. Agresti (Collaborator) is Professor of Physics, Department of Physics, The University of Alabama, Birmingham (UAB). Agresti is the PI for the UAB Raman imaging facility and will work with Schopf on samples containing ancient fossil microbes (§ 7.5)(agresti@uab.edu).

Jonathan Aurnou (Collaborator) is Assistant Professor in the Department of Earth and Space Sciences. He is an experimental fluid dynamicist interested in understanding how conductive fluid flow generates magnetic fields. He will work with numerical modelers (§ 6.3) to explore planetary conditions needed for magnetic fields and plate tectonics, both possibly important for habitability (aurnou@ucla.edu).

Stanley M. Awramik (Collaborator) is Professor in the Department of Geological Sciences at the University of California, Santa Barbara. He studies Precambrian microfossils and stromatolites and will contribute his many years of field experience (awramik@geol.ucsb.edu).

Eric E. Becklin (Collaborator) is Professor in the Department of Physics and Astronomy, and Director Designate of the NASA Stratospheric Observatory for Infrared Astronomy (SOFIA). Becklin's research deals with infrared observations in and beyond the Solar System. Here, he proposes (§ 5.2) the modification of an existing SOFIA instrument to allow for the detection of organic molecules in these environments (becklin@astro.ucla.edu).

Gary R. Byerly (Collaborator) is the Robey H. Clark Professor in the Department of Geology and Geophysics, Louisana State University. He and Lowe have decades of experience mapping the early Archean Barberton Greenstone Belt in Africa. They and their collaborators propose (§ 6.5) to further explore the Archean history of large impacts and their possible effects on early life (gbyerly@geol.lsu.edu).

James Farquhar (Collaborator) is Assistant Professor in the Department of Geology at the University of Maryland. His research applies stable isotope geochemistry to atmosphere-surface interactions, atmospheric evolution, sulfur and oxygen biogeochemistry, meteorite studies, isotopic exchange, and geothermometry. Proposed collaborative research on mass-independent sulfur isotope effects (§ 7.3) is aimed at understanding the unusual chemistry as well as using these effects to probe the Archean environment (jfarquha@essic.umd.edu).

Sorel T. Fitz-Gibbon (Collaborator) will be an Assistant Research Molecular Biologist (from July 1, 2003) in the Institute of Geophysics and Planetary Physics. Having assembled and annotated the genome the hyperthermophile *Pyrobaculum aerophilum*, she became concerned with both whole-genome comparisons and the evolution of methane and sulfur metabolisms. She will contribute to the proposed (§ 7.3) collaborative research on the early history of sulfur cycling (sorel@ucla.edu).

James G. Gehling (Collaborator) is Research Scientist in Palaeontology at the South Australian Museum. His work over several decades on the end-Precambrian Ediacaran biotas will be used (§ 8.3) to examine how global ("snowball Earth") glaciations influenced the evolution of complex multicellular life (Gehling.Jim@saugov.sa.gov.au).

Andrea M. Ghez (Co-Investigator) is Professor in the Department of Physics and Astronomy and the Institute of Geophysics and Planetary Physics. Ghez's work is focused on using and developing high spatial resolution imaging techniques to study the formation of stars and planets. She is Associate Director of Astronomical Science for the National Science Foundation's Technology Center for Adaptive Optics. Here, she proposes (§ 5.2) to investigate grain growth in early evolving planetary systems (ghez@astro.ucla.edu).

Michael Ghil (Collaborator) is Professor in the Department of Atmospheric Sciences and the Institute of Geophysics and Planetary Physics and also holds a chair at the École normale supérieure, Paris. His main research interests are in climate dynamics; here, he will contribute (§ 8) mathematical tools to an understanding of the evolution of complexity (ghil@lmd.ens.fr).

Kathleen Grey (Collaborator) is in the Mineral and Petroleum Resources Branch of the Geological Survey of Western Australia and is a member of the Australian Centre for Astrobiology. She studies Precambrian fossils and microfossils with a view to understanding their biology and time significance. She will collaborate (§ 8.2) on assembling paleontological evidence for the Proterozoic history of eukaryotes (kath.grey@doir.wa.gov.au).

Brad Hansen (Co-Investigator) is Assistant Professor in the Department of Physics and Astronomy and the Institute of Geophysics and Planetary Physics. Hansen is investigating the evolution of planetary systems, including the inward migration of giant planets and the dynamical interactions between asteroids and comets and planets. He proposes (§ 5.4) to investigate theoretically the interaction of

planetismals with the giant planets being discovered in extra-Solar planetary systems as well as the accumulation processes of terrestrial planets in our own and other systems (hansen@astro.ucla.edu).

T. Mark Harrison (Co-Investigator) is Professor in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics, and is currently the Director of the Research School of Earth Sciences at the Australian National University. Harrison applies his expertise in isotope geochemisty to broad problems to fields that include Himalayan tectonics, Earth's oldest materials, and the early evolution of life. He and colleagues aim (§ 7.2) to obtain sufficient material from 4.0+ billion-year-old minerals to allow them to investigate Earth's earliest atmosphere and hydrosphere (mark.harrison@anu.edu.au).

Christopher H. House (Collaborator) is Assistant Professor in the Department of Geosciences at Pennsylvania State University and a member of the Penn State Astrobiology Research Center. He studies geomicrobiology using living and fossil organisms, genomics, and novel analytical techniques. He will collaborate on two aspects of the proposed research - the characterization of Earth's earliest life (§ 7.4) and the evolution of microbial metabolisms (§ 7.5) (chouse@geosc.psu.edu).

David K. Jacobs (Co-Investigator) is Professor in the Department of Organismic Biology, Ecology, and Evolution and a Member of the Molecular Biology Institute. Jacobs studies the role of developmental genes in the animal evolution. His lab will investigate (§ 8.3) therole of genes involved in the production of mineral skeletons as possible triggers for the Cambrian explosion of bilaterian animals (djacobs@ucla.edu).

Patricia J. Johnson (Co-Investigator) is Professor in the Department of Microbiology, Immunology, and Molecular Genetics and a Member of the Molecular Biology Institute. Johnson's work with human parasites led to her interest in the origins of energy producing organelles in the evolution of eukaryotes. Her proposed (§ 8.2) work on the origin of energy-producing organelles will contribute significantly to a collaborative study of the origins of eukaryotes (johnsonp@ucla.edu).

Per M. Jögi (Collaborator) is Programmer/Analyst in the Institute of Geophysics and Planetary Physics. He is using the mathematical methods of condensed matter physics to model the growth of the earliest stromatolites (§ 7.5) (jogi@physics.ucla.edu).

Michael A. Jura (Collaborator) is Professor in the Department of Physics and Astronomy. Jura studies the astrophysics of materials in the interstellar medium as well as debris, comets, and asteroids around other stars. He proposes (§ 5.2) to use ground-based and SIRTF observations to study protoplanetary dust in extra-Solar systems (jura@astro.ucla.edu).

Isaac R. Kaplan (Collaborator) is Professor Emeritus in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics. His enormous experience in stable isotope biogeochemistry and environmental geochemistry will be available to members of the team working on isotope fractionations, organic molecules, and biochemical pathways (§6.6, 7.3, 7.4, 7.5) (irk@zymaxusa.com).

Abby Kavner (Collaborator) is Assistant Professor in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics. She will work on investigations of iron isotope fractionations using electrochemical techniques (§ 7.4) (akavner@igpp.ucla.edu).

Artem Kouchinsky (Collaborator) is a Postdoctoral Researcher in the Institute of Geophysics and Planetary Physics working primarily in the Jacobs lab on the origins of mineral skeletons and their effects on the evolution of carbon cycling during and after the Cambrian explosion (§ 8.3) (akouchin@ucla.edu).

Anatoliy Kudryavstev (Collaborator) is Research Assistant Professor of Physics, Department of Physics, The University of Alabama, Birmingham (UAB). Kudryavstev works in the UAB Raman imaging facility and will collaborate with Schopf on samples containing ancient fossil microbes (§ 7.5) (wdowiak@uab.edu).

Frank T. Kyte (Co-Investigator) is an Adjunct Associate Professor in the Department of Earth and Space Sciences and an Associate Research Geochemist in the Institute of Geophysics and Planetary Physics. Kyte documents (§ 6.5) the geological and geochemical evidence for impacts on Earth ranging in age from the Archean to the present (kyte@igpp.ucla.edu).

James A. Lake (Co-Investigator) is Professor in the Department of Molecular, Cell, and Developmental Biology and a Member of the Molecular Biology Institute. Lake's research is concerned with organismic and genomic evolution, the history of hyperthermophilic prokaryotes (§ 7.6), and the origin of eukaryotes (§ 82) (lake@mbi.ucla.edu).

Donald R. Lowe (Co-Investigator) is Professor in the School of Earth Sciences at Stanford University. Lowe is a sedimentologist who focuses his research on the history of Earth's early environment, particularly the role of large early impacts (§ 65) and the context of the early evolution of life (lowe@pangea.stanford.edu).

James R. Lyons (Co-Investigator) is Assistant Research Geochemist in the Institute of Geophysics and Planetary Physics. Lyons works on the generation and transfer of isotopic signatures in planetary atmospheres. He proposes to study the photochemistry of oxygen and sulfur in the atmospheres of Earth (§ 7.3), Mars (§ 6.6) and the early Solar nebula (§ 5.4) (jrl@ess.ucla.edu).

Kevin D. McKeegan (Co-Investigator) is Professor in the Department of Earth and Space Sciences and Director of the ion microprobe laboratory of the W. M. Keck Foundation Center for Isotope Geochemistry. His research relevant to this proposal includes origins of isotope anomalies in the early solar nebula (§ 5.4), the age of Earth's hydrosphere (§7.2), sulfur cycles (§ 7.3), and paleobiology (§ 75). McKeegan is a Co-Investigator of NASA's GENESIS Discovery mission and a member of the science team for STARDUST (kdm@ess.ucla.edu).

Ian S. McLean (Collaborator) is a Professor in the Department of Physics and Astronomy and Director of the UCLA Infrared Imaging Detector Laboratory. He is known for early work on astronomical polarimetry, both visible and infrared, and recent work on infrared studies of star-forming regions, low-mass stars, the Galactic Center, and primeval galaxy formation. He will work on instrumentation for SOFIA (§ 5.2) (mclean@astro.ucla.edu).

Craig E. Manning (Co-Investigator) is Professor in the Department of Earth and Space Sciences. Manning is an experimental geochemist and petrologist working on a wide range of problems that includes the structure of supercritical fluids, the permeability structure of Earth's crust, and the role that hydrothermal systems may have on the origin and early evolution of life (§ 7.4) (manning@ess.ucla.edu).

Rudolf A. Marcus (Collaborator) is Arthur Amos Noyes Professor of Chemistry at the California Institute of Technology. He received the Nobel Prize in chemistry in 1992 for his contributions to the theory of electron transfer reactions in chemical systems. He will collaborate (§ 7.3) on understanding the chemistry of mass-independent isotopic effects in sulfur compounds (ram@caltech.edu).

Stephen J. Mojzsis (Collaborator) is Assistant Professor in the Department of Geological Sciences and a member of the Center for Astrobiology at the University of Colorado, Boulder. He is a geologist who studies all aspects of the Hadean and Archean Earth from an astrobiological perspective. He will work with Harrison on the earliest evidence for life on Earth (§ 7.2) (mojzsis@colorado.edu).

William B. Moore (Collaborator) is a Postdoctoral Researcher in the Department of Earth and Space Sciences working on the geophysics of the Galilean moons of Jupiter, especially Europa. His work (§ 6.2) will contribute to the understanding of Europa needed for planning a future astrobiological mission (bmoore@avalon.ess.ucla.edu).

Mark R. Morris (Co-Investigator) is Professor in the Department of Physics and Astronomy. He studies galactic nuclei, mass loss from giant stars, and the photochemistry of protoplanetary disks.

Morris will use infrared observational techniques to study photochemistry (§ 5.4) and grain growth (§ 5.2) in protoplanetary disks (morris@astro.ucla.edu).

William I. Newman (Co-Investigator) is Professor in the departments of Earth and Space Sciences, Mathematics, and Physics and Astronomy. Newman's research focus is on the dynamical evolution of the early Solar System, the response of planetary atmospheres to catastrophic impacts (§ 6.5), and problems in astrophysics (win@ucla.edu).

Francis Nimmo (Collaborator) is an Adjunct Assistant Professor in the Department of Earth and Planetary Sciences. Nimmo is a planetary scientist studying the structure and evolution of planets and satellites, especially Mars, Venus, and Europa. His work (§ 6.2) will contribute to the understanding of Europa needed for planning a future astrobiological mission (nimmo@ess.ucla.edu).

David A. Paige (Collaborator) is an Associate Professor in the Department of Earth and Space Sciences. His research is aimed at understanding the role that volatiles have played in the evolution of Mars and the evolution of planetary atmospheres in general. He proposes to analyze existing Mars datasets in order to assist in the planning of future astrobiological missions to Mars (§ 6.6) (dap@mars.ucla.edu).

Susannah M. McG. Porter (Collaborator) is NAI National Research Council Postdoctoral Research Associate in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics. She is incoming Assistant Professor in the Department of Geological Sciences at the University of California, Santa Barbara and proposes collaborative research on the evolution of eukaryotes (§ 8.2) and the Cambrian explosion (§ 8.2-8.3) (suporter@ucla.edu).

Maria C. Rivera (Collaborator) is an Assistant Research Molecular Biologist in the Institute of Geophysics and Planetary Physics and the Molecular Biology Institute. She will investigate the evolution of microbial metabolisms (§ 7.6) and the prokaryotic sources of eukaryote genes (§ 8.2) (rivera@mbi.ucla.edu).

Alan E. Rubin (Collaborator) is Associate Researcher in the Institute of Geophysics and Planetary Physics who works mainly on the originally molten components of meteorites (chondrules). He will help understand sulfur isotope effects in the solar nebula and on early Earth using sulfides in primitive meteorites (§ 7.3) (aerubin@ucla.edu).

Bruce N. Runnegar (Collaborator) is Professor in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics and a Member of the Molecular Biology Institute. He is currently on extended leave, as Director of the NASA Astrobiology Institute, at the NASA Ames Research Center. His research is concerned with the history of early life on Earth using evidence from geology (§ 7.5), paleontology plus molecular biology (§ 7.6,8.3), and stable isotope geochemistry (§ 7.3) (bruce.n.runnegar@mail.arc.nasa.gov).

Stanley P. Sander (Collaborator) is group leader of the Chemical Kinetics group at the Jet Propulsion Laboratory. Sander works on laboratory studies of the kinetics of gas-phase reactions of relevance to the Earth's stratosphere. He has extensive experience with discharge flow tubes, and will provide technical guidance for the proposed (§7.3) sulfur isotope flow tube studies (stanley.sander@jpl.nasa.gov).

Edwin Schauble (Co-Investigator) is an incoming Assistant Professor (from July 1, 2003) in the Department of Earth and Space Sciences who will teach and do research in astrobiology. He proposes exploring the ways (§ 7.4) in which stable isotopes of metals might be used to track transport between biological and inorganic reservoirs (edwin@gps.caltech.edu).

J. William Schopf (Co-Investigator) is Professor in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics and a Member of the Molecular Biology Institute. He directs the IGPP's Center for the Study of the Origin and Evolution of Life (CSEOL). Schopf's research

deals with evidence for the antiguity of life on Earth (§7.5) and the evolution the biosphere during the Precambrian (schopf@ess. ucla.edu).

Gerald Schubert (Co-Investigator) is Professor in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics. He is concerned with the formation and evolution of planetary bodies and understanding their physical states. A recent focus has been the structure and evolution of the Galilean moons of Jupiter, work he proposes to continue here (§ 6.2) (schubert@ess.ucla.edu).

Ralph Y. Shuping (Collaborator) is a Postdoctoral Researcher in the Department of Physics and Astronomy. Shuping has been studying the protoplanetary disks of Orion using near infrared techniques. He proposes to use infrared observational methods to test for self-shielding by CO in protoplanetary disks (§ 5.4) (shuping@astro.ucla.edu).

Inseok Song (Collaborator) is an Assistant Research Astronomer in the Institute of Geophysics and Planetary Physics and the Department of Physics and Astronomy. He is an observer who has been using spectral features to find the youngest, nearest stars to Earth that are targets for proposed investigations (§ 5.3) that may lead to the first direct images of planets (song@astro.ucla.edu).

Paul J. Tackley (Collaborator) is Associate Professor in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics. Tackley uses complex numerical simulations to understand the dynamical and chemical evolution of the interiors of Earth and the other planets and moons. He will work with experimentalists (§ 6.3) to explore planetary conditions needed for magnetic fields and plate tectonics, both possibly important for habitability (ptackley@ess.ucla.edu).

Ference Varadi (Co-Investigator) is an Associate Research Geophysicist in the Institute of Geophysics and Planetary Physics. Varadi studies the long-term effects of planetary orbits on the evolution of bodies in the inner Solar System as well as problems in solar physics, atmospheric sciences, and applied mathematics. His advanced computational models will be used to investigate the habitability of Jupiter's moons (§ 6.2), the orbital dynamics of extrasolar planet systems (§ 6.4), the dynamics of Earth-crossing asteroids (§ 6.5), and the recent history of climate on Mars (§ 6.6) (varadi@atmos.ucla.edu).

Ashwin R. Vasavada (Collaborator) is an Adjunct Assistant Professor (Rubey Fellow) in the Department of Earth and Space Sciences. Vasavada is a planetary scientist concerned with the occurrence of volatiles on Mars (§ 6.6), the Moon, Mercury, and other bodies. (ashwin@ess.ucla.edu).

M. Indira Venkatesan (Collaborator) is a Research Geochemist in the Institute of Geophysics and Planetary Physics who has great experience with the use of organic biogeochemical techniques to understand petroleum occurrence and formation and other paleobiological and environmental aspects of carbon chemistry. Here, she will be involved in the characterization of organic compounds made experimentally (§ 7.4) (indira@ucla.edu).

John T. Wasson (Collaborator) is Professor in the departments of Chemistry and Biochemistry and Earth and Space Sciences, and a Member of the Institute of Geophysics and Planetary Physics. His principal research interest is the study of meteorites as a tool for understanding the early evolution of the Solar System. He will work with others (§ 5.4) on the fate of water in protoplanetary systems (jtwasson@ucla.edu).

Thomas J. Wdowiak (Collaborator) is Associate Professor Emeritus, Department of Physics, The University of Alabama, Birmingham (UAB). Wdowiak is affiliated with the UAB Raman imaging facility and will work with Schopf on samples containing ancient fossil microbes (§ 7.5) (wdowiak@uab.edu).

Mark Webster (Collaborator) is an incoming Assistant Professor (from July 1, 2003) in the Department of Earth and Space Sciences. Webster studies the early evolution of body plans using morphometric techniques applied to fossils, notably Cambrian trilobites. He is part of a proposed collaboration (§ 8.3) aimed at investigating the origins of animal body plans (websterm@citrus.ucr.edu).

Edward D. Young (Principal Investigator) is a Professor in the Department of Earth and Space Sciences and the Institute of Geophysics and Planetary Physics. Young is an isotope geochemist and cosmochemist whose work is directed towards understanding the geological and astrochemical processes attending the formation of rocky bodies in the early solar nebula (§ 5.4) and the characterization of isotope fractionation in both inorganic and organic systems (§ 6.6,7.4) (eyoung@ess.ucla.edu).

Benjamin Zuckerman (Co-Investigator) is a Professor in the Department of Physics and Astronomy. Zuckerman has a longstanding interest in life elsewhere and has chosen research topics in astronomy that might have a bearing on the question of extraterrestrial life. He proposes (§ 5.2,5.3) to continue work on the origins of planetary systems based on the nearby young star database which he and his colleagues have assembled recently (ben@astro.ucla.edu).

Additional UCLA team members: Students, Associates, and Alumni (see § Plan for Strengthening the Astrobiology Community).

3 MAJOR TOOLS TO BE USED BY THE UCLA LEAD TEAM

There are a number of diverse experimental and computational techniques being used by UCLA astrobiologists. In order to facilitate the reading of this proposal, some of the major tools to be used in the coming years are described below and cross referenced to the sections of the text. More detailed descriptions can be found in volume II of this proposal.

The Institute for Genomic Research (TIGR): A non-profit research institute located in Rockville Maryland with laboratories that include large facilities for DNA sequencing, bioinformatics, molecular biology, and biochemistry. UCLA lead-team member Johnson is collaborating with the Institute (§ 8.2).

The Submillimeter Array (SMA): An imaging array at submillimeter wavelengths consisting of eight 6-meter antennas located on Mauna Kea, Hawaii that is a collaborative project of the Smithsonian Astrophysical Observatory and the Academia Sinica Institute of Astronomy & Astrophysics of Taiwan (http://sma www.harvard.edu/). SMA is scheduled to go into full operation in late 2003. Morris and Shuping propose to use it on a collaborative basis with members of the scientific staff (§ 5.2,5.4).

The Combined Array for Research in Millimeter-wave Astronomy (CARMA): An array of radio telescopes (operating at 115 to 345 GHz) located at high elevation (4,000 feet) in California. The facility will be open to the astronomy community on a competitive basis once it is fully operational (expected in 2005). Morris and Shuping propose to use CARMA for measuring carbon monoxide isotopomers in protoplanetary disks (§ 5.4).

The Space Infrared Telescope Facility (SIRTF): The final mission of NASA's Great Observations Program , SIRTF is an infrared telescope that will be launched in April, 2003. It is the largest infrared telescope ever sent to space. The capacity to see deeply into dense clouds of dust and gas makes SIRTF particularly useful for observing phenomena within the protoplanetary disks that surround young stars where planets are made (§ 5).

Stratospheric Observatory for Infrared Astronomy (SOFIA): An airborne infrared light observatory aboard a Boeing 747SP aircraft that will be based at Moffett Federal Airfield, California. SOFIA will fly to stratospheric heights in order to avoid interferences from the atmosphere that are especially problematical for infrared wavelengths. It is scheduled to begin observing in late 2004. It is well suited for observations of dense dust clouds such as those found in circumstellar disks. UCLA's Becklin is the Chief Scientist and Director Designate for the project (§ 5).

W.M. Keck Observatory: Twin telescopes situated at 14,000 feet atop Hawaii's dormant Mauna Kea volcano. The high altitude of the Keck I and Keck II telescopes makes ground-based observations in

the infrared possible and addition of adaptive optics (AO) results in images in the near infrared sharper than those from the Hubble Space Telescope. The system is a crucial new tool for studying protoplanetary disks around young stars (§ 5).

UCLA orbit integrator code: UCLA team member Varadi has developed a specialized code to accurately reconstruct the orbital and rotational history of planets and asteroids for the past 100 million years. The numerical integration scheme is a version of the classical Stormer-Cowell integrator which has been optimized to reduce the long-term effects of numerical round-off errors. This code is used in several studies described herein (e.g., § 6.4, 6.5).

The IBM Blue Horizon: Supercomputer installed at San Diego Supercomputer Center in 2000. Blue Horizon has a parallel architecture with 1152 processors distributed over 144 nodes. A fast internode connection makes the configuration capable of a peak performance of 1.728 TFlops per second. The versatility of the nodes, the fairly mature state of processor resource allocation protocols (e.g., Message Passing Interface), and the rapidly increasing amount of parallel numerical software makes this machine accessible for large-scale simulations. Jögi uses Blue Horizon to model stromatolite growth (§ 7.5).

UCLA stable isotope laboratory: A new stable isotope laboratory is nearly complete at UCLA. It includes two 10 μm (CO_2) infrared lasers for sample heating, a 213 nm (5th harmonic Nd-YAG) ultraviolet laser for in-situ ablation sampling, a Finnigan MAT Deltaplus gas-source mass spectrometer, a Finnigan MAT 253 gas-source mass spectrometer, two gas chromatographs (HP and Varian), two carrier flow interfaces, and two F_2 vacuum extraction lines (one under construction) for O_2 analysis of silicates, oxides, and phosphates. This facility is essential for measurements of oxygen isotopes in meteorites (§ 5.4).

UCLA multicollector inductively coupled plasma-source mass spectrometer: A new Finnigan MAT Neptune multiple collector inductively coupled plasma-source mass spectrometer (MC-ICPMS) has been installed in the Department of Earth and Space Sciences. This instrument is necessary in order to measure the isotope ratios of elements such as Fe and Mg with high precision (0.1 per mil or better). The high-precision obtainable with this instrument is essential for the proposed studies of how elements move between the organic and inorganic realms (§ 7.4).

W.M. Keck National Center for Isotope Geochemistry (ion microprobe): The main instrument of astrobiological significance is the CAMECA ims 1270 high-sensitivity, high-resolution ion microprobe, which is a national facility under the direction of Co-I McKeegan. The high spatial resolution of this instrument (1-30 μm) is key to several of the proposed studies (§ 7,8).

Raman Imaging Facilities: The University of Alabama (UAB) Laboratory for Paleobiological Chemical Imagery provides images from rastered Raman spectroscopy measurements (§ 7.5). The UCLA lead team subsidizes this facility by way of a subcontract but plans are being made to construct a new laser Raman imaging facility (Jobin Yvon Horiba T64000 Modular Triple Raman System) in the UCLA Department of Earth and Space Sciences (funds provided by the University). Both the UAB and new UCLA facilities will serve as national resource for the astrobiology community.

4 HIGHLIGHTS OF PREVIOUS ACTIVITIES

Highlights of research conducted in the UCLA Center for Astrobiology over the past several years are listed below. This list is meant to be representative of the types of work completed. It is not a comprehensive publications list (publications lists are available in NAI annual reports).

- A distorted disk of cold dust surrounding the star Fomalhaut (25 light years from the Sun) demonstrates the existence of a large, Saturn-like planet around the star. This is direct evidence for the existence of the types of planets that maybe necessary for life in extrasolar planetary systems (Holland et al. 2003).

- Acquisition of new isotopic and phylogenetic evidence for the anaerobic use of methane by microbial consortia composed of sulfate-reducing bacteria and methane-consuming Archaea (Orphan et al. 2001).
- A model of mass-independent fractionation (MIF) of the three isotopes of oxygen was developed for the modern atmosphere that makes testable predictions about the distribution of MIF effects in different atmospheric molecules (Lyons 2001).
- The catalogue of youngest, nearest stars, such as the Beta Pictoris moving group (Zuckerman et al. 2001) was expanded to ~ 200 members as a result of ongoing observations of the southern skies at the Siding Springs observatory, Eastern Australia. Several of these stars (less than 30 million years old) are expected to have warm Jupiter-class planets that should be visible in the infrared using current technologies (e.g., the Hubble Space Telescope).
- A major survey using the near-infared spectrometer (NIRSPEC), designed and built at UCLA for the Keck Observatory (Mclean), obtained IR spectra of 62 brown dwarfs. The data obtained will provide a wealth of information for modeling the atmospheres of such cool objects.
- Laser-Raman spectral imaging of micrscopic filaments from the 3.5 billion year old Apex chert was used to demonstrate their carbonaceous composition (Schopf et al. 2002). These filaments have been regarded as the world's oldest microfossils, though controversy surrounds their origin.
- Sulfur isotopic evidence was found for atmospheric but not bacterial processes in the formation of early Archean sedimentary sulfides and sulfates (Runnegar et al. in preparation).
- Reorientation of transfer RNA molecules during protein synthesis may indicate how "proofreading" developed as life emerged from the RNA world (Simonson and Lake 2002).
- All living eukaryotes appear to have mitochondria or to have lost them during the course of evolution. Their last common ancestor must postdate the permanent symbiosis that created the aerobically energized eukaryotic cell from prokaryotic precursors (Roger and Silberman 2002).
- Using genes involved in development, an antecedent of the pituitary gland was identified in early-diverging metazoans (e.g., jellyfish). The pituitary is a relic of a light and gravity detecting structure that may represent the first step in the evolution of the complex sensory and neural organization that characterizes animals (Jacobs and Gates in prep.).
- Calculations showed that a chaotic transition in the dynamics of the inner Solar System around the end of the Cretaceous some 65 million years ago may have disturbed the inner part of the asteroid belt, thus increasing the likelihood of an asteroid hitting the Earth at that time (Varadi et al. 2001).
- Geological mapping of Akilia island, Greenland, confirms an age greater than 3.8 billion years for the oldest sedimentary rocks and the organic material contained within them (Manning et al. 2001).
- Detection of a massive population of asteroids around nearby star ζ Leporis (Jura and Chen 2001).
- Ocean-continental crust interactions approximately 4.3 billion years ago on Earth are evidenced by the isotopic compositions of very old zircon crystals from Western Australia (Mojzsis et al. 2001).

- Horizontal gene transfer is limited by the complexity of gene product interactions (Lake et al. 2000).

- Planetary atmospheres may not be significantly eroded by giant impacts (Newman et al. 1999).

5 GAUGING THE POTENTIAL FOR TERRESTRIAL PLANETS IN EXTRASOLAR PLANETARY SYSTEMS

5.1 OVERVIEW

Observations and models for extrasolar planet formation can be compared with clues about planet-forming processes in the Solar System to answer the question: how typical are the processes that formed our Solar System? By addressing this question one gains insight into the likelihood of Earth-like planets elsewhere in our Galaxy. Collaborations between researchers studying conditions in nearby stellar systems (astronomers Becklin, Ghez, Hansen, Jura, Morris, Shuping, and Zuckerman) and those focused on the history of our Solar System as revealed through the study of meteorites (cosmochemists Lyons, McKeegan, Wasson, and Young) pave the way for new research opportunities related to terrestrial planet formation. These opportunities arise as a result of consultation between workers in fields that have been historically distinct in their approaches to elucidating how planets form.

The 2002 assessment of the NASA astrobiology program by the National Research Council's Committee on the Origins and Evolution of Life noted the weak level of interaction between research in the Astronomical Origins and the Astrobiology programs relative to analogous interactions between the astrobiology community and, for example, the geobiology community. The research outlined in Section 5 addresses this general shortcoming by strengthening, specifically, links between the Astronomical Origins and Astrobiology programs at UCLA.

One area of astrobiology where collaboration between UCLA astronomers and cosmochemists is proving fruitful is description of the first few million years of planet formation. Through studies of young stars surrounded by gas and dust, in the form of “protoplanetary” rings and disks, that could coalesce to form planets, Ghez, Jura, Morris, McKeegan, Shuping, and Young plan to evaluate the time scales over which such structures evolve, and perhaps infer time scales over which planets form.

It has been suggested that the presence of a Jupiter-like (giant) planet in orbit well outside the conventional habitable zone is a requisite for sustaining life on rocky planets similar to Earth. The gravitational field of a giant planet can relatively quickly cleanse a planetary system of the numerous planetesimals that must be part of the planetary formation process and can shield rocky planets from catastrophic impacts. Searches for giant planets orbiting many astronomical units (AU) from their star are therefore relevant to the problem of identifying planetary systems with favorable habitable zones. The most successful technique used to detect extrasolar planets – measuring the wobble of a star due to the pull of an orbiting planet (Marcy et al. 2000) – has revealed mostly giant planets that reside far closer to their central star than does Jupiter. Notwithstanding an occasional exception, it is recognized that systems containing such proximal giant planets are, in general, unlikely to harbor planets that can sustain life. Members of the UCLA lead team are engaged in development and application of new techniques geared to image detection of giant planets located in orbits resembling those of the giant planets of our Solar System.

Advances in astronomy that utilize ground-based telescopes equipped with adaptive optics systems (Beckers 1993), as well as an infrared camera on the Hubble Space Telescope (HST), make it possible to image directly Jupiter mass planets (Macintosh et al. 2001). But a caveat is that such detections must be of thermal emission from young, warm planets rather than of reflected starlight from old, cold planets like Jupiter. For this reason, imaging of giant planets in systems resembling the Solar System requires finding stars within about 50 parsec of Earth and not older than a few tens of millions of years. Recent work on isotopes in the hafnium-tungsten system in chondritic meteorites and in terrestrial samples indicate that Earth’s core formed in < 30 Myrs (Fitzgerald 2003). Thus, to identify

optimum stars at which to image cooling giant planets and to match the timescale for terrestrial planet formation, Zuckerman and Song will continue their compilation of very young stars close to Earth. Identification and cataloging of young, close, solar-like stars was a major part of the CAB activities over the past several years and will continue, with more of a focus on faint, low-mass stars, during the next five years.

Studies of the origins of meteorites can be used to deduce the processes by which Earth-like planets are formed. Although cosmochemistry is concerned with the origins of planets in the Solar System, the relevance of these studies to astrobiology is enhanced considerably if they are informed by astronomical evidence for analogous processes occurring around other stars. Cosmochemists Lyons, McKeegan, Young and Wasson propose to continue their studies of how rocks and water coalesced to form terrestrial planets in our Solar System, but in a manner that makes better use of astronomical information. Their approach will depart from the more traditional research programs in cosmochemistry in that they will use astronomical measurements obtained by other team members to compare and contrast constraints on planet formation in our Solar System with those seen elsewhere. This cooperation has already resulted in new insights into the significance of some vexing features of meteorites in understanding planet-forming processes (see Section 5.4.2).

UCLA astronomers Morris and Shuping propose a new observational program designed to test results from investigations of photochemistry in the early solar nebula (the Sun's protoplanetary disk, extant 4.6 Gyr ago) that may generally occur during rock formation in young circumstellar disks. Such observations are a direct manifestation of the synergy between the astronomical and cosmochemical communities being cultivated in the UCLA Center for Astrobiology.

Some details of the Center's research programs directed towards understanding the potential for terrestrial planets and Solar System-like planetary systems in our Galaxy are described below.

5.2 ROCKS AND ICES IN THE GALAXY- STUDIES OF HOW AND WHEN ROCKS AND ICES ARE MADE THAT COULD COALESCE TO FORM PLANETS (BECKLIN, GHEZ, JURA, MCLEAN, MORRIS, SHUPING, ZUCKERMAN)

5.2.1 Grain growth in young stellar systems

Most, if not all, young solar-like stars are surrounded by circumstellar disks prior to planet formation. Indeed, it is these disks that provide the basic building blocks for future planetary systems. The ways in which sub-μm size particles of dust in the interstellar medium eventually accumulate into kilometer sized, asteroid-like, planetesimals, which in turn aggregate to form rocky planets, are poorly understood. The process starts in the disks. Understanding the time scales and regions of significant grain growth in disks that surround young stars would enhance our understanding of how rocky planets form, and might be used as a tool for identifying those systems with proclivity for terrestrial planet formation.

Some observational and theoretical research on grain growth from sub-micron specks to millimeter size particles has been carried out (e.g., Pollack et al. 1994). But many more direct measurements of circumstellar disks are needed. Ghez and others are engaged in identifying the earliest stages of planet formation (i.e., dust coagulation) in regions surrounding million year old T Tauri stars. Their search for grain evolution focuses on infrared thermal emissivities and scattering/polarization properties which change as grains grow in size. By comparing infrared images between 1 and 10 μm, obtained with the Keck telescope and Hubble Space Telescope, Ghez has recently demonstrated the existence of grains substantially larger than interstellar in the disk that encircles the T Tauri star GG Tau (McCabe et al. 2003).

During the coming years Ghez and coworkers will build on this early success by performing similar observations at mid-infrared wavelengths of disks surrounding T Tauri stars of various ages. By correlating the extent of grain growth with age, this program of observations should paint a clearer picture of time scales for grain growth around solar-like stars. The program will require observing sessions at the Keck observatory extending at least for the next few years.

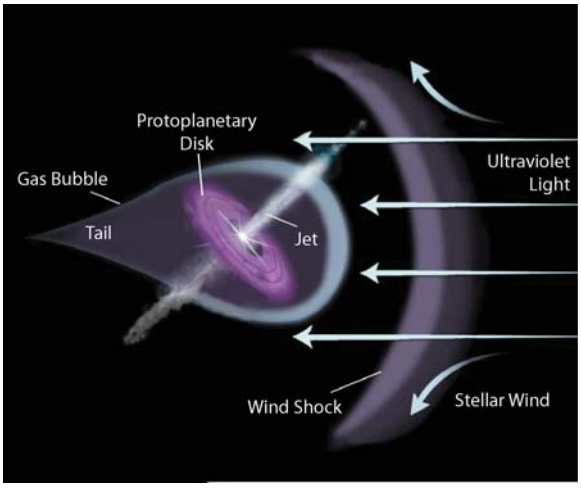

Figure 5.2.1. Schematic of a typical protoplanetary disk in Orion. Ultraviolet (UV) radiation from a nearby massive star eats away at the protoplanetary disk surrounding a young star creating a bubble of warm gas. The outer portions of the gas bubble are then heated and removed by energetic UV radiation. Material falling from the disk onto the central protostar fuels twin gas jets. Artwork: Space Telescope Science Institute.

Ghez's research focuses on the nearest regions of current star formation such as the Taurus dark clouds. While proximity is obviously a virtue when one is investigating planetary system size phenomena, study of more distant youthful star clusters also has advantages. For example, most stars are thought to form in clusters containing many hundreds of stars, including some that are much more massive than our Sun. The closest, well studied, such region is the Orion Nebula Cluster.

Grain growth up to a few μm has been inferred from studies of transmitted light through a circumstellar disk seen in silhouette against the Orion Nebula. But, unlike forming stars in Taurus which are far from any massive, luminous stars, disks in Orion are being evaporated by intense ultraviolet light from four high-mass stars at the cluster's center (Figure 5.2.1). Models indicate that these disks can be evaporated away on timescales of about a million years. The key question is whether or not planets can form before the disks are destroyed by the UV radiation field. Evidence of grain growth in the Orion disks is especially important in addressing this question.

Silhouette and scattered light images reveal the nature of grains in the outer edges of the protostellar disk while thermal radiation from the mid-infrared through radio wavelengths traces grains in the midplane. Morris and Shuping plan to continue their studies of Orion using existing data available from HST archives coupled with new observations of: (1) silhouettes and scattered light in the near- and mid-IR (at Keck Observatory); and (2) thermal emission from the mid-IR through the far-IR, sub-millimeter and radio wavelengths (using Keck, SIRTF, SOFIA, and various radio observatories). The planned observations will require approximately five to 10 nights to complete, and a few years to analyze and publish.

5.2.2 Detecting asteroids and comets in extrasolar systems – precursors to rocky planets elsewhere in the Galaxy

Asteroids and comets are composed of rocks and volatile ices. Since cosmochemical studies of meteorites demonstrate convincingly that planets of the inner Solar System were made from similar objects, the presence of asteroids and/or comets in other stellar systems may point toward the existence also of rocky planets. Although the asteroidal and cometary building blocks of planets are too small to be directly detected around other stars, their presence can be inferred indirectly, and we propose a program of astronomy-based research that will permit indirect detection of these objects.

In our Solar System, asteroids are eroded by mutual collisions while comets disintegrate by passing near the Sun. Microscopic dust particles from these disrupted parent bodies are subsequently distributed throughout the inner solar system. The zodiacal light is produced by sunlight scattering off these dust particles while absorption and reemission of sunlight by this material generates infrared emission. Dust particles near Earth have a typical lifetime of about 100,000 years before spiraling into the Sun under the operation of the Poynting Robertson effect (photon drag).

In 1983, the IRAS satellite discovered dust orbiting many main sequence stars, including the very bright star Vega. Analogous to our own Solar System, it is thought that this dust results from the disruption of parent bodies (e.g., Zuckerman 2001). Within 2 AU of the Sun, the total mass of dust is about 2×10^{17} g (Ney 1982). Around stars like β Pictoris (age ~12 Myrs) and Fomalhaut (age ~200 Myr), the total mass of dust may be 10^{25} g (Zuckerman and Becklin 1993), but as shown by direct imaging in infrared, optical and submillimeter wavelengths and as illustrated in Figure 5.2.2, dust in some systems is detected as far as 100 AU from the central star (Holland et al. 1998, Weinberger et al. 1999, Weinberger et al. 2002). Typically, this corresponds to location in the Kuiper Belt of comets and large icy bodies in our Solar System. At least one star, ζ Lepus, appears to be encircled by an asteroid belt with about 200 times the mass of the asteroids in the Solar System (Chen and Jura 2001).

One of the most significant consequences of the initial IRAS discovery, is the realization that the dusty disks around main sequence stars usually show non-axisymmetric structure (e.g., Zuckerman 2001, Holland et al 2003 and Figure 5.2.2). The submillimeter SCUBA camera at the James Clerk Maxwell Telescope (JCMT) at Mauna Kea Observatory has been the most successful instrument in imaging non-axisymmetric disks around main sequence stars including some of the best known (e.g., Vega and Fomalhaut). The most plausible cause of such structure is the gravitational field of planets of substantial mass with semi-major axes as large as that of Neptune and even larger (e.g., Ozernoy et al 2000). This is the first observational evidence, albeit somewhat indirect, for the existence of planets in such wide orbits. During the coming years, Zuckerman expects to continue his fruitful SCUBA collaboration with astronomers from the United Kingdom.

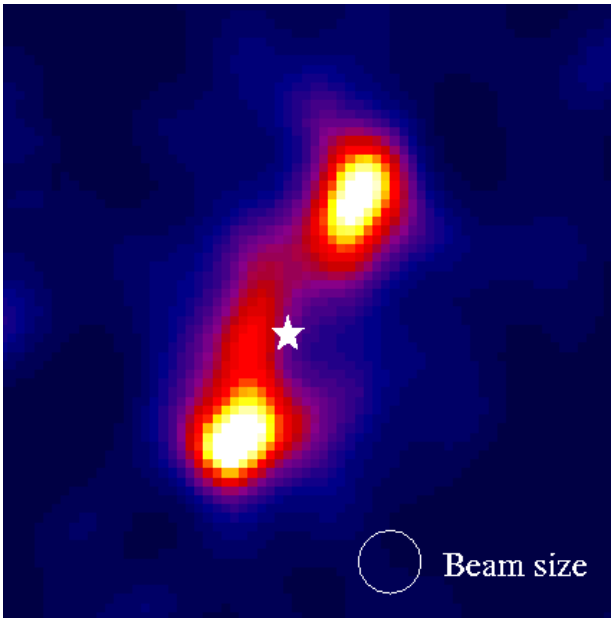

Figure 5.2.2. Image of a dusty debris disk around the bright star Fomalhaut obtained by lead team member Zuckerman and outside collaborators using the SCUBA camera at JCMT, Mauna Kea Observatory . The image, at 0.45 mm wavelength, shows a non-uniform distribution implying the existence of a planet that shepherds the debris.

With a variety of instruments, including the 10 meter Keck telescopes, the HST, SIRTF (launch April 2003) and SOFIA (first light late 2004), Jura, Zuckerman, Becklin, and Hansen propose further studies of debris dust derived from comets and asteroids around main sequence stars with the specific goal of learning more about the formation and evolution of planets in the context of what we know about our Solar System.

For example, for comparisons with the Solar System, the minimum mass, M_{PB}, of the parent bodies of debris can be obtained with the expression,

$$M_{PB} = \frac{4L_{IR}t_{age}}{c^2}$$

where L_{IR} is the observed infrared luminosity of the dust, t_{age} is the age of the star, and c is the speed of light (Chen and Jura 2001). Observation of stars with different ages enables study of parent body mass as a function of time (see Spangler et al. 2001), thus enabling a comparison with what is known about evolutionary time scales in our Solar System as derived from studies of meteorites.

With low resolution spectra obtainable with SIRTF, Jura and colleagues expect to learn about the origin and evolution of the particles that comprise the debris. Grains spiraling inwards under the action of the Poynting Robertson effect produce a spectrum which varies as ν^{-1} independent of the grain size (Jura et al. 1998). Comparison of the data with simple models based on this result will enable Jura et al. to infer where the particles are formed (perhaps in the equivalent of our Kuiper Belt) and whether they spiral all the way into the star or are stopped - as might occur if there is accretion onto a Jovian-mass planet.

New observations made by the UCLA lead team relating to the mass and dynamics of dust vs. central star age will be compared with models for early Solar System evolution by consultations between Jura and colleagues and lead-team cosmochemists McKeegan, Wasson, and Young. The result will be a synthesis of what is known about our Solar System formation in the context of the formation of rocky materials around other stars.

Comparisons between the evolution of our Solar System and that of debris disks around other stars can be taken further. For example, we have the capacity to search for the equivalent around other stars of the era of the Late Heavy Bombardment inferred to have occurred within the Solar System within the first ~800 million years. The development of life in the Solar System is thought to have been delayed by this bombardment. Are such events common in planetary systems? Elsewhere, are they comparable in magnitude to the event(s) recorded in the inner Solar System?

IRAS could only begin to address such questions because its sensitivity limited meaningful observations mostly to stars with twice or more the mass of the Sun. Soon SIRTF will enable astronomers, including Jura and Zuckerman, to investigate stars with masses comparable to that of the Sun. Also, in the Solar System, comets and asteroids produce about 10^6 g s^{-1} of dust which then spirals into the Sun under the operation of the Poynting Robertson effect (Ney1982). SIRTF will be sufficiently sensitive that Jura and coworkers will be able to search for similar dust-production rates around nearby, low mass, main sequence stars of various ages. Do such stars experience asteriodal grinding and comet disruption at the same rate as in the Solar System? Through a new program of SIRTF observations of debris disks, the lead-team members propose to determine whether there are similarities between our Solar System and other regions where tell-tale signs (albeit indirect) of rock and ice formation are present.

5.2.3 Searches for signs of life's essential chemical constituents surrounding young stars

The importance for astrobiology of debris disks surrounding other stars goes beyond garnering indirect evidence for planet-forming processes; the nature of disk material can be constrained from its spectroscopic features. Are there signatures of organics that potentially could be precursors to life? Major organic spectral features are seen in comets in the infrared at 3.3 to 3.4 μm and 5.5 to 8 μm. The former can be studied from the ground with, for example, the Keck telescope. The latter feature must be studied from space (with SIRTF) or from the stratosphere (with SOFIA).

Because of his position as Chief Scientist and Director Designate for SOFIA, lead team member Becklin will concentrate his future efforts on SOFIA. He is currently working with the NASA Ames

Astrobiology lab group to assure that the correct filters and spectrometers become available soon after initiation of SOFIA flights. In addition, UCLA is building a camera and spectrometer for the 1 to 5 μm region. This camera, called FLITECAM (lead team member McLean is the FLITECAM PI), is missing GRISM (GRISM stands for "grating prism") spectrometers in the critical region from 3.0 to 5.0 μm. With these GRISMs we will be able to investigate organic spectral features around 3.3 μm with greater sensitivity than from ground-based telescopes. In addition we will be able to observe the primary carbon containing molecules CO and CO_2, which together with H_2O, are essential to the chemistry that could lead to life.

Becklin proposes as part of this work to purchase the appropriate GRISMs that will enable him to utilize SOFIA for observations relevant to astrobiology. McLean has obtained price quotes from three US vendors for the two necessary GRISMs in the 3 to 5 μm range; typical costs are included in the budget.

5.3 IMAGING EXTRASOLAR PLANETS – REMOVING THE BIAS TOWARDS DETECTION OF GIANT PLANETS CLOSE TO STARS (GHEZ, HANSEN, SONG, ZUCKERMAN)

5.3.1 Development of the youthful star target data base: identification of nearby, youthful stars offers the best nearterm opportunity for imaging Solar System-like planetary systems

The past few years have seen the discovery of numerous massive extrasolar planets (Marcy et al. 2000). All have been detected indirectly, by virtue of their gravitational tug on the star about which they orbit. Only when planets are imaged directly will it be possible to measure their spectra and thus their compositions. Advances in astronomy from the ground, specifically adaptive optics (AO; Beckers 1993), and the employment of an infrared camera on HST, now enable imaging detection of planets with masses comparable to that of Jupiter (Figure 5.3.1; Macintosh et al. 2001). But such detections

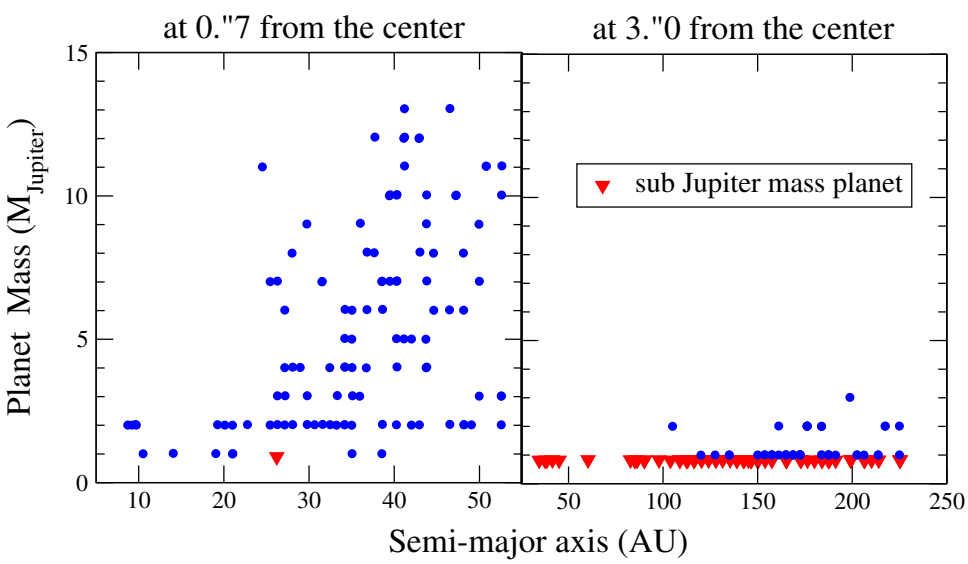

Figure 5.3.1. Plot of masses and star-planet distances for warm, giant planets that would be observable if they were to be in orbit around the 112 stars identified by Zuckerman and Song as very young and near to Earth. The plot shows the minimum masses that should be detectable using the near-infrared camera aboard the Hubble Space Telescope.

must be of thermal emission from young, warm planets rather than of reflected starlight from old, cold planets, such as Jupiter. At wavelengths near a few μm, thermal emission from a giant planet not older than tens of millions of years can be hundreds of times brighter than reflected starlight; the latter

is still much too faint to be detected with any existing imaging system.

The giant planets of our Solar System are 5 to 30 times more distant from the Sun than is Earth. Given the diffraction and instrumental scattered light properties of AO and of HST, imaging of Solar Systemanalogs requires finding stars within about 50 pc of Earth and not older than a few tens of millions of years. During the past few years, Zuckerman and his group have carried out a long term project to find the youngest, closest stars to Earth. Using all-sky survey data in X-rays (ROSAT) and major astrometric catalogs (Hipparcos, Tycho-2, and SuperCOSMOS), they have generated a list of a few 1000 very young star candidates within 60 pc of Earth. This project has been very successful. During the course of our present NAI award, Zuckerman and Song have observed about one thousand stars from which they have identified about 200 young nearby stars including the nearest young stellar association, the β Pictoris moving group (Zuckerman et al. 2001; Song et al. 2003).

5.3.2 Infrared searches for Jupiter-mass planets around low-mass stars

Encouraged by the success of the above project, Zuckerman proposes to continue the search for young nearby stars during the period of CAN-3 with greater emphasis on lower mass stars. This is of special interest for two reasons. First, for imaging faint planetary companions around stars, one can detect lower mass planets around low mass stars. For example, at 10 pc from Earth, with AO or HST, it is possible to detect a Saturn-mass planet at an orbital separation of 20 AU around a 10 million year old M-type star. On the other hand, around a 10 million year old A-type star, only planets with masses of about ten Jupiter masses or higher are detectable at the present state of the art. Second, there are many more less-massive stars than massive stars. In fact, regardless of age, there are fewer than 300 A-type stars within 50 pc of Earth while we expect 100,000 or more M-type stars in the same volume.

Various international teams of astronomers have been imaging young stars with AO and/or HST for evidence of cooling planets. But it is generally impossible to know at which nearby star to search without a preceding survey (like that of Zuckerman and Song) to identify "young stars" and to classify them according to mass and age. Thus, all competing planet hunting groups are dependent in various ways on the results of Zuckerman and Song's search. For example, stars identified in the beta Pictoris moving group will be observed early-on with SIRTF by a variety of teams. Indeed the young stars identified by Zuckerman and Song will be re-observed again and again in coming years, each time a more sensitive spaced-based telescope becomes available to astronomers. Young nearby stars from the UCLA survey will be obvious targets for upcoming NASA missions such as Space Interferometry Mission (SIM, lead team member Ghez is a Co-I), James Webb Space Telescope (formerly NGST), and SOFIA.

With compilation of a young nearby stars catalog, members of the UCLA lead team will contribute to the astronomy/astrobiology community by providing a set of valuable targets while maintaining a leading position in an ongoing race for imaging detection of cooling extrasolar planets.

5.4 COSMOCHEMISTRY IN AN ASTROPHYSICAL CONTEXT– RELATING THE ORIGIN OF THE SOLAR SYSTEM TO PROCESSES OF PLANET BUILDING ELSEWHERE (HANSEN, LYONS, MCKEEGAN, MORRIS, SHUPING, WASSON, YOUNG)

5.4.1 The distribution of H_2O in protoplanetary systems

A key question related to the origin and evolution of terrestrial life is that of how water was accreted to Earth and, more generally, how rocky planets acquire their water. There are two main possibilities. One, the endogenous origin, is that the water accreted together with the planetesimals (ranging from asteroid to Mars in size). The other is that water present on Earth’s surface today is exogenous, having been delivered by comet impacts (e.g. Morbidelli et al 2000). At present there is skepticism about the cometary origin of water because the ratios of the heavy isotope of hydrogen to the light isotope, D/H, in the three comets measured thus far (Halley, hyakutake, and Hale-Bopp) are on the order of 2 times

higher than in Earth's oceans (Bockelee-Morvan et al. 2000). On the other hand, D/H would most likely have varied with radial distance from the Sun in the early Solar System, making the arguments based on D/H uncertain.

One way to distinguish between the exogenous and endogenous origins of water in rocky planets is to characterize the amount of water that was present in planetesimals. Lead-team investigators McKeegan, Wasson and Young have been studying the role that water played in the evolution of rocky precursors to planets in the Solar System through studies of the ways in which carbonaceous chondrite meteorites, vestiges of the planetesimals, have been altered by reactions with liquid and/or vaporous water (Choi et al. 1997; Choi et al. 1998; Young et al. 1999; Young 2001). This work builds on years of studies pertaining to the role of water in the evolution of planetesimals (asteroid-like precursors to planets) as evidenced by these primitive meteorites (e.g., Kerridge and Bunch 1979; Clayton and Mayeda 1984; Clayton and Mayeda 1999).

It is clear that the bodies from which at least some carbonaceous chondrites come from (now represented by the C-type asteroids) had significant amounts of water within them early in the history of the Solar System, but exactly how much water existed in primitive meteorite parent bodies depends on how the data are interpreted. This is an important question because if objects represented by the C-type asteroids had uniformly large amounts of water (as suggested by some workers, e.g. Young et al. 1999), then the implication is that water was plentiful in the building blocks of the planets in the Solar System (C-type asteroids are the most abundant type of primitive rocky body and the largest asteroid 1 Ceres is such an object). In this case, the origin of water in and on the terrestrial planets need not have been exogenous (i.e., from late introduction by comets) but instead could be the residues left over from much larger amounts of water that existed prior to melting and differentiation of the bodies (i.e., endogenous). Alternatively, if large amounts of water were present in only a small fraction of the primitive building blocks of the planets (as implied by other interpretations of the meteorite data, e.g. Clayton and Mayeda 1984), then water would not have been nearly as plentiful during the planet building process and would not be expected to have survived the planet-forming processes in sufficient quantity to explain present-day abundances (on and within Earth and perhaps Mars).

There are two models put forward to explain the mineralogical and oxygen isotopic effects of reactions between water and rocks as evidenced in carbonaceous chondrite meteorites. One, the closed-system model put forward by Clayton and Mayeda (1984), states that the amount of water evidenced by the altered rock materials in a meteorite is an expression of the amount of water that existed in the entire parent body (that is to say, the "water/rock ratio" is a characteristic of the object). The implications of

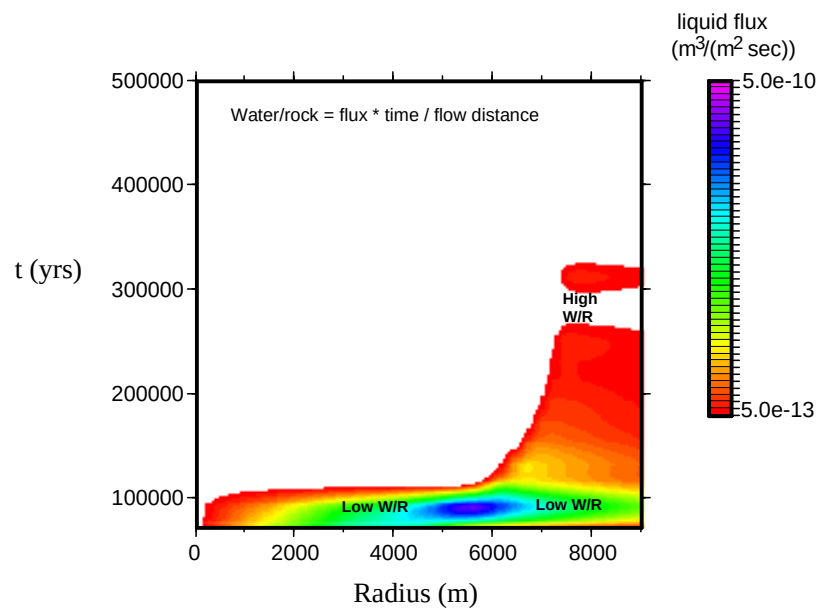

Figure 5.4.1. Results of finite difference model for water flow through an asteroid-like body in the early Solar System (after Young 2001) as a function of radius and time. Colors show intensity of water flux in the body ($m^3/(m^2\ s)$) as it evolves with time. Where water/rock recorded by isotopes and mineralogy is high, flux was protracted. Where water/rock is low, flux was fleeting. The total water content of the original body was uniform at 20 volume % but the water/rock preserved in a rock would depend on the original location it occupied in the body rather than the total water content of the body.

this interpretation of the data is that while a few carbonaceous chondrite parent bodies had on the order of 50 volume % water, most had substantially less(< 10%). The othermodel, the open-system put forward by Young et al. (1999) and Young (2001) is that the amount of alteration of the meteorite depended upon where the particular sample came from in the parent body (Figure 5.4.1). In this model, the parent bodies were heterogeneous in mineralogy and oxygen isotopic characteristics but they all could have had on the order of 20 to 30 volume % water to begin with. If the open-system model is correct, it implies that water was a major constituent of proto-planets prior to their melting and differentiation.

Distinguishing between open-system alteration and closed-system alteration of a chondrite, and thus between large amounts of water in all bodies and large amounts of water in just a few bodies, requires analysis of $^{17}O/^{16}O$ ($\delta^{17}O$) and $^{18}O/^{16}O$ ($\delta^{18}O$) by ultraviolet laser ablation combined with gas-chromatography isotope ratio mass spectrometry (Young et al. 1998). This novel technique is time-consuming but provides a combination of spatial resolution and precision that can not be obtained by any other method. Young and others will carry out oxygen isotope ratio analyses of carbonaceous chonderite meteorites to search for signals that can be used to distinguish between open and closed-system reactions between the rocks and waters. Collection of data for characterization of a reasonable sampling of carbonaceous chondrite meteorites will take several years. The analyses will be carried out in Young's stable isotope laboratory at UCLA.

Another way to examine the likelihood for exogenous and endogeous sources of water is to study the ways in which giant planets affect the delivery of water to regions where habitable, terrestrial-like planets are likely to form. Planetesimals formed beyond the "snow line" where water is in the solid state in a planetary system are harbingers of water (e.g., comets). Planetesimals formed inside of the snow line may be relatively dry. Giant planets interact gravitationally with planetesimals, in effect stirring them up. What is more, it is now clear that some giant planets in extrasolar systems may have migrated radially with respect to their stars, and it has been suggested that early on gaseous and icy giant planets in the Solar System may also have moved closer or further from the Sun (e.g., Thommes et al. 2002). Indeed it has been suggested that a mechanism for stopping the migration of a giant planet towards its central star is for it to encounter a sufficient number of asteroid-like or comet-like planetesimals (Murray et al. 1998). Giant planets are therefore expected to regulate the distribution of water-bearing planetesimals into regions of terrestrial planet formation.

The consequences of this new paradigm of moving giant planets for terrestrial planet formation are poorly understood. Hansen, in collaboration with Young, plans to perform a detailed investigation of giant planet-planetesimal interactions and the implications for the development of solar-like planetary systems. The challenge will be to cover both the large dynamic range in mass and the large number of particles necessary for a realistic description. The results will be examined in the context of the meteoritic evidence for the early evolution of our own Solar System.

5.4.2 The astrochemistry of protoplanetary systems and the meteorite record

An important step in recognizing signs of life, or the essential precursors to life, is characterization of the various abiotic pathways by which organic molecules are produced in protoplanetary environments, including in our own solar nebula (the protoplanetary disk that surrounded the Sun 4.6 Gyr ago). One potentially important process for forming organic molecules is photolysis. Lyons and Young have begun a program of research devoted to elucidating those characteristics of primitive meteorites that might be explained by photochemistry in the solar nebula. The goal is to get a better picture of the role that photochemistry might have played in determining the inorganic and organic chemistry of the nebula.

One of the most important clues to the origin of the Solar System is the presence of an excess of the ^{16}O isotope of oxygen relative to the two heavier oxygen isotopes, ^{18}O and ^{17}O. This unusual distribution of O isotopes (by terrestrial standards), discovered by R.N. Clayton in 1973, was one of the reasons for

suggesting that explosion of a nearby super nova might have triggered the collapse of molecular cloud material to form our Solar System (^{16}O is a product of such an event). However, the connection between an overabundance of ^{16}O inferred to derive from a super nova explosion has not been observed in populations of "presolar" mineral grains that represent pristine ejecta from stars found in meteorites. The variability in ^{16}O apparently has another explanation that remains elusive.

What is clear is that the ^{16}O enrichment is telling us something about conditions that prevailed in the very young solar nebula. Whether these conditions are relevant to the subsequent development of life on Earth is unknown, but they are almost certain to be central to our understanding of other young planetary systems and may be clues to the photochemistry that took place early in the Solar System.

Clayton (2002) made the suggestion that self shielding by CO in the early solar nebula could have been the origin of the anomalous array of $^{18}O/^{16}O$ and $^{17}O/^{16}O$ in primitive Solar System materials (the slope-1 line on a plot of $\delta^{17}O$ vs. $\delta^{18}O$ where $\delta^{17}O$ = per mil deviation in $^{17}O/^{16}O$ relative to standard mean ocean water and $\delta^{18}O$ is defined similarly). The efficacy of the photodissociation of CO as a means for producing slope-1 lines in oxygen three-isotope space is underscored by the detection of slope-1 oxygen isotope ratios in interstellar CO (Sheffer et al. 2002). A major component of the CO self shielding hypothesis is that the starting materials for all solids in the early solar nebula were rich in ^{16}O, with isotopic compositions comparable to the most ^{16}O-rich calcium-aluminum-rich inclusion minerals.

Calculations by Lyons and Young show that self shielding by CO of a stellar flux of UV photons illuminating regions above and below the midplane of the solar nebula (the protoplanetary disk that became the Solar System) should have been sufficient to produce several Earth masses of oxygen with large depletions in ^{16}O relative to the starting over time scales > 10^3 yrs. The most likely sink for the ^{17}O and ^{18}O-rich oxygen liberated by photolysis of CO would have been adsorption onto solid dust grains followed by surface reactions to produce water, as described for molecular clouds by Yurimoto and Kurimoto (2002). Settling of these dust grains and radial transport toward the accreting star (the Sun) brings this source of ^{16}O-depleted oxygen into the nascent inner Solar Systemwhere it can react with gases, minerals, and liquids that form planet precursors.

Experiments show that exposure of ices containing C compounds to UV photons can produce complex organic compounds (Schutte 2002). The model put forward by Lyons and Young implies that ices, primarily composed of water, were important in determining the oxygen isotopic composition of rocky bodies in the Solar System. It also implies that irradiation of condensed materials, including ices, by UV photons could have been an important process, and that the UV photon flux could have come from the central proto-Sun if the ices existed well above the midplane. If shown to be correct, there is the possibility that the origin of organic molecules and ^{16}O anomalies in primitive meteorites are both telling us about the photochemistry of the early solar nebula, and perhaps

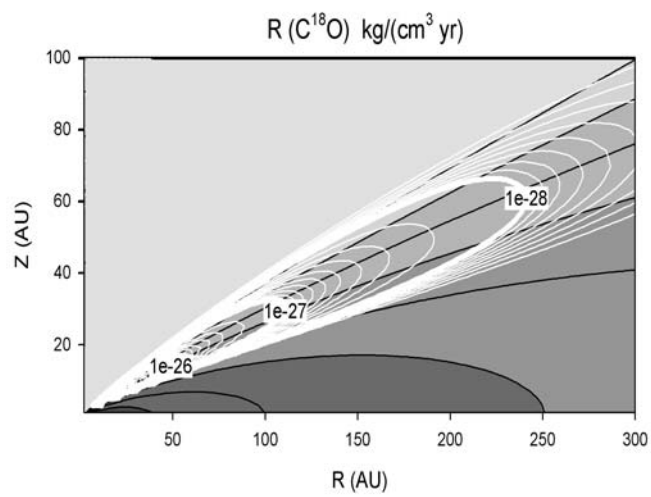

Figure 5.4.2. Calculated rate of $C^{18}O$ photodissociation (white contours) in a protoplanetary disk representing the solar nebula as a function of radial distance from the star (R) and height above the midplane (Z). Grey tones show increasing density in the disk. After calculations by Young and Lyons (2003).

protoplanetary environments in general.

Theoretical tests of this model will be conducted in the next several years. A key refinement will be development of molecular shielding functions. The effects of mutual and self shielding by H, H_2 and CO were included in the original calculations by using the H_2 and CO density-dependent shielding functions of van Dishoeck and Black (1988). These functions were based on calculations appropriate for molecular cloud environments, but should be redone for applications in protoplanetary disks. The calculations will ultimately be extended to investigate the implications for D/H and N isotopes.

If photochemistry at distal regions in protoplanetary disks is common in the Galaxy and recorded in the isotopic compositions of meteorites, then the process should be evident in disks surrounding young low-mass (i.e., solar-like) stars elsewhere. Lead-team members Shuping and Morris propose to test this suggestion by examining the protoplanetary disks in the Orion Nebula. The relative abundances of carbon monoxide isotopomers in the disks can be measured directly using millimeter and submillimeter interferometers. Interferometry is required because the target disks are typically small; spatial resolution on the order of, or better than, an arcsecond is required to match or resolve nearby disks such as those found in Orion and Taurus. Resolution of up to 0.1" is achievable with the Submillimeter Array and with the CARMA array (soon to be in operation).

CO emission has been reported widely in star-forming cores, but only the new generation of interferometers will be capable of measuring the isotopomers of CO in compact protoplanetary disks. By measuring them initially at 230 GHz (the J=2-1 rotational line), where sensitivity is greatest, Shuping and Morris would establish which disks are most promising for measurements of the rarest isotope, $C^{17}O$. Then higher-lying lines would be measured in order to provide information on optical depth and the temperature structure in the disks. The ultimate goal is to determine whether there has been any significant selection, via photodissociative processes, for the more abundant isotopomer relative to the CO abundances in the nearby molecular clouds from which the star and disk formed.

Another approach to take to investigate isotope abundances in disks is to observe the near-infrared (4.6 and 2.3 μm) rotational-vibrational absorption by CO of light from the central star as seen through the disk. While ^{13}CO and $C^{18}O$ are both routinely observed toward protostellar sources at 4.6 μm, the $C^{17}O$ isotope has not yet been detected. Absorption line observations can only be made for protostellar disks with just the right inclination to our line of sight (60 - 80 degrees), and with central stars that are sufficiently bright at 1-5 μm. There are a handful of protostars in Taurus and Ophiuchus which satisfy these conditions. High-resolution near-infrared spectroscopy of the CO lines at both 2.3 and 4.6 μm can be carried out using the Near-Infrared Spectrometer (NIRSPEC) at the Keck Observatory (adaptive optics is not required). In addition to the isotope ratios, these observations will also enhance our understanding of the overall abundance of CO in protostellar disks, both in the gas-phase and as ices condensed onto dust grains (e.g. Shuping et al. 2001, Boogert et al. 2002).

6 CHARACTERIZING THE FACTORS THAT CONTROL THE HABITABILITY OF PLANETS AND PLANETARY SATELLITES

6.1 OVERVIEW

Here we describe research plans in those areas that pertain to the habitability of planets, including Earth. The five areas of proposed research include assessments of the habitability of icy bodies within our Solar System, the potential links between planet-scale dynamics and long-term habitability, the influences of orbital dynamics on prospects for habitable planets in extrasolar planetary systems, the role of impacts on survival and evolution of life, and the evolution of climate on Mars.

The most direct means for investigating the habitability of planets and their satellites is through the study of bodies in the Solar System. Lead-team members Schubert, Moore, Veradi, and Nimmo will

examine the potential for sustaining life within the Galilean moons of Jupiter. Two of these bodies, Europa and Ganymede, may owe their potential for habitability to the immense tidal forces they experience by virtue of their gravitational interactions with Jupiter and the other Galilean moons. These forces produce heat that may be capable of sustaining liquid water beneath their surfaces. The mere prospect of the existence of these subsurface oceans demonstrates that an Earth-like planet in circumstellar orbit is but one of a large number of possible hosts for life in the Galaxy and underscores the need to study non-Earth-like systems for gauging their habitability. Our group will study the implications of tidal interactions for oceans within the icy satellites Callisto, Europa, and Ganymede. They will also refine estimates of the thicknesses of the icy crusts of these bodies.

Lead team members Lyons, Newman, Paige, Varadi, and Young will study the volatile inventory of Mars and the Martian climate. Emphasis will be placed on linking Martian climate to rotational and orbital dynamics of the planet, interpreting the stable isotope data for Mars, and on shaping future missions to Mars.

The issue of long-term habitability of planets is being addressed through numerical simulations of planetary-scale dynamics by lead team members Tackley and Aurnou. Earth has been habitable for billions of years while Mars may have been so for a much shorter interval. Based on comparisons between Earth, Mars, and Venus, it seems likely that the long-term habitability in general could depend on the dynamics of the host planet. Plate tectonics may be necessary to sustain an appropriate level of carbon in the atmosphere, for example, which in turn influences surface temperatures. The long-term stability of an atmosphere suitable for life might depend on the presence of a magnetic field of sufficient intensity. Tackley and Aurnou envision establishing criteria that will afford predictions about the likelihood for plate tectonics and magnetic fields based on size and perhaps ages of extrasolar planets. The result of this research has been likened to a rocky planet analogue to the Hertzsprung-Russell diagram (HR diagram) that summarizes the evolution of stars. The HR diagram is used to portray manifestations of stellar evolution. The rocky planet version of the HR diagram would serve an analogous role and would be a useful tool for assessing the potential habitability of extrasolar rocky planets.

Impacts of asteroids and comets must play a fundamental role in the origin, evolution, and extinction of life. Impacts are a primary mechanism of planetary accretion and are possibly responsible for the delivery of water and organic matter to young planets. Large-body impacts may inhibit the formation of life in the early history of planetary formation. Once life has taken hold, impacts can play an important role in the path followed by evolution, such as the mass extinctions that are now known to be coincident with the Chicxulub impact event at the Cretaceous-Tertiary (K-T) boundary. This is not just a terrestrial problem. If life exists on Mars, Europa, or other planets outside our Solar System, impacts must have played a fundamental role there as well. Impacts may even play a role in transporting organisms between planetary objects.

Orbital and rotational dynamics is a central theme in several on-going studies by UCLA lead team members. The factors that cause changes in the frequency of asteroid impacts in the inner Solar System, coupling between Mars' orbital and rotational dynamics and climate, and the influences of giant planet orbital eccentricities on prospects for life in extrasolar planet systems are all being investigated by lead team member Varadi and colleagues using computer codes developed at UCLA. These specialized codes accurately reconstruct the orbital and rotational history of planets and asteroids for up to 100 million years. The physical model is successively refined to take into account small corrections in the equations of motion due to General Relativity, the finite size of the lunar orbit, and so forth. Varadi and Runnegar are using this code to investigate the possibility that impacts that caused mass extinctions might be the result of inner Solar System orbital chaos resulting in asteroid impacts. It might be said, albeit with a hint of frivolity, that because the dynamics of the inner Solar System depend on subtle interactions involving relativistic effects, the extent to which the geological

record of extinctions on Earth can be explained by changes in planet orbits serves as a test of General Relativity.

The details of these habitability-related research plans are described below.

6.2 HABITABILITY OF JUPITER'S GALILEAN MOONS (MOORE, NIMMO, SCHUBERT, VARADI)

6.2.1 Tidal forces and the implications for oceans within the icy satellites

There is strong evidence for the existence of a subsurface liquid water ocean on Europa and evidence for the existence of oceans buried in the deep interiors of Callisto and Ganymede as well. A liquid water ocean, at least near the surface of Europa, is a possible habitat for life. It is important therefore to understand how liquid water oceans can exist on Jupiter's icy moons in spite of the tendency for the satellites to cool and the oceans to freeze. The answer lies in a source of heat that offsets the tendency to freeze. It is possible that this heat source is simply the radiogenic heat supplied by the rocky material in the moons (e.g., Callisto, Spohn and Schubert 2003), but especially in the case of Europa, the crucial heat production might originate in the tidal flexing of the satellite by Jupiter. We are therefore motivated to study the role of tides in establishing and maintaining oceans of liquid water beneath the surfaces of the icy Jovian satellites.

From a more general perspective, it is evident that the large satellites of the giant planets have experienced significant orbital, rotational, and thermal evolution due to tides. Tidal dissipation makes Io the most volcanically active body in the solar system, but at the same time, Io clearly demonstrates that too much tidal heating can desiccate a body and render it inhospitable to life. In order to identify the boundaries of a tidally supported habitable zone we must study the orbital, rotational, and thermal evolution of systems of bodies around giant planets. The need to study giant planet moons as a system, even if interest lies mainly in the icy bodies, is clear from the coupling of Io, Europa, and Ganymede in the Laplace resonance.

Schubert, Moore, Varadi and colleagues are developing a numerical model that will calculate the coupled orbital, rotational and thermal evolution of satellite systems of giant planets and will apply the model to the Jupiter, Io, Europa, and Ganymede system. The orbital and rotational evolution of these satellites is obtained by following the dynamical equations forward in time using both symplectic and traditional integrators. We take a primitive-variables approach that does not arbitrarily restrict the orbits or rotations. Thermal evolution models using parameterizations also lead to differential equations that may also be integrated by these methods.

The key to coupling the orbital dynamical and thermal models is development of a dynamical approach to the tidal and rotational deformation of the satellites that represents the deformation equations within each body as a system of ordinary differential equations in time (Hanyk et al.1996). The entire coupled system can then be advanced forward in time using the same integrator. Novel heat transport parameterizations must be developed to adequately describe the magmatic and convective transport of heat in Io; similar parameterizations are necessary for the rocky mantles and icy shells of the other Galilean satellites. The result will be quantitative estimates for the times and places that sufficient heat and liquid water combine to yield potential habitats for life in the icy Galilean moons. The model includes the tidal interactions among the bodies and the tidal dissipation within them. It accounts for the feedback between tidal heating and heat transport in the bodies through the temperature-dependence of viscosity; this is a fundamental aspect of the dynamics of close satellites. Since tidal heating might be the only heat source capable of sustaining geologic activity on the moons for billions of years, understanding this process will be critical for evaluating their habitability. Tidal dissipation is also the mechanism driving orbital evolution and therefore it connects the interior dynamics to the observable orbital state, allowing precise astrometric measurements to constrain the thermal state of the interior of a satellite using only telescopic observations. Since we will not be able to visit any extrasolar satellite systems directly, the development of this theory will be vital to interpreting observations of such

systems in terms of astrobiological potential.

For example, by incorporating the tidal models of Schubert and colleagues into ephemeris computations, these workers will be able to tell how much the orbits of the Galilean satellites have changed over recent decades for different internal satellite structures. These predictions will be compared to past observations of the mutual events of the Galilean satellites. This model will also predict the physical librations of the satellites, i.e., departure from rotation around a principal axis of the moments of inertia tensor. These predictions can be tested against observations of the librations, perhaps by a future orbiter (for example, the planned Jupiter Icy Moon Orbiter) to constrain the interior structures of the satellites.

From results already obtained on the physical librations of the Galilean satellites, it has become apparent that improvements in our understanding of the dynamics of synchronous rotation are necessary. Straightforward numerical simulations of rigid satellites reveal that physical librations can have very large amplitudes, comparable to those of spin axis precession. We expect that tides would damp such motions, but it is not obvious how. Nevertheless, the heating rate implied by this dissipation is potentially significant, and could explain the excess heat flow observed from Io relative to that expected from Keplerian tides alone. Europa may also be subject to this excess tidal heating, and additionally may experience differential rotation between its ice shell and rocky interior. Measuring the amplitude of such librations (from an orbiting spacecraft) would provide an excellent constraint on the thickness of the ice when compared with the predictions of the theory being developed as part of this project.

6.2.2 Estimating the thickness of Europa's icy crust

The thickness of the icy crust of a Galilean satellite has major implications for its thermal history, habitability, and suitability for future missions. On Ganymede and Callisto, the crust is thought to be O(100 km) thick; on Europa, the crust is perhaps O(10 km) thick or smaller, but there is considerable uncertainty in this estimate (Pappalardo et al. 1999). Some inferences of crustal thickness from interpretations of the surface geology have the crust only a few kilometers thick (Geissler et al. 1998; Hoppa et al. 1999; Greenberg et al. 2000) As discussed more thoroughly in § 6.2.3, the thickness of Europa's crust is key to the transport of nutrients from the surface to an underlying liquid water ocean. So much depends on how close Europa's ocean is to the surface that it is essential to explore all avenues at our disposal for estimating the thickness of Europa's icy crust.

One approach to estimating the thickness of Europa's crust is to construct theoretical models of its internal structure as part of a thermal history investigation. This has been done by Hussmann et al. (2002) and Spohn and Schubert (2003). These models predict ice crusts that are a few tens of kilometers thick. The coupled orbital dynamical, rotational, and thermal evolution models discussed in § 6.2.1 contain more physics and will provide improved theoretical estimates of ice crust thickness.

Another way of estimating the crustal thickness is to measure its rigidity, or effective elastic thickness. Ice retains its elastic strength only at relatively cold temperatures. Since the temperature gradient within the ice crust depends on its total thickness, the measured elastic thickness can be used to infer the crustal thickness. The elastic thickness measured is the lowest since the deformation occurred.

On the Galilean satellites, stereo topography and flexural analysis can be used to derive the elastic thickness (Nimmo et al. 2002). This has been done for topographic profiles across two rifts on Ganymede, with the result that both profiles give an effective elastic thickness of about 1 km, implying a total crustal thickness of about 3 km at the time of loading (Nimmo et al. 2002). The crustal thickness at present is much larger. However, these calculations indicate that Ganymede once had an ice crust only a few km thick, probably due to an episode of tidal heating.

Nimmo and coworkers propose to extend this approach to Europa. In a preliminary analysis of several topographic profiles across Europa they find an elastic thickness of 6 km, suggesting that the present-

day crustal thickness is at least 15 km. This is larger than some estimates, but it agrees with evidence from impact crater studies (Schenk 2002) and predictions from thermal history models (Hussmann et al. 2002; Spohn and Schubert 2003). Possible links between the surface and the internal ocean through such thick ice will be studied in the project described in the next section.

6.2.3 Transport of nutrients through an icy crust – feeding an isolated world

The ice shell covering Europa (§ 62.2) impedes the transfer of nutrients and energy (e.g., sunlight) from the near surface to the interior ocean that most likely lies beneath. The extent to which material from the surface can be transported to the interior, and vice versa, is crucial for assessing the likelihood for Europan life.

Nutrients could be produced by either ^{40}K decay within the ice shell (Chyba and Hand 2001) or the collision of particles accelerated by Jupiter's magnetic field with the surface (Carlson et al. 1999). the latter may be efficacious because Europa is located at the outer edge of Io's plasma torus. The co-rotating plasma from Jupiter's magnetosphere continually overtakes Europa and bombards it with plasma with energies ranging from 100 eV to several MeV. The particles that have a significant effect on the surface chemistry and morphology have energies exceeding tens of keV.

The chemical products formed from the radiolysis of water and CO_2 ice by charged particles include H, OH, H_2O_2, O_3 and many C-H-O compounds like CH_3OH, H_2CO, and CH_2CO etc (Delitsky and Lane 1998). The yield factors for these chemical products are not very well known but yields of 0.01 for O_2 molecules and 0.2- 0.4 for H_2O_2 molecules for each 100 eV of deposited energy have been reported (Brown et al. 1982; Moore and Hudson 2000). Carlson et al. (1999), using infrared spectroscopy from the Galileo spacecraft, estimate that hydrogen peroxide (H_2O_2) abundance is 0.13% relative to water ice on the surface of Europa.

Because photosynthesis is severely inhibited by the thick ice covering Europa, and because the primary energy from geothermal and chemical weathering processes would be quite limited there, Gaidos et al. (1999) argue that most metabolic pathways that power the life cycle on Earth would be denied to organisms on Europa. Therefore, many authors (Chyba 2000; Chyba and Phillips 2001; Cooper et al. 2001; Chyba and Hand 2001) have considered the potential of the radiolysis-produced oxidants to power life in an oceanic ecosystem. The rate at which the oxidants produced at the surface are transferred to the liquid ocean depends on the primary yield from radiolysis, erosion of surface from sputtering and impact gardening, the ultraviolet processing of the surface and the oxidants, and the overturn rates of the surface material by endogenic geological processes.

The radiation not only creates oxidants, it also destroys them and through sputtering can eject a large fraction of the radiolysis products from the surface altogether. Chyba and Phillips (2001) have analyzed the competition between particle sputtering and impact gardening in creating, destroying and preserving (through regolith burial) oxidants on the surface of Europa and suggest that if the regolith is well mixed and communicating with the deeper layers, as much as 2.5×10^{25} molecules of H_2O_2 would be produced per square centimeter of the surface over a time period of 10 Myr. Depending on the models of ice thickness and subsurface geology a wide variety of scenarios can be supposed for the delivery of these oxidants to the liquid ocean. Using fairly conservative estimates of oxidant creation and transfer to the ocean, Chyba and Phillips (2001) suggest that the ocean would be able to support ~ 10^{23} to 10^{24} prokaryotic-analog cells in the oceanic biomass. However, if the upper layers of ice could be constantly replenished with material exchange from the interior so that a maximum transfer of the oxidants occurs to the subsurface ocean, the biomass estimate would increase by a factor of 10^3 and the level of oxygen in the Europan ocean could be comparable to that in the Earth's ocean (Cooper et al. 2001).

The solid ice shell of Europa consists of two parts, a near-surface cold region in which deformation occurs by brittle processes, and a deeper warmer region in which ductile deformation predominates. The latter region may experience solid-state convection (see below) and is likely to be the area in which heat is generated by tidal deformation of the ice shell.

Nimmo and coworkers propose to investigate possible transport mechanisms in both these regions, and focus in particular on the likelihood of melting the ice. Melting is attractive because it forms an efficient transport mechanism, and there is observational evidence for it, such as the surface chaos regions.

Melting within the brittle near-surface layer is difficult to achieve, but one potential mechanism is shear heating on strike-slip faults (Nimmo and Gaidos 2001). This model suffers some drawbacks, notably its inability to calculate the thickness of the brittle layer self-consistently. We propose to remedy this deficiency in order to better establish the conditions under which near-surface melting can occur. A further consequence of the shear-heating model is that it may produce linear diapirs, which can potentially advect material towards the surface. It is well known that Europan ridges are darker than the surrounding material (Fagents et al. 2000). Nimmo et al. propose to investigate whether this observation is consistent with diapiric activity.

Transport of material within the convective zone of the Europan ice crust is estimated to occur on timescales of about 10^3 yr to about 1 Myr. If nutrients can be transported through the brittle lid, such timescales are probably sufficient to sustain a modest oceanic biosphere (Chyba and Phillips 2001). Previous convection models of Europa have generally assumed Newtonian behavior (e.g., Pappalardo et al. 1998), but the ice may actually be behaving in the non-Newtonian regime. An important consequence of non-Newtonian behavior is episodicity, which means that previously calculated transport timescales could be incorrect. Furthermore, convection might lead to discrete diapirs (e.g., Nimmo and Manga 2002) rather than the steady currents previously envisaged (e.g., Pappalardo et al., 1998). Nimmo and colleagues propose to carry out a suite of convective model calculations that will incorporate non-Newtonian behavior and tidal heating and examine the consequences for material transport and melt generation. While they intend to focus on Europa initially, the equations are general so that one can also investigate convective processes on Ganymede, Callisto and the Saturnian icy satellites.

A key feature of the convection models will be the incorporation of a realistic composite ice rheology (Goldsby and Kohlstedt 2001), including the different creep deformation mechanisms (dislocation, superplastic, and diffusional) that come into play at different stresses and grain sizes. The former two of these are non-Newtonian, which can lead to deformation that is episodic and localized (e.g., Larsen and Yuen 1997), as mentioned above. The models will also include a simple parameterization of brittle failure of the lid using a pseudoplastic yield stress, which can also lead to highly episodic behavior, as well as mobile-lid-like features (Tackley 2000). This modeling can be performed using an existing code, Stag3D, which has previously been used for modeling silicate convection in 2-D and 3-D and already includes the necessary rheological capabilities. Failure of the brittle layer would be especially interesting in this context as it would permit advective transport of materials from/to the surface.

The convection models will be coupled to a tidal dissipation calculation, in order to self-consistently treat the feedback between viscosity variations caused by convection, and tidal dissipation (Sotin et al. 2002). Tidal flexing is a long-wavelength phenomenon so it couples mostly to long-wavelength viscosity variations, and a global treatment is necessary to calculate this correctly. The main interest in such heating is that it is another possible melt-generating (and hence nutrient-transporting) mechanism.

6.3 THE STRUCTURE AND DYNAMICS OF ROCKY PLANETS (AURNOU, TACKLEY)

Cycling of volatiles (particularly water and carbonate) between the interior and surface of a terrestrial planet could play a major role in the evolution of the fluid envelope, and hence the long-term habitability of the surface environment. This cycling is strongly influenced by tectonic mode (i.e., plate tectonics, rigid lid or episodic plate tectonics). For medium-sized planets, a magnetic field, when present, greatly reduces solar wind-induced escape of the atmosphere, aiding long-term habitability. Previous habitability analyses have included only highly idealized, parameterized models of planet interiors (bringing into question their veracity) and have not included the generation and consequences of a planetary magnetic field. Tackley and Aurnou propose to study the interaction of the interiors of

terrestrial planets (mantles and cores) with their fluid envelopes using advanced numerical models, with the goal of formulating predictions for the evolution of habitability of terrestrial planets as a function of size, stellar flux and initial conditions. The assumed criterion for planetary 'habitability' will be the usual: the existence of liquid water at the surface (e.g., Kasting et al. 1993b) which requires a surface temperature of between 273K and 373K (at 1 bar or 10^5 Pa).

Both the mantle and the core of a terrestrial planet play important roles in determining the evolution and composition of its atmosphere. The fluid envelope of a planet in turn influences the dynamics of its interior, resulting in a system of feedbacks that requires careful analysis. Such an analysis requires consideration of coupled mantle-core-atmosphere systems to determine the likelihood of surface liquid water (hence habitability). Habitability is clearly time-dependent, with planets such as Mars possibly passing through a short-lived (e.g., 100s of Myr) habitable phase before reaching a long-term, non-habitable (too-cold) condition, while planets such as Earth experiencing a long-term, slowly-evolving habitable condition. It is notable that the known planets without plate-tectonics-related interior-atmosphere feedback (i.e., Mars and Venus) appear to have undergone a transient one-way evolution to a non-habitable mode.

The mantle of a rocky planet influences the planet's fluid envelope by contributing volatile components (e.g., water, carbon dioxide gas) to the surface by way of volcanic activity, and by as a sink for volatiles from the surface where tectonic processes are capable of delivering surface material to the mantle. Indeed, a long term cycle that moves carbonate to and from the mantle (volcanic outgassing, tectonics) may provide a critical feedback mechanism that maintains surface temperature in a habitable range (Kasting et al. 1993b; Sleep and Zahnle 2001). Volatile recycling also affects the redox (reduction/oxidation) state of the mantle, which in turn, through outgassing, influences the redox state of the atmosphere (Delano 2001; Kasting et al. 1993a; Lecuyer and Ricard 1999), and may have been responsible for the rapid rise in atmospheric oxygen ~2 Gyr ago (Kump et al. 2001).

Plate tectonics appears to be a crucial component of such planet-scale cycles and feedbacks. It is by way of plate tectonics that carbonate, water and other volatiles are returned to the mantle by the process of subduction. Such an efficacious return mechanism is not possible in a planet with a rigid, unyielding outer lid (lithosphere). Apparently, the existence of plate tectonics may be important for long-term planetary habitability. The conditions necessary for plate tectonics to exist as a feature of other rocky planets of variable sizes and distances from their stars, and the scaling of key rates (e.g., outgassing, subduction) associated with plate tectonics on other planets, are not understood. In addition, the details of how recycled water and other volatiles circulate in the mantle to be returned to the shallow melting zones beneath volcanic centers are poorly understood even for Earth. The uncertainties are exacerbated by the fact that the mantle of a planet may be partly stratified in convection or in composition, and this stratification will have evolved with time (Tackely 2000a). Tackley and colleagues propose to study these aspects using numerical models of mantle convection and lithosphere dynamics, as discussed below.

Based on the known terrestrial planets of our Solar System, the core of a rocky planet is responsible for the generation of the magnetic field that shields the planet's atmosphere from the stellar wind emanating from its star, thereby greatly reducing the rate of atmospheric escape (Shizgal and Arkos 1996; Yung and DeMore 1999). Existence of a magnetic field is particularly important for retaining atmospheres around smaller terrestrial planets such as Mars (larger ones such as Venus are more able to hold on to their atmospheres by gravity alone) and is also important for protecting primitive organisms from potentially lethal charged particles (Horneck et al. 1994). Existence of a magnetic field may be important for maintaining a habitable atmosphere over billions of years for smaller planets, but not all terrestrial planets have an internal dynamo of long duration.

Defining those characteristics of a rocky planet that lead to its ability to generate a magnetic field (by virtue of a dynamo in the core) is of the utmost importance for determining the likelihood that the planet could sustain life over the long term. A first-order criterion for the existence of a dynamo is that

the heat flux extracted from the core by the mantle must be greater than that conducted down the core adiabat. However, it may be more complicated than this criterion implies. For instance, studies by (Kutzner and Christensen 2002) and (Olson and Christensen 2002) suggest that the existence and behavior of the magnetic field depends strongly on the strength and pattern of core heat loss as well as the planetary rotation rate. Assuming that the heat flux out of the core is sufficient to drive a dynamo (Gubbins 2001; Nimmo and Stevenson 1992), these studies find that generating an Earth-like dipole dominated magnetic field is sensitive to the ratio of buoyancy forces and rotational forces. It is not understood how this ratio evolves over the thermal history of a planet. In addition, the solid inner core tends to germinate and grow outward at the expense of the liquid outer core during the lifetime of a terrestrial planet (Labrosse et al. 1997). It is not well understood how the magnetic field generation process changes as the core solidifies (Al-Shamali et al. 2002). Tackley and Aurnou propose to investigate these aspects using three-dimensional numerical geodynamo models and parameterized models of coupled core-mantle evolution, as discussed below.

Tackley, Aurnou and lead-team colleagues propose to investigate the coupled system comprising the fluid envelopes, mantles and cores of terrestrial planets by first performing fundamental research on key aspects of the problem (e.g., magnetic field generation, generation and scaling of plate tectonics convection) using multidimensional numerical models and planetary evolution calculations using either numerical modeling of the mantle with parameterized core and atmosphere models, or fully parameterized models. This improves on previous research which has used only simple parameterizations, about which there is considerable uncertainty, for planetary interior behavior. The new models will be applied to known terrestrial planets prior to extrapolating to all possible terrestrial planets. Terrestrial planets from Mars size to ~10 Earth masses will be considered, with the core making up different proportions of the total mass.

A key output of this will be to establish a predicted domain diagram of exoplanet habitability as a function of size, incident stellar flux (related to steller brightness and distance from the star) and time. The sensitivity of this to initial conditions (e.g., initial concentration of carbon dioxide in the atmosphere) will also be investigated. A preliminary example of a hypothesized domain diagram at a time corresponding to the present-day Solar Systemis shown in Figure 6.3.

Numerical modeling (in two- and three-dimensions) of mantle convection in a plate tectonic regime will be performed. The models will include chemical transport and differentiation associated with crust production. This numerical modeling is necessary because there is considerable doubt as to how convective quantities scale with convective vigor in a plate tectonic regime due to the dissipation associated with subduction (Conrad and Hagar 1999), to the changing compositional buoyancy of oceanic plates (Davies 1992), and to changes in internal dynamics associated with phase transitions (Christensen and Yuen 1985; Davies 1995; Tackley 1995). The basic modeling technology already exists (Tackley 2000b; Tackley and Xie 2002) but will be expanded to include tracking of the subducted volatiles carbonate and water. Tracing these key volatiles will permit Tackley to monitor the evolution of the redox state and water-dependent viscosity of the mantle.

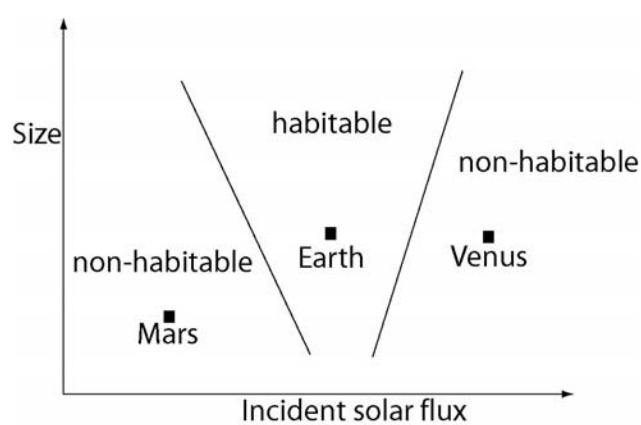

Figure 6.3. Plausible domain diagram of present-day planetary habitability (i.e., after billions of years of evolution) as a function of planet size and solar flux. Domain boundaries will be time-dependent.

A parameterized atmosphere-ocean 1-D radiative model will be overlain on these calculations in order to account for feedbacks between the two systems. A parameterized model of core heat loss (e.g., Labrosse et al. 1997) will also be included to evolve the lower boundary condition and determine core heat loss. Key goals are to establish the regions in parameter space where plate tectonics is expected when the fluid envelope of the planet is taken into account and to establish how feedbacks between the fluid envelope and the interior affect the time-dependent dynamics of the planet interiors.

Fundamental research into core dynamics of prospective terrestial planes will be performed via 3-D numerical dynamo simulations (Wicht 2002) to understand the effect of the main parameters on the type and strength of dynamo generated. The main parameters are the size of the core, the size of the solid inner core, cooling rate, and rotation rate. These experiments will give us a systematic understanding of how a planetary dynamo is likely to vary in strength and form (e.g, the relative importance of quadropole and higher components) for different sized planets at different stages of their evolution. Using these results, the rate of escape to space of different atmospheric species can be calculated, and this will form an important component of the atmosphere evolution model.

The abundances of the main atmospheric and oceanic constituents will be tracked over a planet's evolution, with inputs coming from mantle outgassing, and outputs to rock formation and subduction, and escape to space. A parameterized, 1-D atmospheric model will be used (as in e.g., Kasting et al. 1993b) to determine surface temperature and any other necessary quantities. Tackley and Aurnou do not plan to develop more sophisticated atmospheric models because this is being done by other NAI groups (e.g., Meadows et al. at JPL-2) and thus Tackley and Aurnou will collaborate with them if more sophistication is required. The initial condition (after major impacts, etc.) may have an important influence on subsequent planetary evolution, and this will be a major focus. Clearly, if life starts it will also exert a major influence, so the parameterized models will also include this possibility and compare the signature of planets with and without life.

6.4 ORBITAL DYNAMICS OF HABITABLE EXTRASOLAR PLANETS (VARADI, RUNNEGAR)

The known giant extrasolar planets have wide ranges of orbital semi-major axes and eccentricities. A giant planet with even moderately large eccentricity encounters a wide section of space. Accordingly, such planets can affect perturbations in the orbits of smaller bodies. Can habitable terrestrial planets survive in such orbital environments? Varadi et al. have unique capabilities to locate orbitally stable regions in extrasolar planetary systems. As opposed to the straightforward trial-and-error numerical simulations, they map out the stable regions of orbital resonances by computing the location and stability of resonant periodic orbits. They already have extensive results for the 2:3 (Varadi 1999; Figure 6.4) and the 1:2 (Haghighipour et al. 2003) orbital resonances. In the case of the former, they find stable, nearly circular orbits for small planets which cross the highly eccentric orbit of a giant planet. The configuration is the reverse of the Neptune-Pluto system. Their technique will also be used to find stable planetary orbits in multiple-star

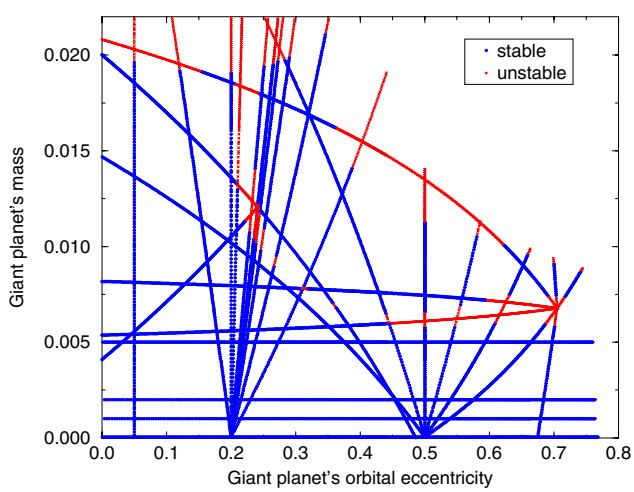

Figure 6.4 The stability of orbits of hypothetical small planets in 3:2 orbital resonance with a giant planet. Each dot represents a particular resonant periodic orbit found by varying the mass and orbital eccentricity of the giant planet. The results define paths along which resonant periodic orbits of small planets were determined.

systems and to constrain the evolutionary path of multi-planet systems in orbital resonances. Varadi and colleagues collaborate in this onging project with the Carnegie Institution of Washington NAI lead team.

6.5 STUDIES OF ASTEROIDS, IMPACTS, AND THEIR EFFECTS ON THE DEVELOPMENT OF BIOSPHERES AND THEIR PLANETARY ENVIRONS (BYERLY, KYTE, LOWE, NEWMAN, VARADI)

Members of the UCLA Center for Astrobiology are directly involved in exploring several areas of this important subject. This work involves direct studies of terrestrial impact events and critical events in Earth history, analytical studies of the evolution of planets and asteroids through time to explore the possibility of planetary forcing of clustered impacts, and modeling large impacts to understand their influence on the evolution of planetary volatile inventories (i.e., oceans and atmospheres).

6.5.1 Extraterrestrial impact history on Earth

This area of research is led by Frank Kyte, an established expert on the study of sediment deposits formed by large-body impacts and co-chair of the NAI Impact Focus Group. Kyte plans to engage in collaborative research with several research groups to expand our understanding of the impact history on Earth and potential links to perturbations in the Earth's evolutional history. This research involves Kyte's expertise in characterizing an impact signature using chemical and mineralogical analyses, in collaboration with experts in diverse fields ranging from sedimentology to isotope geochemistry. Several of the collaborations are described briefly below.

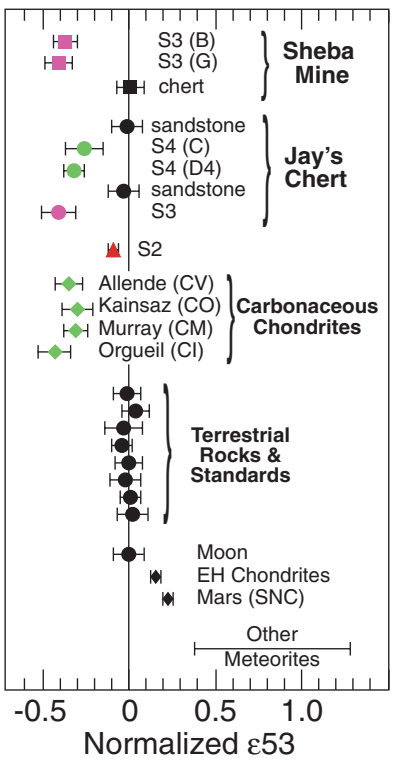

Figure 6.5.1. Cr isotope data for terrestrial rocks, carbonaceous chondrite meteorites, with $\varepsilon 53 < 0$, and K-T boundary, and Archean spherule beds (S2-S4). The spherule beds have isotopic compositions similar to the carbonaceous chondrites.

Early Archean (3.5 to 3.2 Gyr) impact deposits that were first reported by Lowe and Byerly (1986) in sedimentary rocks from the Barberton Greenstone Belt are now clearly established as derived from mega-impacts, based on their anomalous Cr-isotopic compositions (Figure 6.5.1, Kyte et al. 2003b). These rocks provide the earliest confirmed record of impacts on Earth, and provide a link to the Late Heavy Bombardment impacts that were recorded on the Moon. This research includes field and laboratory studies with UCLA lead team members Lowe (Stanford) and G.R. Byerly (LSU), as detailed below.

The Triassic-Jurassic boundary is one of the "Big Five" mass extinctions in the Phanerozoic. Detailed studies of new stratigraphic sections have demonstrated a significant stable isotope shift (Ward et al. 2001) and a small Ir anomaly (Olsen et al. 2002), suggesting a possible impact link to these extinctions. Kyte and the rest of the impact lead team members are engaging in analyses of new sections with P.D. Ward (University of Washington lead team) and other workers, to further examine this record.

Multiple Late Eocene impacts might be caused by a comet shower, according to Farley et al. (1998) who reported an increased flux of ^{3}He to sediments at that time. Kyte and colleagues are using chemical, mineralogical, and isotopic studies of these ejecta deposits to sort out the provenance (e.g., cometary,

impact plume, interplanetary dust?) of various physical components at several sites around the world. This work should help to constrain the comet shower hypothesis.

The late Pliocene impact of the Eltanin asteroid is the only known deep-ocean (5 km) impact (e.g., Gersonde et al. 1997) and is characterized by high-energy deposits with high concentrations of meteoritic materials (Kyte et al. 2002a-c). A recent oceanographic expedition to the impact site recovered 17 new sediment cores with ejecta deposits. This unique deposit might be used to constrain models of impacts on early Earth that are proposed as a potential source of organic matter for the origin of life (Pierazzo and Chyba 1999; Kyte et al. 2003a). Kyte and colleagues are also attempting to obtain precise ages on this impact to link it to the climate record at 2.4 Myr before present, a time of rapidly deteriorating climate.

6.5.2 Exploring the early Archean impact record and the consequences for early life

Over the next few years, the UCLA lead team plans to investigate three main aspects of the Barberton Greenstone Belt (BGB) impacts: (1) Archean impact rates; (2) the effects of large impacts on surface environments; and (3) the effects of impacts on putative biological materials and communities.

Lowe (Stanford), Byerly (LSU), and colleagues propose to constrain the Archaean frequency of impacts by providing additional geochronological constraints on critical sections within the BGB, especially within the upper Onverwacht, which is composed of over 100 million years of sedimentary and volcanic rock with no internal age dates. The Stanford-USGS SHRIMP (ion microprobe) will be used on several samples previously collected from the upper Onverwacht. Within the upper Onverwacht they will also collect closely spaced samples, and in places obtain continuous cores, from the condensed sedimentary sections. Preliminary analyses for chromium anomalies will be used to screen for possible impact layers. Subsequent analyses for iridium anomalies and possibly chromium isotopic anomalies will be used to distinguish chondritic impact materials from the very similar komatiitic volcanic materials.

In shallow-water and shelf environments, impact spherules have generally been extensively reworked by impact-produced tsunamis and, in some areas, subsequent environmental currents and waves. In deeper-water settings, the spherules accumulated as direct fall deposits (Lowe and Byerly 1986; Lowe et al. 2003). By tracing beds along individual outcrop belts and among the various structural belts, it will be possible to document the effects of tsunamis and fall-deposition of enormous volumes of impact-produced debris on local environments and to determine how new environmental conditions that were established following the impacts differed from those prevailing before the impacts. The latter should provide key evidence regarding the long-term effects of such large impacts. Lowe and Byerly will also examine the sedimentary record immediately above well documented impact layers for indications of major compositional anomalies that might be associated with impact-induced modifications of the atmosphere and shallow hydrosphere.

It has been suggested that impacts that deposited some BGB spherule-containing layers were large enough to boil away the surface layer of the oceans (Sleep and Zahnle 1998). Such a profound environmental effect may be reflected in the character of organic matter across the impact horizons. In a number of localities, black organic chert occurs below and immediately above impact layers. The types, abundances, distribution, and isotopic composition of organic grains and mat-like layers (e.g., Walsh and Lowe 1999) should provide clues about the changes in organic materials and perhaps the organisms that produced them across the impact layers.

Lowe and Byerly will spend approximately 3 weeks each summer conducting field studies in the Barberton and other Kaapvaal greenstone belts, South Africa, and/or the greenstone belts of the Pilbara, Western Australia. One week each summer will be spent with analytical studies. Byerly will visit Stanford for one week each summer to do geochronology in the SHRIMP Lab. The initial two years will focus on Barberton, and subsequent years will be spent in the Pilbara looking for correlative impact layers.

6.5.3 Solar-System chaos and the frequency of asteroid impacts

Chaos in the motions of the inner planets of the Solar System causes episodic transitions in the way their orbits interact. While chaos makes it difficult to reconstruct or predict these transitions far from the present, state-of-the-art simulations indicate that a major transition took place about 65 Myr ago, coinciding approximately with the Chicxulub asteroidal impact (Kyte, 1998) that is believed to be responsible for the Cretaceous-Tertiary (K-T) mass extinction event. So the question is: was chaos in the inner Solar System the ultimate cause of the extinction of the dinosaurs? Or, more generally, can chaotic disturbances of inner Solar System dynamics lead to an increased probability of a sizeable asteroid hitting the Earth?

These questions have to be considered in the general framework of asteroid orbital dynamics. The largest changes in the orbits of the major planets and asteroids are regular rather than chaotic. From time to time, the orbits of some asteroids (e.g., 1750 Eckert) are strongly perturbed by the major planets due to certain orbital alignments (see Williams and Hierath 1987). Furthermore, some regions of the asteroid belt are chaotic independently of chaos in the motions of the major planets (Lecar et al. 2001). The result may be collision with another body, ejection from the Solar System, or scattering within the asteroid belt. Most of the current asteroids are the survivors of this constant perturbative erosion. However, chaotic transitions in the motions of the inner planets may change the locations of regions of strong erosion in orbital parameter space (Figure 6.5.3) and, as a result, long-term survivors may become vulnerable.

In order to determine the magnitude of any chaos-induced increase in asteroid impact probability, Varadi and colleagues propose to carry out a variety of long-term numerical simulations. First, they will determine the types and ranges of possible chaotic transitions by simulating only the motions of the major planets. Next, they will add thousands of randomly placed hypothetical asteroids into the model in order to locate regions in parameter space which enable the test asteroids to survive. Finally, Varadi will drive the survivors through regime transitions. One way to approach this problem in the shorter term (which we shall also use) is to take a set of the most vulnerable existing asteroids - those which are already or nearly planet crossing, such as 433 Eros and 1750 Eckert - and simulate their behavior both backwards in time through the observed major transition near the K-T boundary and through an equally sizeable transition that is expected to occur about 30 Myr from now.

Varadi will use a numerical integration scheme that is a version of the classical Stormer-Cowell integrator but optimized to reduce the long-term effects of numerical round-off errors. The UCLA lead team has obtained an improved analytical representation of the lunar orbit which is a significant step toward increased accuracy. The simulation results are used not only to investigate a possible connection between inner Solar Systemorbital chaos and asteroid impacts, but also to couple the orbital and

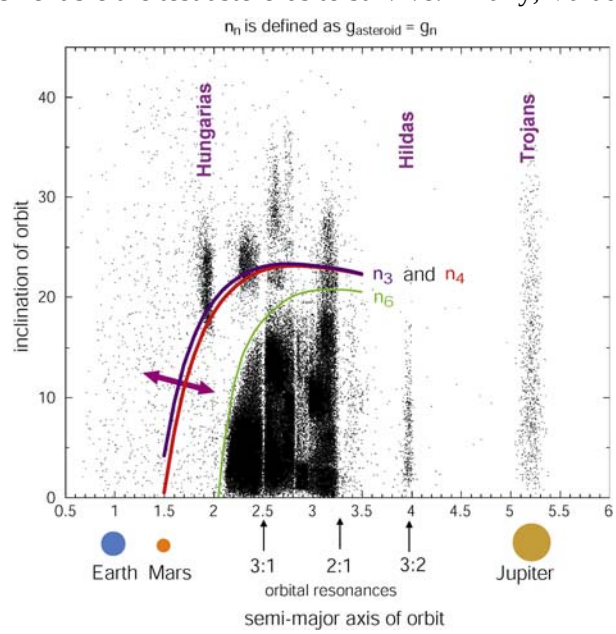

Figure 6.5.3. Plot of asteroids (dots) as a function of distance from the Sun (semi-major axis) and inclination of orbits. The n_3, n_4, and n_6 refer to secular resonances resulting in very large perturbations of the asteroid orbits. As the rates of Earth's and Mars'orbits change due to chaos, so too do the locations of these unstable orbits of the asteroids depicted by n_3, n_4, and n_6.

rotational dynamics of Mars in order to understand long-term changes in Martian climate (§ 6.6.1).

6.5.4 Impacts and the evolution of atmospheres

Newman and colleagues will consider the energetics of large bolide impacts (10 times the K/T event and greater) on atmosphere-free planets to determine whether such impacts will contribute to the planet's volatile inventory.

It appears that it is possible to acquire volatiles through very large impacts even in the absence of degassing of surface volatiles and mantle outgassing. This is evident by considering an impactor that strikes a planet at 20 km/s and vaporizes approximately three times its own mass in target (i.e., surface) material (O'Keefe and Ahrens) (O'Keefe and Ahrens 1977). The vapor cloud has, therefore, four times the mass of the impactor. Excluding all other sinks of energy (i.e. the impact has perfect conversion of kinetic energy from bolide to vapor cloud), the mean speed of the cloud will be reduced to 10 km/s, less than Earth's gravitational escape velocity. If one adds more realistic sinks of energy (i.e., latent and sensible heat, seismic radiation, etc.), the expansion velocity of the cloud will decrease further, and more vapor will be retained. Newman et al. are therefore calculating a strict lower bound on retained material. More inclusive representations of energy sinks will increase the amount of vapor retained.

Newman and coworkers have created a simple, physical model derived from the self-similar model of vapor cloud density of Zel'dovich and Raizer (1966) for expansion of gas in a vacuum, the model used previously by Vickery and Melosh (1990). They also assume the amount of target material excavated by an oblique impact based on the findings of O'Keefe and Ahrens (1977) as well as Pierazzo and Melosh (2000). By considering both the expansion speed of the cloud and the bulk drift of the cloud due to lateral motion of the oblique impactor, they solve for the fraction of the expanding cloud for which the total (vector) velocity is less than the escape velocity of the planet. They have observed (Mischna and Newman, in prep) that most impact events on airless worlds provide substantial inventories of volatiles, thereby producing the atmospheres and hydrospheres necessary for life.

Newman et al. propose to perform first-principles hydrodynamic simulations of oblique impacts for a variety of impact speeds (i.e., Solar System positions of origin), target planet, impact angles, crustal volatile content (ranging from ocean-impacts to dry, Venus-like lithospheres). The simulations will be performed in collaboration with Los Alamos National Laboratory (LANL) collaborator Eugene Symbalisty using LANL and Sandia National Laboratory codes, some of which have now been ported to desktop workstations, to develop more accurate quantitative estimates of the efficiency of the volatile retention process. Finally, Newman will consider how these results can be applied to extrasolar planetary systems.

6.6 MARTIAN CLIMATE AND VOLATILE INVENTORIES THROUGH TIME (LYONS, NEWMAN, PAIGE, VARADI, VASAVADA, YOUNG)

In the search for life on Mars, either present or past, it is necessary to develop comprehensive understanding of the inventory and behavior of Martian volatiles and the Martian climate. Volatiles and climate on Mars are intimately linked to a wide range of dynamical phenomena, including: (1) the dynamics of the Solar System and Mars' spin axis; (2) the dynamics of impactors and their interaction with the Martian surface and atmosphere; (3) the dynamics and chemistry of the Martian crust and interior; (4) the dynamic interaction between the Martian atmosphere and the space environment; and (5) the dynamics of the Martian atmosphere and polar caps. During the next five years, the UCLA NAI Mars Volatiles and Climate subgroup will address four focused problems directly relevant to Mars volatiles and climate studies.

6.6.1 Orbital and axial dynamics of Mars

Varadi, Paige, Vasavada and colleagues proposes to develop new, state-of-the-art models for orbital and rotational dynamics and couple them to low-dimensional climate models to better understand how

large-scale quasi-periodic variations in Mars orbital and axial elements are coupled to Martian climate change. Successively refined numerical simulations of the orbits of the planets are expected to provide a more accurate orbital history of the Solar System for the past 70 million years (see § 6.5.3).Beyond this time scale, the uncertainties due to chaos make the results of individual simulations unreliable. They are still meaningful, however, in terms of understanding the general features of orbits over hundreds of millions of years. Rotational dynamics, on the other hand, needs to be fundamentally reconsidered.

According to the conventional view, planets rotate around a principal axis of the moments of inertia tensor and the spin axis precesses in space due to torques by the Sun, other planets, and also satellites. This is a convenient approximation which is not consistent with the equations of rotational dynamics. The torques responsible for spin axis precession also cause the planet to deviate from exact principal-axis rotation. Our recent results demonstrate that the deviation can be comparable to the amplitude of spin axis precession in the case of synchronously rotating satellites (§ 6.2.1). Could similar phenomena be important for Mars? Once the body is allowed to deviate from principal -axis rotation in our models, there can be a number of resonances between the period of the wobble and short-period orbital forcing from other planets.

UCLA lead team members are already developing new analytical machinery in the form of perturbation theory, to obtain accurate equations for the long-term behavior of obliquity. While rotational variations can affect climate, the reverse is also true. The seasonal deposition and sublimation of large polar caps on Mars perturbs the moments of inertia (as measured by Mars Global Surveyor through the gravity field) of Mars, providing a means for feedback between climate and rotation. Other internal processes such as the formation of Tharsis also modify the inertia tensor and therefore influence the rotational state of Mars. The team is developing a model for following the time-dependent deformation of Mars under various loads. This model will be coupled to both the climate model (through the polar cap loading/unloading) and the orbital and rotational dynamics through the time-dependent inertia tensor. The dynamics of such a system have not been analyzed, and this feedback may have a significant effect on the obliquity history of Mars.

The orbital and rotational dynamics will be coupled with simplified models of the behavior of the Martian climate system that will calculate the state and mass distribution of the polar caps and atmosphere. Such models will be used to understand potential coupling between the dynamics and of the Solar Systemand the Martian climate, which may be recorded in the Martian layered deposits. We will develop and use sophisticated time series analysis techniques based on Singular Spectrum Analysis to extract potential climate signals that can be compared to observations of Martian layering patterns as demonstrated by Laskar et al (2002). Our studies will include polar layered deposits as well as mid-latitude layered sedimentary deposits recently identified in Mars Global Surveyor Images (Malin and Edgett 2000). The analysis will employ a recently-developed “denoising” technique called Random-Lag Singular Cross-Spectrum Analysis. This technique provides not only cleaned-up signals, but also cross-filters with phase information between signals to detect time lags between astronomical forcing and changes in the rates of deposition.

6.6.2 Oxygen isotope fractionation and the crysosphere of Mars

The ratios of the isotopes of oxygen in Earth’s atmosphere reveal much about the functioning of our planet. In particular, the fact that the $^{18}O/^{16}O$ of oxygen in tropospheric air is grossly out of equilibrium with $^{18}O/^{16}O$ in the oceans is a first order consequence of the existence of a biosphere; disequilibrium in oxygen isotopes between air and oceans is the result of respiration, a phenomenon known as the “Dole effect” (Dole 1935). Similarly, the oxygen isotope ratios of the various oxygen-bearing reservoirs on Mars are potentially useful as first-order indicators of processes that operate in the outer, volatile-rich envelope of the planet. Lead team investigators Young and Lyons propose to investigate oxygen

isotope fractionation processes applicable to Mars with an eye towards elucidating the meaning of the measurements that exist for that planet.

Oxygen represents a major component of the Martian atmosphere, cryosphere, lithosphere, and perhaps its biosphere if it exists. With respect to the ^{16}O, ^{17}O and ^{18}O isotopic system, data from Martian meteorites (SNC meteorites) suggest unusual fractionation patterns that have yet to be fully explained. Clues come in the form of similar, though far from identical, effects in Earth's troposphere.

Oxygen in Earth's troposphere has $\delta^{17}O$ values ($\delta^{17}O$ = per mil deviation in $^{17}O/^{16}O$ relative to standard mean ocean water) that are ~ 0.3 per mil lower than rocks and waters at the same $\delta^{18}O$ value. Differences in $\delta^{17}O$ relative to $\delta^{18}O$ values are referred to as differences in $\Delta^{17}O$ where $\Delta^{17}O = \delta^{17}O - 0.52\ \delta^{18}O$. This definition of $\Delta^{17}O$ is based on the assumption that the so-called mass-dependent fractionation among the three isotopes of oxygen follows the relation $\delta^{17}O = 0.52\ \delta^{18}O$. In fact the "slope" factor 0.52 is actually an exponent in a fractionation law and takes on a range of values from about 0.53 to 0.51depending upon the physicochemical process involved (Young et al. 2002). This factor, referred to as β, is predicted to be lower for kinetic processes than it is for equilibrium processes (Young et al. 2002), though only a few direct measurements to confirm or contravene this assertion have been made to date. The convention of assuming that $\beta = 0.52$ for the purpose of defining $\Delta^{17}O$ values is well ensconced in the literature and is retained.

Luz et al. (1999) attributed the $\Delta^{17}O$ value of tropospheric O_2 to photochemistry in the stratosphere. Young et al. (2002) suggested that the $\Delta^{17}O$ value of tropospheric O_2 is instead an expected consequence of the Dole effect; it represents a steady state between two processes acting with near constant rates. The two processes are photosynthetic production of O_2 and extraction of O_2 by respiration. Although the control that these competing biological processes exert on $\delta^{18}O$ in atmospheric oxygen is well known, until recently there had been no characterization of β values for respiration (photosynthesis results in O_2 with the same $\Delta^{17}O$ as ocean water because the fractionation associated with the process is small). Young et al. (2002) made the prediction that the β value for the kinetic process of respiration should be close to 0.508, and that this value is sufficient to explain the $\Delta^{17}O$ of tropospheric O_2 with out the need to invoke mixing with photolysis products from the stratosphere. The competing-rates hypothesis has gained momentum with new measurements showing that β values attending photorespiration and dark respiration are 0.506 and 0.518, respectively (Luz et al. 2002). The measured values are close to the predicted value of 0.508.

From the preceding discussion it is clear that the cause of the measured $\Delta^{17}O$ of O_2 in the troposphere is uncertain but the existence of the $\Delta^{17}O$ difference between rocks ($\Delta^{17}O$ ~ 0 similar to waters) and tropospheric O_2 means that there are two distinct reservoirs of oxygen maintained by some phenomenon that affects the lower atmosphere but not the rocks. The phenomenon is either a steady state imposed by the relative rates of O_2 consumption (respiration) and production (photosynthesis) or it is photochemistry that occurs elsewhere in the atmosphere. It is likely that both explanations are valid. In any case, before we can use $\Delta^{17}O$ as a tool for tracing material transfer to and from different near-surface reservoirs on Earth, it will be necessary to understand all of the factors that can influence $\Delta^{17}O$ values, both mass dependent (variable β) and mass independent (photochemistry).

Similar arguments pertain to Mars. The atmosphere of Mars is dominated by CO_2, making the oxygen isotopic compositions of carbonates from the planet especially useful tracers of atmospheric processes there. Carbonates from one SNC meteorite (apparently formed on Mars approximately 3.6 billion years before present near the Hesperian time interval, McKay et al. 1996) have $\Delta^{17}O$ values ~ 0.5 per mil higher than the igneous minerals from all measured SNCs. The difference between the two mineral types suggests that just as on Earth, there are, or were, at least two oxygen reservoirs on Mars. One in the atmosphere (sampled by SNC carbonate) and the other represented by the regolith (sampled by the

SNC igneous minerals). This is an important clue to the way that the Martian atmosphere behaved in Hesperian time, but the cause of the observation is uncertain.

Farquhar et al. (1998), the workers who made the measurements of $\Delta^{17}O$ in carbonate from SNC meteorite ALH 84001, suggested that the difference in $\Delta^{17}O$ between carbonate and igneous minerals was the result of exchange between CO_2 and electronically excited O liberated by photodecomposition of ozone. Another possibility is that the $\Delta^{17}O$ in the carbonates reflects an anomalous, partly non-mass dependent fractionation caused by gravitational separation of the isotopes above the homopause and isotopically selective escape of isotopomers at the exobase (Jakosky 1993).

Yet another explanation for the difference in $\Delta^{17}O$ between ancient Martian carbonate and igneous minerals is that competing processes with different mass-dependent β values, analogous to the situation that controls $\Delta^{17}O$ of tropospheric O_2 on Earth, are operating near the surface. In this case (Young et al. 2002) a steady state between sublimation of CO_2 ice with a low value for β, consistent with a kinetic process, and condensation of CO_2 with a higher β value approaching equilibrium values (since condensation implies partial pressures approaching equilibrium), might be the cause of the disparate carbonate $\Delta^{17}O$ values, as shown in Figure 6.6.2.

Young and Lyons propose to test the hypothesis that $\Delta^{17}O$ can be affected by passage of CO_2 between the cryosphere and the atmosphere on Mars through a program of experiments that will characterize the β values attending condensation and sublimation. The first experiments will be on O_2 liquid and vapor (since the three isotopes of oxygen are readily analyzed in this system). Young and Lyons will freeze O_2 in a vacuum line and analyze the residual gaseous O_2 as a function of fraction of gas frozen. Techniques will be similar to those described by Eiler et al. (2000) but with the exception that $\delta^{17}O$ as well as $\delta^{18}O$ will be measured using the stable isotope laboratory at UCLA. These early studies of O_2 will serve as the basis for new experiments on CO_2 in which fluorination will be used to liberated O_2 from C for isotopic analysis.

Lyons will simultaneously reexamine the suggestion by Jakosky (1993) that escape from the top of the Martian atmosphere could have caused significant shifts in $\Delta^{17}O$ in CO_2. He will evaluate the mass

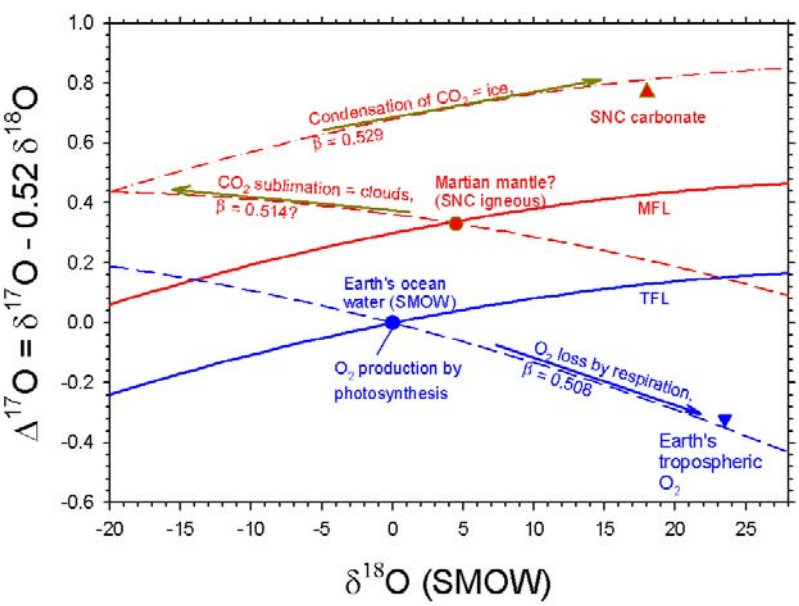

Figure 6.6.2. Oxygen three isotope plot showing the mass-dependent isotope fractionation mechanisms that may have caused negative $\Delta^{17}O$ values for O_2 in Earth's troposphere and positive $\Delta^{17}O$ in Martian carbonates.

independent component of diffusive escape processes in the Martian homopause. The problem requires examination of how O, CO, and CO_2 behave above the homopause and the physics of escape of O from the exobase. These processes will be examined in the context of the competing processes of non-mass dependent photochemical reactions that liberate electronically-excited oxygen.

The product of this research will be a better understanding of the meaning of variable $\Delta^{17}O$ values (at the ~ 0.5 per mil level) both on Earth and on Mars.

6.6.3 Liquid water on Mars

Understanding when and where liquid water may have existed on Mars is crucial for assessing the biologic potential of the planet, as well as for guiding our efforts to explore Mars with robotic spacecraft. The selection of promising landing sites is of particular importance for future missions in order to maximize their potential scientific return.

Paige and Shebenski have developed a new approach for studying the distribution and stability of Martian near-surface ground ice by observing the thermal effects of the presence of ground ice on diurnal and seasonal surface temperature variations using Mars Global Surveyor Thermal Emission Spectrometer data (Shebenski and Paige 2002). This approach can produce maps of the spatial distribution of ground ice at much higher spatial resolution than has been possible with the gamma ray data, while simultaneously providing key constraints on the depth, temperature and thermal properties of the ice itself. With these new data, Paige and colleagues can study correlations between the present distribution of Martian ground ice and geological and topographical features, as well as construct detailed models for the distribution and behavior of ground ice through time. Of particular interest are periods during the past 50 million years when the obliquity of Mars' spin axis has increased to as high as 45 degrees. During these high obliquity periods, the high latitude regions of Mars experience much higher summertime temperatures that could result in the transient melting of near-surface ground ice deposits (Costard et al 2002). Paige and coworkers propose to use their new constraints on ground ice distribution to map the most favorable regions for ice melting and correlate these with high-resolution imaging and topographic data. The results of this work will provide an important test of the notion that aspects of the geomorphology of Mars can be understood through models of past thermal and hydrologic behavior. It will also identify promising landing sites for future exploration.

7 EARLY EARTH'S ATMOSPHERE, OCEANS, AND LIFE

7.1 OVERVIEW

Knowledge of Earth's earliest life, and the environment in which it evolved, is incomplete and controversial at the present time. Here, cutting-edge, microscale, isotopic geochemistry ($^{131}Xe/^{136}Xe$, $\delta^{18}O$, $\Delta^{33}S$, $\Delta^{36}S$) of Earth's oldest materials (>4.0 Gyr-old zircon crystals) and Archean (3.9-2.5 Gyr-old) sulfur minerals (sulfides and sulfates) will be used to explore early atmosphere-ocean evolution and sulfur cycling. In addition, proposed theoretical and experimental studies are aimed at understanding the extraordinary gas-phase chemistry responsible for mass-independent isotope effects in oxygen and sulfur compounds. Other advanced geochemical techniques (laser Raman and optical tomography, in-situ carbon isotopic analysis) are to be used to scrutinize the microfossil evidence for Earth's earliest life. The complementary macrofossil evidence (stromatolites) will be assessed through the use of numerical simulations based on the mathematics of condensed matter physics. The pooled results from these disparate studies will provide new information about the antiquity of life on Earth and provide a firm basis for life-detection on other bodies in the Solar System.

7.2 WHEN DID EARTH BECOME SUITABLE FOR HABITATION? (HARRISON, MCKEEGAN, MOJZSIS)

The earliest evidence of life on Earth comes from graphitic inclusions within >3.83 Gyr marine sediments from West Greenland that were found by ion microprobe analysis to contain isotopically light carbon (Mojzsis et al. 1996; cf. Fedo and Whitehouse 2002). As this places the emergence of life prior to the end of a period of intense bombardment in the inner Solar System (3.8-3.9; Ryder 1990), this result raises the possibility that life originated during the Hadean Eon (4.5-4.0 Gyr) – a period of Earth history for which there is no known rock record. That being the case, how can we determine whether the criteria thought necessary for biopoesis (i.e., energy source, organic molecules, liquid water) were extant during this time? Since the necessary energy sources and molecular building blocks for life were surely available during the formative stages of planetary evolution (Chyba and McDonald 1995), our question reduces to: When did suitably quiescent conditions and liquid water first appear at the Earth's surface?

Attempts to trace Earth history back in time via the rock record reach an impasse around 4 billion years ago. However, the discovery of detrital zircons (Compston and Pidgeon 1986) from the Jack Hills, West Australia, as old as 4.3 to 4.4 Gyr offers the prospect of gaining unprecedented insights into surface environmental conditions during the earliest phase of Earth evolution (Mojzsis et al. 2001, Wilde et al. 2001; Peck et al. 2001). For example, oxygen isotope compositions of these ancient zircons suggest the presence of a terrestrial hydrosphere and stable continents only 200 Myr after accretion (Mojzsis et al. 2001). These preliminary results challenge the traditional view that continental formation and development of a hydrosphere were frustrated by meteorite bombardment and basaltic igneous activity until ~4.0 Gyr. We propose to utilize these ancient zircons – the only tangible record of the Hadean Eon – to seek insights into the origin of the atmosphere, hydrosphere, and the geodynamo during the earliest stages of Earth evolution.

7.2.1 The age of the atmosphere

The Earth's atmosphere is thought to have been derived from mantle degassing (Brown, 1952). Although cometary water has more recently been suggested as a possible source (e.g., Delsemme 2001), D/H measurements of comets Halley, Hyakutake, and Hale-Bopp are inconsistent with this hypothesis (Bockelee-Morvan et al., 2000). While comets formed in the Jovian region might have a similarly low D/H ratio to the Earth's oceans (Mumma et al. 2001), this idea remains untested.

Excesses of ^{129}Xe in mantle-derived samples relative to the atmosphere have been interpreted to indicate the presence of live parent ^{129}I in the deep Earth following early degassing of the atmosphere (e.g., Staudacher and Allegre 1982; Allegre et al. 1983) since ^{129}I decays to ^{129}Xe with a half life ($t_{½}$) of only 16 million years. If true, then it is argued that the present atmosphere and hydrosphere must have formed by ~4.4 Gyr (Podosek 1970).

However, the isotopes of Xe offer additional constraints on the age of the atmosphere. The plutonium isotope ^{244}Pu has a short half life of 82 million years ($t_{½}$=82 Myr). Spontaneous fission of ^{244}Pu produces ^{131}Xe, ^{132}Xe, ^{134}Xe, and ^{136}Xe in characteristic relative abundances. The component of mid-ocean ridge basalts associated with fission decay of ^{244}Pu implies a source that is 100 times lower than the chondritic plutonium/uranium ratio of 0.007 (it is assumed that Pu/U ratios equivalent to chondrites should be "primordial", i.e. solar) (Kunz et al. 1998). This appears paradoxical as other noble gas isotopic data are interpreted to indicate the presence in the deep Earth of a primitive reservoir of solar composition (e.g., Honda et al. 1991) that presumably would be associated with chondritic Pu/U (Azbel and Tolstikhin 1993). In the absence of a mechanism to fractionate Pu from U during planetary formation, the possibility remains that ^{129}Xe differences simply reflect inherited heterogeneities in the I/Xe ratio of terrestrial reservoirs. If this is the case, then the atmosphere and hydrosphere could be substantially younger than ~4.4 Gyr (Caffee et al. 1999). Alternatively, since the absolute amounts of ^{129}Xe excess to fissiogenic ^{136}Xe (fission of both ^{244}Pu and ^{238}U produces ^{136}Xe) in the atmosphere and

their relative proportions are comparable to what would be left behind in the Earth after an early outgassing, such as that resulting from a Giant Impactor event, xenon isotope systematics may carry little information regarding the age of the atmosphere.

The place to begin to address this issue is to determine the terrestrial Pu/U. There are no firm constraints on this parameter, but existing data are suggestive of a chondritic Pu/U for the Earth (e.g., Caffee et al., 1999; Honda et al., 2000). The fundamental problem in elucidating this parameter is illustrated in Figure 7.2.1, which shows the expected $^{131}Xe/^{136}Xe$ due to Pu and U fission during the first 900 Myr of Earth history. Clearly, zircons < 4 Gyr in age contain very little signal of ^{244}Pu (i.e., ^{131}Xe). Materials formed between 4.4-4.1 Gyr provide the best opportunity to characterize terrestrial Pu/U and thus better utilize Xe isotope studies to understand the evolution of the atmosphere.

Harrison and his colleagues in Manchester, England (outside collaborators I. Gilmour and G. Turner) have recently discovered the very first evidence for extinct ^{244}Pu in individual 4,150 Myr old zircons from Jack Hills and propose to carry out a research program to determine the Pu/U ratio in the Earth and the early crust and investigate the implications for the earliest differentiation of crust and atmosphere. Their ability to analyze the isotopic composition of xenon in individual zircons relies on the development in Manchester of a uniquely sensitive mass spectrometer based on the principle of laser resonance ionization (Gilmour et al. 1994). The instrument, RELAX (Refrigerator Enhanced Laser Analyser for Xenon), is capable of analyzing samples of only a few thousand atoms, some two orders of magnitude smaller than conventional noble gas mass spectrometers, permitting single zircons to be measured. In the first four RELAX analyses one has a $^{131}Xe/^{136}Xe$ composition consistent with a chondritic Pu/U ratio, but three others have lower values. The preliminary interpretation of this result is that the lower ratios reflect partial xenon degassing subsequent to the extinction of ^{244}Pu. What this means is twofold: analysis of an aggregate of old zircons would lead to a highly ambiguous result due to mixing of degassed and un-degassed populations, and assessment of the terrestrial Pu/U will require a large number of measurements using single crystals varying in age from 4.1 to 4.4 Gyr to define the upper bound of the population thus far discovered.

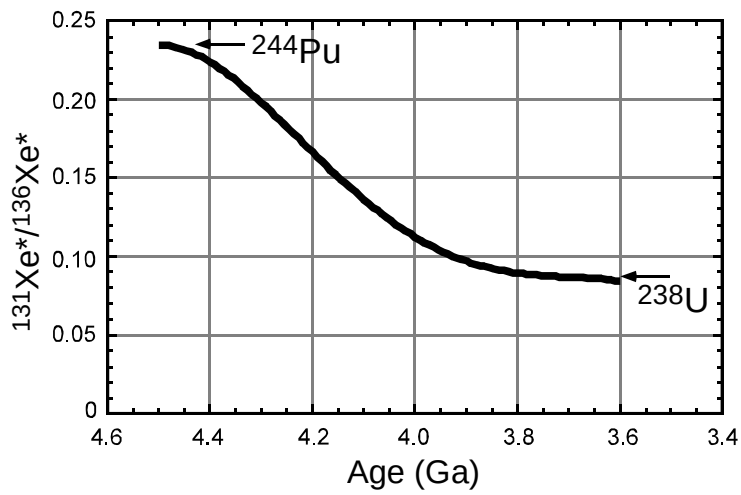

Figure 7.2.1. The changing $^{131}Xe/^{136}Xe$ due to fission of ^{244}Pu and ^{238}U over the first 900 Myr of Earth history. >4.1 Gyr zircons are ideal to assess the initial terrestrial Pu/U ratio.

7.2.2 The age of the hydrosphere (and redox state of Earth's surface)

Oxygen isotopes in rocks and minerals provide a means to discriminate among possible sources (i.e., mantle, metasedimentary, hybrid) of granitoids (Taylor and Sheppard 1986). Zircon appears promising in this role as exchange rates for O are very slow (Watson and Cherniak 1997), potentially permitting

preservation of the protolith signature through high-grade metamorphism (Valley et al. 1994). For this reason Mojzsis et al. (2001) and Wilde et al. (2001) used the $\delta^{18}O$ values of Jack Hills zircons to infer crust-hydrosphere interactions at ca. 4.3 Gyr. These preliminary results warrant further study because of the surprising conclusion that a liquid hydrosphere (as opposed to steam atmosphere) was in place by ~4.3 Gyr. We propose to expand the approach to focus also on the "microrock" (i.e. polymineralic inclusion) environments encapsulated within the zircons.

Igneous zircons typically contain inclusions that reflect the parent melt (Chupin et al. 1988). 10-100 μm-sized inclusions have been found in >4 Gyr zircons from Jack Hills quartzites (Maas et al. 1992; Wilde et al. 2001), including assemblages of quartz, K-feldspar, biotite, chlorite and muscovite. One inclusion containing quartz, feldspar, apatite, and monazite (Maas et al. 1992) is significant as it is typical of S-type granitoids (Rapp and Watson 1986) where S-type refers to granitic rocks derived ultimately from sediments. Mojzsis et al. (2001) noted that peraluminous inclusions (those with high Al concentrations relative to alkali elements) encapsulated in zircons enriched in ^{18}O are suggestive of the melt having originated from rocks at the Earth's surface. This is because Al-rich compositions occur today by virtue of weathering and so indicate early development of a sedimentary cycling environment, and because high $\delta^{18}O$ (relative to mantle rocks, for example) is also an indication of interactions with a hydrosphere. While specific circumstances could be convolved to create this signal in the absence of surficial processes, they would be exceptional.

In rare cases, melt inclusions are also preserved in zircon. If the encapsulating zircon shielded a melt inclusion from changing external conditions since formation, then it may be feasible to extract information regarding the fO_2 (fugacity of oxygen) of the melt from which the zircon crystallized. If the melt protolith originated at the Earth's surface (e.g., normative corundum, meaning Al-rich and so having experienced a weathering cycle, and heavy ^{18}O), then it remains possible that a faint record of life at the Earth's surface might be detectable. As an example of how this might be inferred, consider why I-type granites are pink in color and garnet-bearing S-type granites are white. In a subduction environment where I-type (I for igneous) granitic rocks are made beneath island arcs, fO_2 is maintained above the hematite-magnetite buffer (Zen 1988) and Fe^{3+} is soluble in K-feldspar, leading to a pink coloration (Zen 1985; Zen 1988). In S-type granitoids (again, S for sedimentary) of SE Australia, the rocks are white because graphite in the Paleozoic source rocks controlled the fO_2, producing more reducing conditions that stabilize Fe^{2+} in the evolved magmas that derive from them. The ultimate source of reduced C responsible for lowering fO_2 in S-type granitoids is fossil biomass within sediments that formed at the rock-hydrosphere or atmosphere interface. While a surprising concept, the color of a granitoid can relate to the presence or absence of reduced C at the Earth's surface!

In the microrock environment, the intrinsic fO_2 of melt inclusions can be determined via knowledge of the Fe^{3+}/Fe^{2+} ratio (Delaney et al., 1998) and a thermodynamic model (Kilinc et al. 1983). The μXANES (X-ray absorption near edge structure) method is capable of determining Fe^{3+}/Fe^{2+} on a 3 μm spot with ± 5% accuracy(Bajt et al. 1994). Because zircon solubility in melts (i.e., melt inclusions trapped within zircon are zircon saturated) is both a function of Fe^{3+}/Fe^{2+} (i.e., network former vs. network modifier) and temperature (Baker et al. 2002), both fO_2 and peak melting temperature can in theory be determined by knowing Fe^{3+}/Fe^{2+} and dissolved Zr. Thus it may be possible to assess whether conditions consistent with the presence of reduced carbon at the Earth's surface – potentially bearing on the presence of life – were extant between 4.4 to 4.0 Gyr.

7.2.3 The initiation age of the geodynamo

The earliest evidence for geodynamo activity comes from the intrinsic magnetism of 3.5 Gyr volcanics in southern Africa. Because Jack Hills zircons are hosted in low grade sediments, they could retain the oldest record of the Earth's magnetic field. Preliminary measurements using an ultra-sensitive SQUID magnetometer (Baudenbacher et al., 2002) show that a Jack Hills zircon carries a weak (10^{-13} Am^{-2}) intrinsic remanent magnetism (J.L. Kirschvink pers. comm.). Provided the detrital grains were not re-

magnetized, the presence or absence of intrinsic magnetism could be our only signal of geodynamo activity during the earliest period of Earth history. For example, should Harrison discover that all zircons younger than, say, 4.2 Gyr are magnetic but that all older zircons are not, this could be evidence that the terrestrial geodynamo initiated at ca. 4.2-4.3 Gyr. While admittedly a long shot, a positive result could potentially place a profound constraint on the dynamical evolution of the planet.

Harrison and colleagues will provide an outside paleomagnetics laboratory (Kirschvink's laboratory at Caltech is the lead candidate at this writing) 4.0-4.4 Gyr zircons that have been dated without exposure to strong magnetic fields. If age analysis is found to remagnetize the grains, the grains will be characterized before dating (this is feasible as ~100 grains can be measured in a 24-hour period). It may be necessary to undertake limited drilling in the Jack Hills quartzites to obtain unweathered cores. $^{40}Ar/^{39}Ar$ dating of inclusions and single crystal xenon isotopic may analyses may be useful in establishing zircon thermal histories and thus age of magnetization.

7.2.4 Measurements of ancient zircons- building the database

While the discovery of >4.3 Gyr Jack Hills zircons provides unique new opportunities to gain insights into the earliest evolution of the atmosphere, hydrosphere, and continents, zircons older than 4.2 Gyr make up only ~0.5% of the detrital population (Amelin 1998). To overcome this hurdle, Harrison and coworkers have refined a method to survey $^{207}Pb/^{206}Pb$ ages of large numbers of zircons. Using a high-resolution ion microprobe in multi-collector mode, an age with ±1% precision can be obtained in less than one minute. In this way, these workers have thus far analyzed a total of 15,000 grains thereby increasing the number of dated Jack Hill zircons by a factor of 15 (Compston and Pidgeon 1986; Maas et al. 1992; Amelin 1998; Mojzsis et al. 2001; Wilde et al. 2001). Two clear age peaks are evident at ~3.3 Gyr and ~4.0 Gyr (Figure 7.2.4) tailing off at older ages. From this population, Harrison and others have thus far identified 105 zircons in the age range 4.1 to 4.2 Gyr and 31 >4.2 Gyr including three that are >4.35 Gyr. One ~8 μg zircon is 4.37 Gyr. We are confident that we know the yield of the most ancient zircons and therefore can predict the quantities required for future experiments from the database statistics.

Harrison estimates that the experiments proposed above require about three times the present complement of >4.1 Gyr zircons and thus he will need to age characterize another 30,000 zircons. The approach will be to rapidly survey large numbers of zircons using our established multi-collector approach at UCLA. However, this effort is coordinated with the group at the Research School of Earth Sciences at the Australian National University who will undertake about half the analyses. When sufficient quantities of the old zircons have been acquired, they will be disbursed to our collaborators

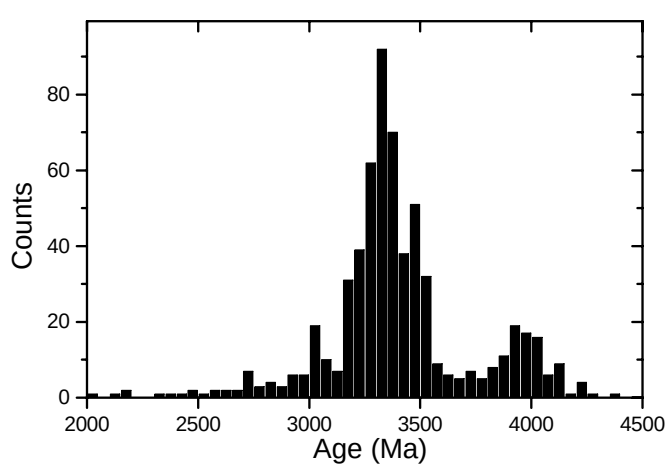

Figure 7.2.4. Historgram of lead-lead ages for ancient zircons from Jack Hills sample 634. Two peaks are evident at ~3.1 Gyr and ~4.0 Gyr, tailing to ages as old as 4.37 Gyr.

for xenon isotopic and magnetic measurements. Oxygen isotopic and inclusion studies will commence at UCLA immediately with material already in hand.

7.3 SULFUR CYCLING ON THE EARLY EARTH (FARQUHAR, FITZ-GIBBON, LYONS, MARCUS, MCKEEGAN, MOJZSIS, RUBIN, RUNNEGAR, SANDER)

The recent discovery by Farquhar et al. (2000) and Bao et al. (2000) that mass-independent isotope effects (§ 7.3.1) maysurvive in terrestrial geological materials has invigorated sulfur and oxygen isotope geochemistry. As these effects are thought to be solely due to gas-phase reactions (Gao and Marcus 2001), their geological record provides an unprecedented source for atmospheric history. Most obviously, the anoxic atmosphere of the early Earth (older than ~2.2 Gyr) promoted UV-induced sulfur photochemistry whereas the subsequent oxygen-rich atmosphere was, and is, one in which ozone is a principal photochemical product of UV radiation (Lyons 2001). The "Great Oxidation Event" (Holland, 2002), which took place about 2.2 Gyr ago, is believed to represent the transition between these two states and is recorded by the sudden disappearance of mass-independently fractionated (MIF) sulfur compounds (principally $BaSO_4$ and FeS_2) from the geological record. In contrast, the preservation of the MIF sulfur signal in rocks older than ~2.2 Gyr results from the fact that there were at least two main products of the atmospheric reactions, elemental sulfur particles and sulfate aerosols, each having a different MIF signature (Figure 7.3). As the elemental sulfur particles were insoluble in surface waters, these two components did not mix, and their positive and negative MIF signatures were transmitted to minerals formed from them in sedimentary environments.

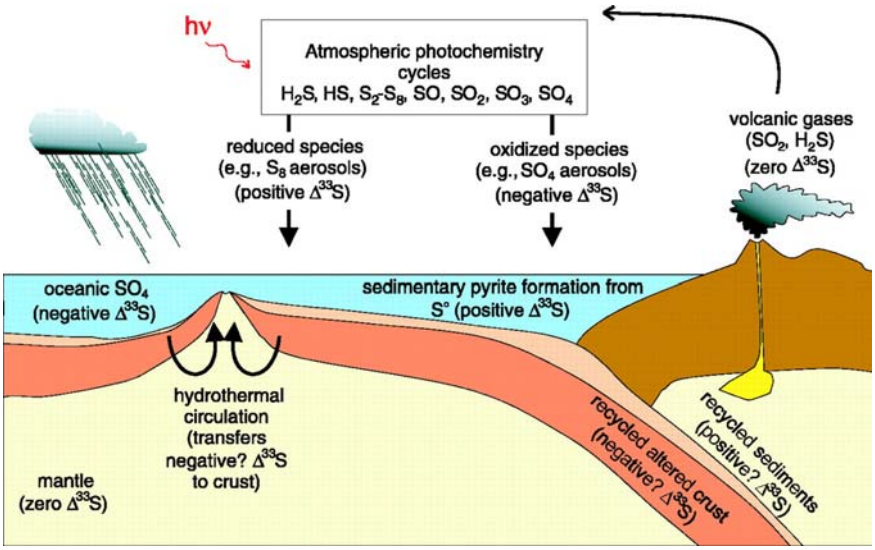

Figure 7.3. The Archean sulfur cycle (Farquhar et al. 2002). The principal products of atmospheric chemistry were ultimately pyrite (positive $\Delta^{33}S$) and sulfate (negative $\Delta^{33}S$) as shown in Fig. 7.3.2. The atmospheric photochemistry (box) is one target of the research proposed here.

7.3.1 Ion microprobe measurements of the four stable isotopes of sulfur

Our role in the development of this fruitful area of research has been the following: (1) The demonstration that the effect is real, and not an artefact of sample preparation as had been argued by Ohmoto (2000), by making $^{33}S/^{32}S$, $^{34}S/^{32}S$, and $^{36}S/^{32}S$ measurements in a completely different way using the UCLA Cameca ims 1270 ion microprobe in monocollector and multicollector modes (§ 3; Runnegar et al. 2002); (2) The development of new sulfide (FeS, FeS_2, $CuFeS_2$) and sulfate mineral

($BaSO_4$) standards for ion microprobe analysis (Greenwood et al. 2000; Runnegar et al., in prep.); (3) Evidence that early Archean sulfate minerals traditionally regarded as barite ($BaSO_4$) replacements of primary gypsum ($CaSO_4.2H_2O$) evaporites were, in fact, originally hydrothermal barite; this bears on the composition of the Archean ocean (Runnegar et al., 2001b, in preparation); (4) The recognition of three different sulfur reservoirs in Archean sedimentary rocks based on their three and four sulfur isotope compositions (Figure 7.3.1), results that argue against widespread microbial sulfate reduction during the Archean (Runnegar et al. 2001a, in preparation) in contrast to work based on only the two most common isotopes (Shen et al. 2001); (5) The use of MIF sulfur signatures as tracers of base metal sources and hydrothermal fluid circulation in Archean ore deposits (Runnegar et al. 2002); (6) A survey of Precambrian and Phanerozoic sulfates and sulfides of many different ages and environments to provide a background for the interpretation of MIF sulfur signatures throughout Earth history (Runnegar et al., in preparation); (7) The development of a quantitative understanding through numerical modeling, of the role that ozone plays in acquiring and transmitting a substantial MIF signature to oxygen-bearing compounds at all levels in the modern atmosphere (Lyons 2001); and (8) Application of the photochemical understanding gained in (7) to the important problem of self-shielding and the production of MIF oxygen in the solar nebula (§ 5.4).

Given these discoveries, and the excitement of complementary studies by our NAI colleagues (Hiroshi Ohmoto, Pennsylvania State University; Douglas Rumble, Carnegie Institution of Washington; Andrey Bekker, Harvard University), we propose to proceed over the next five years on the following fronts:

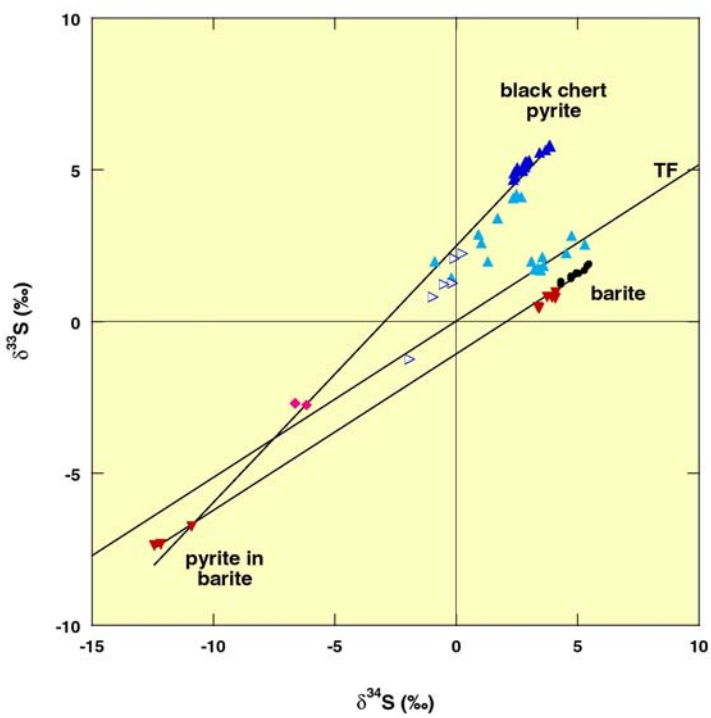

Fig. 7.3.1. Sulfur isotopic compositions of 3.5 Gyr-old sulfides and sulfates, North Pole area, Western Australia. Pyrite in organic-rich cherts are enriched in ^{33}S relative to normal terrestrial materials ($\Delta^{33}S > 0$), indicating their orign from the reduction of elemental sulfur (Fig. 7.3.1). Pyrites that are intimately associated with bedded and cross-cutting sulfates (barites) are depleted in ^{33}S ($\Delta^{33}S < 0$), as are the sulfates. The 15‰differe nce in $\delta^{34}S$ between the barite and the barite-associated pyrite has been regarded as evidence for bacterial sulfate reduction (Shen et al. 2001). However, in the expected setting for sulfate-reducing bacteria (black cherts and shales) the pyrite has a $\Delta^{33}S$ signature which cannot have come from sulfate.

(1) Explore sulfur cycling during and following the Archean by investigating key sedimentary environments in greater detail than has been done in our current survey that involved approximately 300 multiple sulfur isotope measurements on ~50 samples ranging in age from 3.85 Gyr to the Miocene. We are particularly interested in syngenetic pyrite in organic-rich shales and banded iron formation, in sulfates and sulfides associated with volcanic-hosted massive sulfide (VHMS) deposits and ancient "back smokers", and sulfate evaporites. Each of these environments, if tracked through time, has great potential for the preservation of sulfur isotope biosignatures.

(2) Investigate the disappearance of MIF sulfur from the sedimentary cycle at or after the Great Oxidation Event (GOE). Although this problem is being explored effectively by others in a primary stratigraphic context, there is the need to consider other avenues of investigation that involve the recycling of previously MIF sulfur compounds. These include ore deposits formed by meteoric hydrothermal actvity (Runnegar et al. 2002) and material recycled mechanically from older terrains. A combination of geochemical exploration and numerical modeling will be used to investigate the history of sulfur cycling on a global scale during the GOE transition.

(3) We have preliminary data, that needs to be confirmed and supports an earlier report (Rees and Thode 1977), of a small MIF effect in sulfide-bearing phase in the Allende carbonaceous chondrite. Rubin and McKeegan propose to explore this anomaly using the numerous Allende and other carbonaceous chondrite samples in the UCLA meteorite collection maintained by Wasson.

7.3.2 Laboratory experiments and theoretical analysis of the kinetics and photochemistry of mass-independent sulfur isotope effects

The chemical origin of the MIF in sulfur is still unclear. Farquhar et al. (2001) have shown in laboratory experiments that photodissociation of H_2S, SO_2, and $SO_2/CO_2/H_2O$ mixtures all produce elemental sulfur with a wavelength-dependent MIF signature. However, the experimental results that best reproduce MIF in sulfur-bearing rocks older than ~2.2 Gyr involve photodissociation at *single* wavelengths < 200 nm In a real atmosphere, MIF would not be produced at a single wavelength, since solar radiation is broadband. Instead, we believe that MIF in sulfur arises, at least in part, from the reaction $S + S_2 \rightarrow S_3$. It has been clearly shown (Mauersberger et al. 1999) that MIF in O_3 is produced during the ozone formation reaction, $O + O_2 \rightarrow O_3$. Because O and S are isovalent atoms, the ozone formation reaction and the S_3 (thiozone) formation reactions are also isovalent. It is therefore likely that formation of S_3 isotopomers occurs in a mass-independent manner, just as is true for the isotopomers of O_3. We propose experiments to test this hypothesis and to determine the rate coefficients for formation of several isotopomers of S_3.

The first experiment we propose is to pass an electrical discharge through flasks of H_2S and SO_2. Elemental sulfur has been observed previously in such experiments. We will collect elemental sulfur and SO_2, and measure ^{32}S, ^{33}S, ^{34}S, and ^{36}S relative to the initial gas isotope values. The measurements will be done in collaboration with Farquhar. Although very simple, the discharge experiments are an essential first step. If MIF is observed in the collected SO_2 and elemental sulfur residue, then we have demonstrated that an inherently broadband process (discharge) can produce MIF of sulfur. Conversely, if no MIF is observed, then it is likely that photodissociation alone (and not subsequent reactions of photoproducts) is the principal cause of the MIF.

Assuming that MIF is observed in the sulfur residue collected in the discharge experiments, then flow tube experiments will be undertaken to determine the isotope-specific rate coefficients for the formation of several S_3 isotopomers, for example:

$$^{32}S + {}^{34}S^{34}S \rightarrow {}^{32}S^{34}S^{34}S$$

$$^{33}S + {}^{32}S^{32}S \rightarrow {}^{33}S^{32}S^{32}S$$

We shall also determine the rate coefficient for sulfur atom exchange with S_2,

$$^{34}S + {}^{32}S^{32}S \rightarrow {}^{32}S + {}^{32}S^{34}S$$

It may also be necessary to measure isotope-specific rate coefficients for the S_4 formation reaction, $S_2 + S_2 \rightarrow S_4$, and for the SO dimer reaction, $SO + SO \rightarrow (SO)_2$, since these are important pathways to elemental sulfur and sulfate formation (Kasting et al. 1989).

A flow tube will be used because the variable-length reactant port allows for the determination of accurate rate coefficients. Additional ports are for reactant S_2 and the He flow gas. Atomic sulfur will be generated by microwave discharge of isotopically-pure carbonyl sulfide (OCS). Diatomic sulfur will be generated by heating isotopically-pure solid sulfur. The flow tube will be quartz (~1 meter in length), and will be heated to > 150 ºC to prevent condensation of S_2 and S_3. Neutral products will be converted to positive ions by electron impact ionization and will be detected with an Extrel MAX 500 quadrupole mass spectrometer purchased for this purpose. Flow tube measurements will be done in collaboration with Sander.

Lyons also proposes to investigate the kinetic theory of the thiozone formation reaction in collaboration with Marcus. This will be an extension of the non-RRKM (i.e., non-statistical) unimolecular dissociation theory developed for the $O + O_2 \rightarrow O_3$ reaction by Marcus and his colleagues (Gao and Marcus 2001). The sulfur kinetic theory will provide an important link to the much better understood chemistry of gas-phase oxygen isotopes, and may be applicable to higher-order polysulfur compounds such as S_4 and S_8. With knowledge of the isotope-specific rate coefficients for thiozone isotopomers, full photochemical models of Archean sulfur chemistry can be developed.

7.3.3 Genomic approach to understanding of the evolution of sulfur metabolisms

Easily cultured organisms are estimated to constitute a small fraction of all microbial species and are rarely numerically dominant in the communities from which they were obtained (Hugenholtz 2002). Thus, much remains to be learned about the distribution and diversity of microorganisms and their geobiological activities. This can be overcome by purifying total DNA from environmental samples, constructing random DNA fragment libraries, and then using random sequencing or probing for genes of interest. Fitz-Gibbon and colleagues propose to use these exploratory methods to search for novel genes and novel genetic settings for sulfur metabolism.

Genes involved in dissimilatory sulfate reduction (ATP sulfurylase, APS reductase and sulfite reductase) are known from a wide range of Bacteria. Phylogenetic trees based on these genes tend to follow conventional taxonomic groupings (Wagner et al. 1998; Friedrich 2002). On the other hand, few of these genes have been identified from members of the Archaea, hampering efforts to determine whether the ability to reduce sulfate was a property of the last common ancestor of Bacteria and Archaea. Dissimilatory sulfate reduction is well studied in the euryarcheal genus *Archaeoglobus* but may be more widespread within the Archaea. For example, growth with sulfate as an electron acceptor has been reported for *Caldivirgas* (Itoh et al. 1999) and possibly also *Thermocladium* (Itoh et al. 1998), both members of the Crenarchaeota. Moreover, a set of sulfate reductase genes was found in the genome of the crenarchaeote *Pyrobaculum aerophilum* (Fitz-Gibbon et al. 2002), although frameshift mutations have made some of the genes non-functional.

The identification of a small number of novel archaeal sulfate reduction genes would be sufficient to determine whether they will produce phylogenetic trees follow the 16S rRNA pattern. If so, and if the genes were broadly distributed across the Archaea, it would be likely that sulfate reduction had been present in the common ancestor of Bacteria and Archaea. Conversely, the novel archaeal genes may show a clear pattern of more recent horizontal transfer from the Bacteria, and thus the possibility that sulfate reduction postdates the Archaea-Bacteria divergence. Sulfur metabolism genes tend to cluster in many genomes (Fitz-Gibbon et al. 2002). For example in *Pyrobaculum* a 26.5 Kb-long section of the

genome carries approximately 35 genes, eleven of which are clearly involved in sulfur metabolism. Some of the other 24 genes appear to be in operons that also contain the sulfur metabolism genes, suggesting that they may also be involved in sulfur biochemistry. Thus, the identification of one sulfur metabolism gene by random sequencing can be followed by further sequencing along the clone (BAC clones are ~250 Kb in length) in the expectation of discovering additional genes for sulfur metabolism.

7.4 GEOCHEMICAL CONTEXT FOR EARLY LIFE (HOUSE, KAPLAN, KAVNER, MANNING, SCHAUBLE, VENKATESAN, YOUNG)

7.4.1 Chemical feedbacks

Hydrothermal systems in the early Earth were likely hosted by olvine-rich ultramafic igneous rocks. In such systems, the most important silicate-hydrolysis reaction is the conversion of the mineral olivine to serpentine and magnetite (serpentinization). Because this reaction produces large amounts of H_2 gas, CH_4 gas, and basic solutions (e.g., Janecky and Seyfried 1986; Coveney et al. 1987; Abrajano et al. 1988; Rona et al. 1992; Charlou and Donval 1993; Kelley 1996; O'Hanley 1996; Kelley et al. 2002), sites of active serpentinization should have been favorable environments for chemotrophic organisms on the early Earth. Manning and coworkers are testing this hypothesis by conducting experiments on serpentinization in the presence of microorganisms.

Manning and House propose an experimental program that will investigate model ecosystems involving primitive microorganisms, olivine, and hot seawater. Preliminary experiments have been conducted using the hyperthermophilic methanogen *Methanopyrus kandleri* in 100 °C solutions with olivine, serpentine, and magnetite. *M. kandleri* is used because its molecular phylogeny suggests minimal evolution away from the hypothesized universal ancestor of life on Earth, it thrives at 100 ˚C in oceanic hydrothermal vents, and its metabolism utilizes H_2 during chemoautotrophic production of CH_4. *M. kandleri* also facilitates reduction of aqueous Fe(III)-bearing organic compounds, which could release O_2 and create positive feedback for additional olivine-sourced Fe(II) oxidation.

Results at 100 °C, 3 bar H_2+CO_2, show that *M. kandleri* is readily cultured during hydrolysis of olivine (Herrera et al. 2003). Concentrations of Fe, Mg, and Si in solution were higher in the presence of *M. kandleri* than in abiotic systems, implying enhanced olivine dissolution rates and strong chemical feedback. The olivine-rich ultramafic-hosted hydrothermal systems that were abundant in the early Earth are favorable environments for this microorganism. Further experiments are planned, including those involving isotopically-doped Mg and Fe for tracing the exchange of Mg and Fe between phases in the system using the isotopes of these elements using multiple-collector inductively coupled plasma-source mass spectrometry (MC-ICPMS, see next section).

7.4.2 Abiotic pathways to complex organics

The prebiotic Earth contained abundant organic matter that was produced by abiotic chemical pathways. The standard model for the origin of life hypothesizes that the earliest organisms arose from this complex, abiotic, organic brew. Careful assessment of this hypothesis relies on two factors: (1) completeness of the inventory of the prebiotic organic mix, and (2) development of appropriate tests to assess the contributions of different pathways. We are conducting studies in both these areas. To address the completeness of the hypothesized organic inventory, we are exploring alternative pathways for abiotic organic production. Numerous mechanisms have been proposed for abiotic organic production, including electrical discharge, cometary and meteorite delivery, and production in early surficial hydrothermal systems. However, a major additional pathway in the early Earth that has not been considered is the generation of methane, n-alkanes, and potentially simple organic acids and N-bearing compounds at elevated pressure and temperature during tectonic burial of nascent hydrated ultramafic plates in Hadean time. Substantial amounts of organic compounds are produced by this process in the modern Earth. The larger volumes of ultramafic material at the surface of the early Earth motivate chemical reactive flow modeling to investigate organic production by this mechanism.

7.4.3 Role of metals in early biology

A second line of investigation involves using intermediate-weight stable isotopes (e.g., isotopes of Mg, Fe, Cu, and Si) to investigate the role of metals in the early organic chemistry of the Earth. The first application is as tracers of biologically and non-biologically mediated reactions between rocks and waters relevant to organic synthesis. Manning and Young will investigate the partitioning of the stable isotopes in laboratory simulations of water-rock reactions in the presence and absence of microbes to establish isotopic criteria for distinguishing biologically from non-biologically mediated reactions. The other line of study focuses on isotope fractionation attending fundamental physicochemical processes, with the goal of understanding the extent to which stable isotopes of transition metals and alkali metals might be used as biomarkers. In particular, transition metal isotope fractionation attending redox reactions will be monitored using electrochemical techniques. Kavner and Young plan to use potentiostatic techniques to study the fractionation of iron, nickel, and chromium isotopes during redox processes relevant to organometallic chemistry. Studies by Kavner and Young will focus not only on the magnitude of the partitioning of the isotopes, but also on the "slopes" defined by three-isotope systems (see § 6.62). The slopes, under favorable circumstances, can be used to distinguish equilibrium steps from kinetic steps in a fractionation process. The expected outcome will be a quantitative tie between the driving potential for chemical oxidation/reduction processes, and corresponding isotope fractionation signatures. This will help elucidate the mechanism by which microorganisms generate specific isotope signatures and allow us to isolate discrete fractionating steps in both biological and non-biological redox systems. The results can be used for comparisons with fractionation in more complicated systems.

7.4.4 Equilibrium Fe isotope fractionation between Fe^{2+} and Fe^{3+} - an experimental approach

Mass-dependent fractionations of the stable isotopes of iron have recently been discovered, and it is observed that large isotopic fractionations are largely restricted to precipitates formed in low-temperature natural and laboratory environments. This property of the Fe-isotope system suggests that it may be ideally suited for the identification of ancient low-temperature environments on Earth and other planets. Major unanswered questions remain, however, regarding the causes of environmental Fe-isotope fractionations and the possibility of using observed signatures in rock samples to unequivocally identify ancient biological activity. There is evidence to suggest that the largest fractionations (1‰ to 3‰) typically occur when iron is partially oxidized or reduced in the presence of liquid water. These fractionations are preserved in the rock record when the different oxidation states (Fe^{3+} and Fe^{2+}, typically) are separated, for instance by precipitation of $Fe^{3+}O(OH)$. Fe-redox transformations are commonly mediated by microorganisms, and it has been suggested that the biological redox fractionation (~1.5‰) is characteristically smaller than the inorganic fractionation (~3‰). Significant uncertainty in the equilibrium organic fractionation persists, however, due to the difficulty of separating Fe^{3+} and Fe^{2+} reversibly. One way to avoid this problem is to allow an easily separable phase (an immiscible organic solvent such as diethyl ether) to equilibrate with mixed solutions with a range of Fe^{3+}/Fe^{2+} ratios. Such a technique can, in principle, be applied to many exchange-labile chemical species in aqueous solutions.

Schauble and Young propose fundamental laboratory studies to develop techniques for determining Fe-isotope fractionations between different species in aqueous solutions. The project will examine the potential of using an immiscible organic solvent phase (diethyl ether) as a rapidly equilibrating and easily separable reservoir of dissolved iron. In Fe^{3+}-Fe^{2+}-HNO_3-HCl solutions, $[FeCl_4]^-$ is the only ether-soluble species. It is expected, therefore, that the isotopic partitioning behavior of iron in the ether phase will be nearly constant over a range of aqueous Cl^- activities, Fe^{3+}/Fe^{2+} ratios, and pH. Measured fractionations between the iron dissolved in this organic reservoir and aqueous solutions of varying chemistry (for instance, a series of increasing Fe^{2+}/Fe^{3+} ratios) will reflect changing isotopic behavior in the aqueous phase, allowing indirect measurement of fractionations between ether-solvated $[FeCl_4]^-$ and aqueous $[Fe(H_2O)_6]^{3+}$, Fe^{2+} (i.e. $[Fe(H_2O)_6]^{2+}$), Fe^{3+}-chloro complexes such as $[Fe(H_2O)_5Cl]^{2+}$, and

Fe^{3+}-hydroxyl complexes. Relatively fast equilibration (~minutes to hours) between ether and aqueous phases is expected, based on bulk iron partitioning experiments from the literature.

7.5 DETECTION AND GEOCHEMICAL CHARACTERIZATION OF EARTH'S EARLIEST LIFE (AGRESTI, HOUSE, JÖGI, KUDRYAVSTEV, MCKEEGAN, RUNNEGAR, SCHOPF, WDOWIAK)

Although the fossil record of Archean (>2,500-Myr-old) life is notably sparse (Schopf and Walter 1983; Schopf 1992a), it is both better known and biologically more diverse than is generally appreciated. Indeed, from even among the oldest deposits of the Archean, rocks ~3,200 to ~3,500 Myr in age, eight fossil-bearing units have been described (by some 40 workers from 7 countries), containing *in toto* both stromatolites and spheroidal and filamentous microfossils backed both by laser-Raman and carbon isotopic analyses of their kerogenous components. Despite this body of evidence, however, the biological origin of the most thoroughly documented of these assemblages - fossils from the second oldest of the eight units, the ~3,465-Myr-old Apex chert of Western Australia (Schopf and Packer 1987; Schopf 1992a, 1993) - has been questioned (Brasier et al. 2002), doubt that has recently been extended to include *all* reports of particularly ancient evidence of life, anything "older than, say, 3.0 billion years" (M.F. Brasier, quoted in the NASA-sponsored *Astrobiology Magazine*, January 2003).

Such doubt raises a severe problem for Astrobiology. If current techniques are inadequate to establish the existence of early life on Earth, how can we expect them to uncover evidence of ancient life on other worlds? Research proposed in the following sections addresses this issue.

7.5.1 How can biogenicity be established?

In a general sense, the answer to the question of biogenicity was shown decades ago when early workers in the field (Barghoorn and Tyler 1965; Cloud 1965; Barghoorn and Schopf 1965) first demonstrated that "Precambrian microfossils" are, indeed, true fossils. Namely, the biological origin of fossil-like microscopic objects can be established by demonstrating that they possess a suite of traits that are unique to life, traits that taken together are shared by fossils and living organisms but not by inanimate matter. Thus, the solution to the problem is to insist that claims of ancient fossils be backed by data that show what the fossil-like objects actually *are* – rather than what they seemingly might be or apparently are not – positive lines of evidence that when considered as a whole comprise a signature unique to living systems.

Of the various traits thus used, three have been shown to be particularly useful: (1) the μm-scale morphology of the objects in question; (2) the carbon isotopic composition of organic matter associated with and/or comprising the fossil-like structures; and (3) the chemical (molecular) makeup of the fossil-like objects. In the case of organic-walled fossils, this is particulate carbonaceous kerogen. Each of these factors can yield strong evidence consistent with a biogenic interpretation. Yet none of them, if considered alone, has proven definitive and use of morphology alone has led to numerous errors of interpretation (Schopf and Walter 1983; Mendelson and Schopf 1992). Reliance on carbon isotopic evidence, by itself, has proven inconclusive (van Zuilen et al., 2002); in and of themselves, analyses showing that such objects are composed of geochemically mature organic matter establish only their carbonaceous makeup, not their biological origin (Schopf et al. 2002a).

Nevertheless, other than biology, no mechanism is known that can yield communities of fossil-like objects that have cellular morphologies, exhibit a biologically distinctive carbon isotopic signature, and are themselves composed of particulate carbonaceous matter. Thus, if the three factors are taken together, as a suite of biologically indicative traits, the lines of evidence become mutually reinforcing and a biogenic interpretation, compelling.

In principle, therefore, the question of biogenicity can be easily answered. But in practice, the answer has proven elusive, primarily because of a lack of analytical techniques having sufficient power to provide the high resolution three-dimensional morphological information needed to definitively address the question, and an absence of means by which to directly link, in individual microscopic specimens,

morphological information to elemental-isotopic and structural-molecular compositions. Means are now at hand to solve this problem, due primarily to advances pioneered during the past three years by UCLA astrobiologists (House et al. 2000; Kudryavtsev et al. 2001; Kempe et al. 2002; Schopf et al. 2002b). Exploitation of these advances, coupled with development of the new analytical techniques outlined below, will provide a firm basis by which to establish or to refute the biogenicity of putative ancient fossils.

7.5.2 Microbial morphology as evidence for early life

The fossil record of life's early microbial history is based primarly on "morphology," a term that subsumes a great many variables: organismal shape (e.g., coccoid or filamentous); cell shape, size, and surface ornamentation; structure and thickness of an encompassing sheath, if present; and many others (Schopf 1992b), and in taxonomic studies, routinely includes quantitative (morphometric) analyses of intra-taxon variability and population structure. But interpretation of morphology is notoriously subjective; a microscopic object regarded as a good fossil by one investigator may be considered to be a nonfossil artifact by another. Clearly, there is a need for hard and fast criteria by which to separate the *bona fide* from the bogus. For three-dimensionally permineralized (petrified) organic-walled fossils, the most life-like of all types of structurally preserved microscopic biologic remnants, a prime criterion is the mineral-infilled spheroidal or tubular structure defined by their enclosing carbonaceous cell walls, a character by which they differ decisively from solid mineralic pseudofossils (Ruiz et al. 2002). Backed by analyses establishing the chemical composition of the enclosing walls, such cellularity, combined with other morphologic features, can be used to unambiguously distinguish true fossils from mineralic look-alikes.

At the current state of the science, high-resolution optical microscopy can demonstrate the requisite three-dimensional cellularity. In a fossil microbial trichome, for example, the presence of surrounding cell walls and regularly spaced transverse septa that enclose mineral-infilled cell lumina. But, because of the shallow depth of field of the high magnification (e.g., 100x) required to obtain such information, and the limitations imposed by scientific journals on space allotted for illustrative figures, the existence of such cellularlity cannot be effectively conveyed in published form without use of interpretive drawings (e.g., Schopf 1993). This is an unacceptably subjective means of data presentation. To remedy this deficiency, Schopf and coworkers plan to generate high resolution three-dimensional optical images of individual petrified microscopic fossils, an easily achievable technological advance that, combined with the chemical analyses outlined below, will provide data crucial to answering the question of biogenicity.

7.5.3 In-situ isotopic analysis

The carbon isotopic composition of microfossil-associated Precambrian organic matter is known from thousands of measurements in hundreds of Precambrian deposits (Strauss and Moore 1992), studies that have traced isotopic signature of microbial photosynthesis to at least 3,500 Myr ago (Hayes et al. 1983; Strauss et al. 1992). Such analyses of bulk samples, however, yield average isotopic values of carbon derived from a mix of sources, including microfossils of diverse types and states of preservation as well as sapropelic carbonaceous particles of multiple origins. By making use of the spatial resolution afforded by ion microprobe mass spectrometry (e.g., the Cameca 1270 at UCLA), isotopic analyses have now been extended to the kerogenous materials comprising specific microscopic fossils (House et al. 2000; Ueno et al. 2001), an analytical advance that links morphology and carbon isotopic composition in individual fossil microorganisms. The potential of this technique has been barely tapped, and the analytical advance first developed at UCLA (House et al. 2000) has yet to be applied to all but one of the eight earliest fossil assemblages known. Schopf and colleagues therefore plan to combine this newly established method with three-dimensional optical and Raman imaging techniques to evaluate the putative biogenicity of ancient microscopic fossils in rock samples already on hand.

These samples represent some of the oldest reputed fossiliferous units known and include those of the ~3,375 Myr old Kromberg and ~3,465 Myr old Apex deposits.

7.5.4 Molecular composition and geochemical alteration of organic matter

Another important key to establishing the biological origin of ancient microscopic fossil-like objects is to link their morphology to their chemical composition. Studies of the molecular-structural makeup of the organic matter comprising such fossils have only recently begun, most effectively by laser-Raman imagery and atomic force microscopy (Kudryavtsev et al. 2001; Kempe et al. 2002; Schopf et al. 2002b). These analytical techniques have only just recently been applied to the kerogenous components of Precambrian organic-walled microfossils and associated sapropelic debris, yet they hold great promise. In particular, Raman imagery provides the means to correlate optically-discernable morphology with molecular structure in individual carbonaceous microfossils (Figure 7.5.4, Kudryavtsev et al. 2001; Schopf et al. 2002b). Atomic force microscopy studies of carbonaceous microfossils permit visualization of the sub-µm-scale micromorphology of their preserved kerogenous constituents (Kempe et al. 2002). Results thus far have demonstrated a one-to-one two-dimensional correlation between cellular morphology and chemical composition. But to answer the question of biogenicity unambiguously and to rule out the possibility that fossil-like objects represent some sort of solid mineralic (e.g., graphitic) sports of nature, these composition and structure-dependent visualization techniques must be extended to three dimensions. This will be achieved by the installation of a new, state-of-the-art laser Raman imaging facility at UCLA.

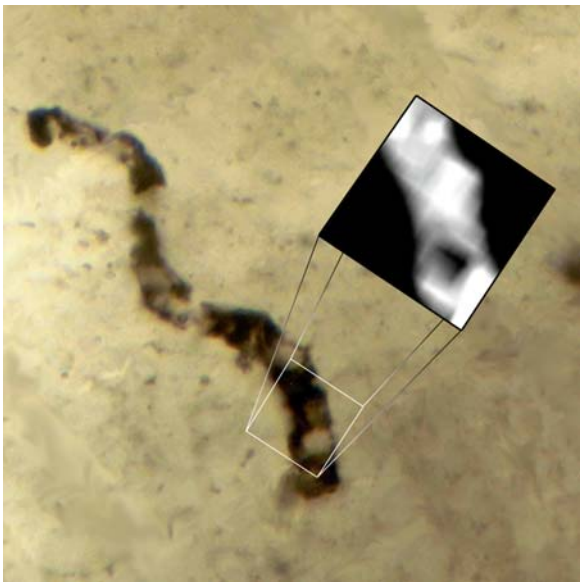

Figure 7.5.4. Photographic image and laser Raman image (inset) of ~3.47 billion year-old microfossil from the Apex chert of Western Australia. Dark material is demonstrated to be carbonaceous by the laser Raman image.

Schopf and colleagues recently used laser-Raman imagery (Kudryavtsev et al. 2001; Schopf et al. 2002b) to conduct a comprehensive study of the chemistry of carbonaceous microscopic fossils permineralized in 25 fine-grained chert units ranging in age from Devonian to Archean. Results demonstrate that the structure of the spectra acquired varies systematically with the metamorphic grade of the fossil-bearing rock units sampled, the fidelity of preservation of the fossils studied, the color of the organic matter analyzed, and with both the H/C and N/C ratios measured in kerogens isolated from bulk samples of the fossil-bearing cherts (Schopf et al. in press). To compare quantitatively the systematic variations observed among the various spectra, this work introduces the concept of the Raman Index of Preservation (RIP), an approximate measure of the degree of geochemical alteration of the 25 kerogens analyzed. Deconvolution of the various spectra, facilitated by comparisons with spectra obtained from experimentally heated fossil specimens, has provided insight into the molecular and chemical makeup of ancient kerogens and the changes that accompany organic metamorphism. To refine and extend this work, arrangements have been made to obtain additional samples of kerogens that are well characterized as to maximum temperature (by vitrinite reflectance, palynomorph color, H/C ratios, etc.), from U.S. petroleum companies (via Dr. W. Dow) and from the Institut Français du Pétrole (via Dr. Mireille Vandenbroucke). Schopf and

collaborators proposes to analyze these ~400 specimens by Raman spectroscopy in order to provide a firm basis for calibrating the thermal (catagenic) history evidenced by their RIP values and to determine the activation energies that have resulted in loss of various chemical moieties as a function of their geologic (or laboratory-simulated) thermal histories.

7.5.5 Quantitative methods for evaluating the biogenicity of fossil stromatolites

Spectacular, conical stromatolites arranged in egg carton-like arrays were reported recently from 3.45 Gyr-old Warrawoona Group strata in Western Australia (Hofmann et al. 1999). These stromatolites are arguably the best evidence for the nature of early life on Earth because they may record some aspects of the behavior and ecology of early Archean microorganisms. However, it is first necessary to be convinced that these structures are, at least in part, biogenic constructions. Understanding their morphogenesis also serves as a prelude to lander and rover explorations of the ancient terrains of Mars because distinctive, large, and widely-distributed sedimentary structures of this type are an obvious target for astrobiological missions.

The case for an abiotic origin for at least some Precambrian stromatolites was advanced by Grotzinger and Rothman (1996), who used a power spectral analysis to quantify the three-dimensional shape of some Paleoproterozoic stromatolites as observed in outcrops and in sawn sections. They believed that the stromatolite growth process could be modeled in 2+1 dimensions by the classic KPZ interface equation of condensed matter physics (Kardar et al. 1986). Although numerical show that this is not correct (Jögi and Runnegar 2002), Grotzinger and Rothman made a very important advance by showing how the terms in the KPZ equation (upward growth, surface-normal growth, diffusion, gaussian noise) can represent processes that are meaningful in sedimentological and biological contexts (sediment fallout, spherulitic crystallization, downslope movement, and environmental fluctuations, respectively).

Simulations are carried out in 1+1 and 2+1 dimensions using code written by Jögi over the past three years. For small problems (say, 256 x 256 grid points), the calculations are performed locally on Sun workstations; larger arrays have been run using parallelized code on the San Diego Supercomputer Center's IBM "Blue Horizon" (§ 3).

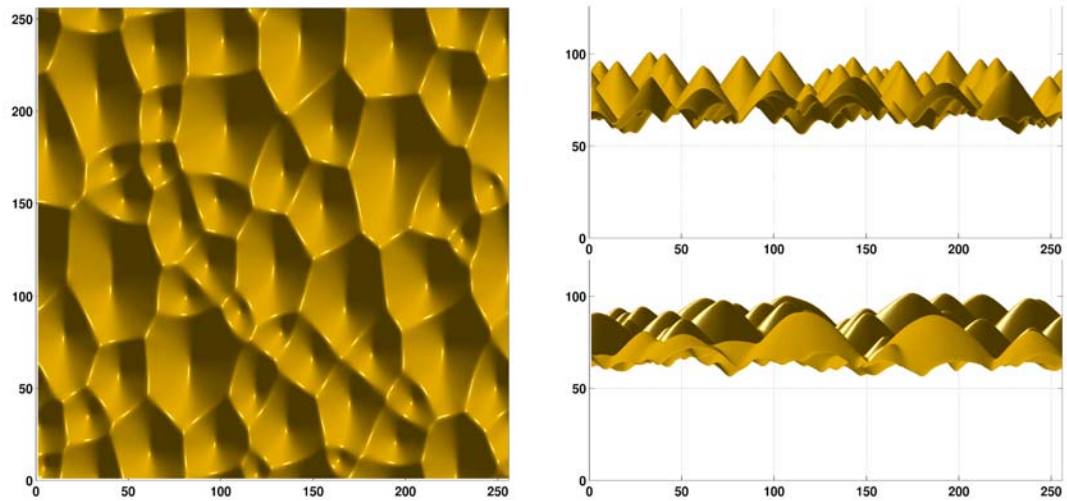

Fig. 7.5.5. Simulation of 3.45 Gyr-old coniform stromatolites, Warrawoona Group, Western Australia using a 256 x 256 grid with periodic boundary conditions (600 timesteps). Left image is a plan (Z) view; note asymmetry and preferred orientation parallel to Y; right images represent views parallel to the Y and X axes.

Results to date have shown conclusively that the KPZ equation, and others like it (EW; Edwards and Wilkinson 1982) are incapable of producing simulated structures that resemble the Archean coniform stromatolites from Western Australia. On the other hand, an equation that is important in the physics of metal atom sputtering (Smilaeur et al. 1999) simulates conical structures effectively (Figure 7.5.5). The reason is that the process being modeled (electron beam epitaxy) has an uphill component caused by an edge effect at the atomic scale. Thus, models constructed using this equation (SRK) incorporate an upslope diffusion term that is not present in KPZ-based models. As upslope diffusion is a process that is easily attributable to life but not to other non-vital environmental agents at anything larger than atomic scale, this parameter may provide a definitive test for biogenicity.

There are other features of the Warrawoona stromatolites that need to be explored mathematically. The SRK equation produces cones that either slowly coalesce ("coarsen") or exhibit rounded tops and froth-like behavior in long simulations. Similar behaviors are seen in experimental and industrial settings when metals are deposited using these methods. Jögi and Runnegar have discovered that there is a narrow zone of stability between these two realms that corresponds to a phase transition between regimes controlled by the endmember EW (frothy) and SRK (coarsening) equations. Simulations that occupy this transition zone generate suitably conical structures (Figure 7.5.5) that do not coalesce. After numerous experiments of this kind, it is now possible to accurately specify the slope angles (in two orthogonal directions as in the stromatolites; Figure 7.5.5) and grow cones to a specified size. It should be emphasized that the only "environmental" input to these simulations is uncorrelated random noise. This and the values given to the various diffusion terms are the controlling parameters. They provide the beginnings of a precise understanding of the physical and biological factors that may have generated these ancient structures.

The next step is to try to incorporate features not captured by the current model (down-dip asymmetry, higher-frequency wrinkles, etc.) and then to make careful, quantitative comparisons between the modeled stromatolites and the field exposures. This will require the development of more sophisticated metrics than the method used by Grotzinger and Rothman (1996), the production of 3D physical models using rapid prototyping technology, and additional analysis of the natural objects using digital images of outcrop and sawn sections, and, possibly, computerized X-ray tomography. Ultimately, the goal is to extend this approach to other distinctive stromatolite types.

7.6 GENOMICS, GEOLOGY, AND THE TREE OF LIFE (AWRAMIK, FITZ-GIBBON, HOUSE, LAKE, RIVERA, RUNNEGAR)

Phylogenetic trees can serve as DNA-derived windows into Earth's past. They offer a framework upon which one can map the atmospheric, paleontological, geological, and climatological records and thereby test scenarios for evolution on a planetary scale. Here, phylogenetic reconstructions will be used to address a central set of questions relating to life's early history: What were environmental conditions like early in the evolution of life on Earth? Did life start in a hot environment? What compounds were being made in the first half billion years of life on earth? What was the role of horizontal gene transfer during these early times? Was methanogenesis developed quickly and only once? How did environmental variables such as temperature, pH, sulfur availability, and oxygen affect the evolution of life? Did heterotrophy evolve before autotrophy, or *vice versa*? The research proposed in this section will focus on these, and related, questions using a combined geological and genomics approach.

7.6.1 Mapping microbial metabolisms on to the tree of life

Parsimony analyses of phylogenetic trees, can allow one to extrapolate from the environments in which extant prokaryotes are currently living to the environments in which their distant ancestors lived. This process (Williams and Fitch 1989) is illustrated in Figure 7.6.1 using a standard reference tree of eight prokaryotes of diverse ancestry. Six environmental parameters of present day organisms are indicated by the colors at the tips of the branches and the inferred parameter values are shown by colors

continuously distributed across the trees. This example is inconclusive because few taxa have been sampled, but serves to illustrate the method. Using trees constructed in this way, but which contain many prokaryotic taxa, it will be possible to trace back the evolution of different environmental parameters into the interior of the tree and thereby relate the tree of life to the geological record.

Phylogenetic reconstruction is central to interpreting the geological record, but obtaining realistic reconstructions of the prokaryotic branches of the tree of life is a daunting task. At present, prokaryotic relationships remain poorly resolved. There is now an emerging consensus that prokaryotic trees, whether based upon ribosomal RNA gene sequences or upon other molecules, fail at depths greater than the "phylum" level (Garrity and Holt 2001). Although it is usually known to which phylum any particular bacterium belongs, it is generally not known how the various phyla are related to each other. This deficiency has obvious implications for understanding the origins and evolution of planetary-scale metabolic activities such as, for example, oxygenic photosynthesis. For example, at present, the relationships among the various kinds of photosynthetic prokaryotes are unknown. Solving this problem is important if we are to understand fully the early evolution of life on Earth. Here, several steps towards obtaining better, more robust trees are proposed.

New computational tools and approaches for genome analysis, that specifically address horizontal gene transfer, have and are being developed (Lake and Moore 1998). HGT can distort phylogenetic relationships since different genes within a given genome will have different histories and therefor give rise to different trees when compared across organisms. Thus, gene trees do not necessarily correspond to organismal trees (Brown and Doolittle 1997; Feng et al. 1997; Koonin et al. 1997; Rivera et al., 1998). However, more recent studies suggest that horizontal gene transfer, while rampant, is not random, since it predominantly affects only "operational" genes, those primarily involved in housekeeping functions (Lake et al. 1999). By using only "informational genes", which are less affected by horizontal gene transfer (e.g., those involved in protein synthesis and RNA transcription), one should be able to derive correct organismal trees and also resolve deep phylogenetic divergences among prokaryotes. Ribosomal RNA genes belong to the informational class, but because they are short (2,000 to 4,000 nucleotides) they cannot accurately place prokaryotic phyla relative to each other. However, sequences constructed from concatenated informational protein genes (>100,000 nucleotides) obtained from public genome databases, are expected to be able to resolve these deep prokaryotic divergences. Lake and Rivera propose to use these methods to greatly refine the prokaryotic (bacterial and archaebacterial) sectors of the tree of life and then to use these refined trees to explore the history of environmental tolerances, as described above. This information will be integrated with the results of geological and geochemical studies (§ 74, 7.5, 8.2) that provide evidence for the time of appearance

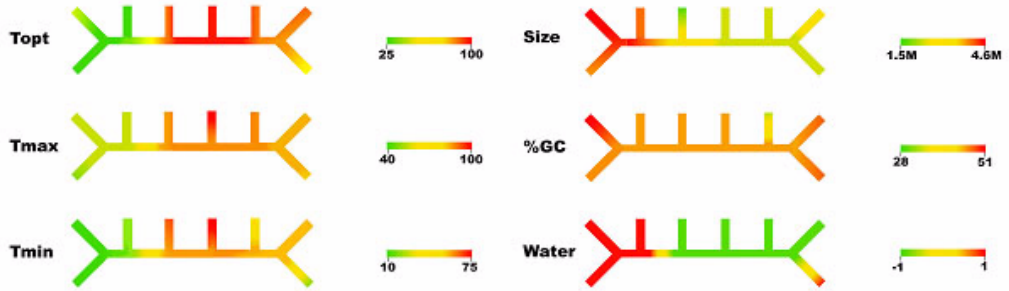

Figure 7.6.1. The distribution of environmental and genomic parameters during prokaryotic evolution as calculated from extant taxa. The parameters illustrated include minimum (Tmin), optimal (Topt), and maximum (Tmax) growth temperature, genome size, genome composition (%GC), and salinity (Water). Clockwise from lower left to lower right, the taxa are: *Escherichia coli* (g proteobacterium), *Bacillus subtilis* (gram positive eubacterium), *Synechocystis* (cyanobacterium), *Aquifex* (eubacterial extreme thermophile), *Pyrococcus* (archaebacterial extreme thermophile), *Methanococcus* (thermophilic methanogen), *Methanobacterium* (moderately thermophilic methanogen), and *Archaeoglobus* (a thermophilic archaebacterium).

of key metabolic activities.

8 EVOLVING COMPLEXITY

8.1 OVERVIEW

Progress in the evolution of life on Earth is measured by an increase in biological diversity (biodiversity), by an increase in morphological disparity, and by progress towards increasing complexity. This are presumably general principals which apply to any living system and so studies of the processes that result in these advances should have universal application. Of the three metrics for evolutionary progress, complexity is the most difficult to define, measure, and understand. In this section, biologists and paleontologists attempt to grapple with this difficult issue by focusing on two outstanding biological events: The origin of eukaryotes and the radiation of animals during the "Cambrian Explosion".

8.2 Origins of eukaryotes (Fitz-Gibbon, House, Johnson, Lake, Porter, Rivera)

Szathmá ryand Maynard Smith (1995) identified eight major milestones in the history of life on Earth: cells (compartments), chromosomes (genomes for cells), nucleic acids and proteins (separation of function at the molecular level), endosymbiosis (combination of function), sexuality (genetic recombination), multicellularity (separation of function at the cellular level), social organization (separation of function at the organismic level), and civilization (communication via language). The rise of eukaryotes began before the fourth of these thresholds was crossed, but eukaryotes progressively passed all of the other milestones and are the only branch of terrestrial life to have done so. Understanding the early part of this progressive history may therefore reveal general principles that are applicable to the growth of complexity in any living system. In this section, biologists and geologists propose to study early evolution of eukaryotes using information from molecular biology and the fossil record. The goals are to better understand the order in which important universal properties of eukaryotes (nucleus, sterols, cytoskeleton, endoplasmic reticulum, organelles, multicellularity, etc.) were acquired, and to try to time these events using both the fossil record and molecular clocks.

Unless ongoing horizontal (lateral) gene transfer has completely obliterated deep historical information from modern genomes (§ 7.6.1), one clear possibility is that the last common ancestor of the primary line of descent leading to eukaryotes – the so-called eukaryote “host” – was also the last common ancestor of the Archaea (Cavalier-Smith 2002; House et al., submitted) or, perhaps, some members of the Archaea, if the Archaea is not a monophyletic group (Rivera and Lake 1992). In this scenario, this event (the origin of the eukaryote total group) would be unrecognizable in the microfossil and biomolecular fossil records because both descendant species would have been essentially identical at the time of divergence. Only subsequently, did they independently acquire those metabolic and other attributes that allow them to be differentiated into distinct biological categories.

A second class of hypotheses for the origin of eukaryotes invokes biological convergence – an amalgamation of two or more fundamentally different kinds of organisms instead of an ordinary evolutionary divergence (Gupta et al. 1994; Lake and Rivera 1994; Martin and Müller 1998; Horiike et al. 2001; Hartman and Federov 2002). If this were true, the originating event would represent a genomic singularity in the tree of life and, perhaps, also be visible in the fossil record. In terms of molecular sequence comparisons, the amalgamation event can, in principal, be seen as a time when a signifcant number of foreign genes entered the genome of the “host” organism.

The nature of the “host” organism and the source of the prokaryotic “donations” to the genome(s) of the last common ancestor of all living eukaryotes are outstanding problems in evolutionary biology that will be addressed by the research proposed here. There are obvious contributions from the endosymbiotic events that gave rise to mitochondria and plastids (chloroplasts etc.), but it is still not

clear whether mitochondria and other similar energy-producing organelles known as hydrogenosomes were incorporated before the origin of the crown group (Roger and Silberman, 2002), and have been lost subsequently in some members (notably *Giardia* and its relatives), or are characteristic of only some groups of eukaryotes. It is even more uncertain whether the "host" organism was a stem or crown group archaebacterium (A), an unusual primitive bacterium (B; Philippe 2002), or a member of an extinct lineage (C) that diverged prior to the universal last common ancestor of all life on Earth (Hartman and Federov 2002).

When these relationships are better understood, it should be possible to use the major amalgamation events as timelines across the tree of life. For example, if all mitochondria are derived from a unique obligate endosymbiosis between a *Rickettsia*-like a proteobacterium, and if mitochondria were acquired by stem group eukaryotes, then the last common ancestor of all living eukaryotes must postdate the differentiation of the proteobacteria (Gray et al. 1999). Combining this approach with molecular clock estimates of divergence times and evidence from the fossil record will ultimately lead to an understanding of the early history of eukaryotes in a planetary context.

8.2.1 Investigating the prokaryotic sources of eukaryotic genes

The increasing availability of eukaryotic and prokaryotic genomes, and of new phylogenetic methods, promises to help understand which prokaryotic groups are the antecedents of eukaryotes. Knowledge of eukaryotic ancestors and their metabolisms, may in turn tell us about the environment in which eukaryotes arose and suggest what conditions on Earth facilitated their evolution. Furthermore, knowing whether eukaryotes are descended from anaerobes or from aerobes may greatly constrain possible scenarios for their evolution. At present, there is increasing interest in proposals that eukaryotes are chimeric and have multiple origins (Gupta et al. 1994; Lake and Rivera 1994; Martin and Müller 1998). Evidence from whole-genome analyses indicates that those eukaryotic genes coding for translation and transcription have come from archaebacterial hyperthermophiles whereas genes used for amino acid biosynthesis and other cellular processes have come from several different groups of eubacteria (Koonin et al. 1997; Rivera et al. 1998; Martin et al. 2002).

Johnson and her collaborators will focus on the steps by which eukaryotes have obtained and lost their genes and organelles (mitochondria and hydrogenosomes). Using methods developed previously, Lake and Rivera will will search for those prokaryotic "phyla" (Garity and Holt 2001) that have made the largest genetic contributions to the eukaryotic genome. In preliminary studies, it was found that several prokaryotic genomes have made significant contributions to eukaryotes. However, by using representative members of the ~80 currently available prokaryotic genomes and procedures similar to those used for studies of horizontal gene transfer (§ 7.6.1; Jain et al.1999), they expect to determine which of the major bacterial groups have been the sources of the eukaryotic genes. The plan then is to identify which classes of genes (e.g., amino acid biosynthesis genes, cell envelope protein genes, energy metabolism genes) have come from which prokaryotes. These will then be compared with the metabolic properties of the contributing prokaryotes in order to test existing hypotheses for the origins of eukaryotes.

In a complementary study, Fitz-Gibbon and House propose to carry out additional whole genome comparisons in order to obtain more fully resolved phylogenetic trees. They score individual genes as being either present or absent, just as any particular nucleotide is scored as being in one of four possible states (A, G, C or T) in DNA sequence comparisons. This method of whole genome comparison has been successfully applied previously (House and Fitz-Gibbon 2002; House et al. submitted).

Since the method of whole genome analysis is in its infancy, Lake and Rivera propose to investigate some of the potential problems. When genomes differ considerably in size, whole genome analyses tend to put adjacent taxa together even if the organisms are not closely related. This effect, "big genome attraction", is similar to the long branch attraction artefact of gene and protein sequence comparisons (Philippe 2002). It can become important because eukaryotic genomes differ so greatly in

size. For example, trees were constructed using the following eukaryotes, listed according to increasing genome size: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophilia melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, and *Homo sapiens*. Invariably, the taxa clustered together according to the size of their genomes as expected from the "big genome attraction" concept. This problem needs further exploration.

8.2.2 Origin and evolution of eukaryotic respiratory organelles

Modern eukaryotes are viewed as a consortium of ancient associations between the nucleus plus cytoplasmic "host" and endosymbiotic bacteria that gave rise to energy-generating organelles, the mitochondria, chloroplasts, and hydrogenosomes. Competing hypotheses speculate about the role these ancient endosymbioses had in shaping the early eukaryotic cell. One view, the "hydrogen hypothesis" (Martin and Müller 1998), suggests that it was an association between a methanogenic host and a hydrogen-producing bacterial symbiont that led to the establishment of mitochondria in extant eukaryotes. Other theories hold that the mitochondrial symbiosis occurred substantially after the origin of the eukaryotic nucleus, pointing to the existence of ancestral amitochondriate eukaryotes (Andersson and Kurland 1999). Each of these hypotheses implies a different time of origin and selective force for the establishment of symbiont-derived organelles and has implications for the origins of anaerobic and aerobic eukaryotes and their metabolic pathways.

The credibility of various hypotheses concerning eukaryotic organellar origins depends upon knowledge of hydrogenosomes (complex organelles involved in anaerobic carbohydrate metabolism) and mitochondria, the relationships between these organelles, and the origin of their protein components (Roger 1999; Dyall and Johnson 2000). To better understand the origin and nature of the hydrogenosomes, Johnson and her collaborators will probe the evolutionary and biochemical properties of this organelle in the anaerobic protist *Trichomonas vaginalis*, using a combination of genomics, proteomics and phylogenetic approaches. They propose to identify all genes encoding hydrogenosomal proteins by scanning the soon-to-be-completed genome using a highly conserved part of all matrix protein-coding genes. A proteomics approach, using mass spectrometry and genome sequence mining, will also be used to directly identify organellar proteins. Proteins identified by both methods will be subjected to phylogenetic analyses and their presence in the hydrogenosome will be confirmed using a variety of cell biological techniques. By combining genomics, proteomics, molecular evolutionary and cell biological approaches, they will attempt to elucidate the ancestry of the trichomonad hydrogenosome, an organelle that is central to energy metabolism in diverse anaerobic, and possibly other primitive, eukaryotes. The data generated will be used to assess alternative hypotheses for the origin and evolution of energy-generating organelles in early eukaryotes.

Only six proteins from hydrogenosomes have been subjected to phylogenetic analyses and the results are ambiguous (Dyall and Johnson 2000; Horner et al. 2000). While the analyses of four of these genes are consistent with a common ancestry for hydrogenosomes and mitochondria, the other two genes suggest an ancestry from certain kinds of anaerobic bacteria. Whether any or all of these genes arose via an endosymbiotic event or via lateral gene transfer is unclear. The central aim of the proposed work is to identify most, if not all, proteins that are targeted for import to the hydrogenosomes. In brief, it is proposed: (1) to identify genes encoding hydrogenosomal matrix proteins as described above; (2) to identify all proteins in the hydrogenosome; (3) to test whether the putative hydrogenosomal proteins found are localized to the organelle *in vivo*; and (4) conduct phylogenetic analyses on newly-identifed hydrogenosomal proteins. Johnson's laboratory is currently collaborating with TIGR (The Institute of Genomic Research) to obtain the complete genome of *Trichomonas vaginalis*. The sequencing is expected to be completed by July, 2003.

These studies, which directly test whether hydrogenosomes and mitochondria arose from a common endosymbiont, will improve understanding of how organelles and eukaryotic cells first arose, and shed light on ancestral metabolic associations that led the evolutionary specialization and complexification of eukaryotic cells. They will explore the ancestry of organelles which are central to energy metabolism in

eukaryotes, and will highlight processes by which cells gain complexity and evolve. If hydrogenosomes arose from an ancestor different from the mitochondrion, then hypotheses concerning the nature of living eukaryotes will be narrowed to those postulating endosymbiotic associations among a consortium of eubacterial and eukaryotic progenitors. The proposed combination of phylogenetics, genomics and proteomics to assess homologies among organelles sets this part of the proposal apart. However, Johnson's work and that proposed by Lake and Rivera (§ 8.21) are intimately related as they both address the potential contribution of bacterial consortia in the formation of the eukaryotic cell.

8.2.3 The fossil record of eukaryotic diversification

The diversification of eukaryotes coincided with dramatic changes in Earth's climate, ocean chemistry, atmospheric oxygen levels, and tectonic configuration. Mechanisms that link eukaryotic diversification to changes in the physical environment have recently been proposed (e.g., Brasier and Lindsay 1998; Anbar and Knoll 2002), but, with a few notable exceptions (Javaux et al. 2001), the stratigraphic and paleontologic datasets needed to test these ideas are not available. Recent work in well preserved, widely separated successions in western North America offers an unparallelled opportunity to gain insight into the interaction between biology and environmental change during the early to middle Neoproterozoic interval of Earth history (1,000 to ~600 Myr ago). Sequence stratigraphic, chemostratigraphic, geochronologic, and tectonic studies are in progress or have already been completed for two basins: the ~770-742 Myr-old Chuar Group, Grand Canyon, and its likely correlative, the Uinta Mountain Group, Utah (Weil et al. 1999; Karlstrom et al. 2000; Dehler et al. 2001; Timmons et al. 2001; Dehler et al. 2002; C. Dehler and L. Crossey, pers. comm. 2002). These studies provide a detailed record of environmental change with which paleobiological studies can be integrated. Initial work in the Chuar Group indicates a tantalizing connection between increasing carbon isotope variability and the first appearance of heterotrophic protists within the basin (Porter and Knoll 2000; Dehler et al. 2001; Porter et al. 2003). This possible causal connection will be explored using studies that investigate the abundance, diversity, and paleoenvironmental distribution of organic walled microfossils (acritarchs), and also the relationship of these variables to environmental parameters. In addition, ultrastructural and ion microprobe analyses of individual acritarchs will help elucidate the affinities of these early eukaryotic fossils, aiding in the calibration of branch points in the eukaryotic tree. Ion microprobe carbon isotope analysis of individual acritarchs may also prove useful for inferring paleoecological or paleoclimatic information; these possibilities and others will be explored in the proposal period. Chuar Group samples have already been collected and processed; funding is requested for fieldwork in the Uinta Mountain Group and for the processing of samples.

8.3 Understanding the assembly of animal body plans in the context of the Cambrian explosion (Gehling, Jacobs, Kouchinsky, Porter, Runnegar, Webster)

The Cambrian Period witnessed one of the most significant events in the history of life: an exponential increase in animal diversity and complexity, commonly referred to as the "Cambrian explosion". Despite recent advances in our understanding of animal relationships (Peterson and Eernisse 2001) and Cambrian geochronology (Bowring and Erwin 1998), important questions remain, perhaps the foremost of which is, what triggered the Cambrian explosion, and what makes it so unusual? Addressing these questions requires that we understand not only the steps by which early animal body plans evolved, but also the extrinsic and intrinsic controls on that evolution. We propose to study four topics that will elucidate these issues: (1) The stratigraphic record of the first multicellular (Ediacaran) animals (Gehling, Runnegar); (2) The nature of the developmental program controlling skeleton formation (Jacobs, Kouchinsky); (3) The evolution of developmental constraints in early Cambrian trilobites (Webster); (4) The sequence of body plan evolution in the lophotrochozoan phyla (Porter, Runnegar). These studies will combine insights from evolutionary developmental biology and data from the fossil record to obtain a clearer understanding of the Cambrian explosion.

8.3.1 Ediacaran biodiversity: Prelude to the Cambrian explosion

Ediacaran organisms have been biologically enigmatic since they were first discovered (and thought to be pseudofossils) in Newfoundland during the 19th century (Gehling et al. 2000). However, recent discoveries of numerous new fossils at remarkably rich sites in the Flinders Ranges of South Australia, in southern Namibia, on the shores of the White Sea, and in Newfoundland have demonstrated that the Ediacaran biotas really do record the initial diversification of multicellular animal life. What is particularly striking, is that the older assemblages (ca. 565 Myr old) lack evidence in the form of body fossils and trace fossils for animals of bilaterian grade (mobile, bilaterally symmetrical, cerebral). In contrast, there is ample evidence for bilaterian grade animals in the younger assemblages worldwide. Ediacaran fossils are no longer limited to single stratigraphic horizons that are isolated by unfossiliferous strata from the succeeding Cambrian explosion. In Newfoundland, for example, Ediacaran fossils have been found beneath virtually every volcanic ash layer (~65 in all) over some 2.5 km of stratigraphic thickness (Narbonne and Gehling 2003). Furthermore, the oldest of these fossiliferous levels is not far above the Gaskiers Tillite, arguably the youngest (580 Myr; Bowring et al. 2002) of the Neoproterozoic snowball Earth glacial events.

The time has come to put all of this globally-distributed paleontologic and biostratigraphic information together in order to: (1) Test the idea that the last one or two Snowball Earth glaciations were implicated in the appearance and diversification of the Ediacaran biota. (2) Document the progressive evolution of biocomplexity through the terminal Proterozoic period (soon to be formally named the Ediacaran). (3) Explore further the connections with the Cambrian explosion. The modest funds requested for this research ($5k/year) will be used by Gehling for field studies of the most important sites outside Australia.

8.3.2 The evolution of mineral skeletons

The evolution of skeletons is thought to have fed back through predator-prey evolution to increase specificity of interactions that contributed to the rapid diversification of Cambrian bilaterian animals. Preliminary work (Jacobs et al. 2000) suggests that aspects of the growth of shells and other mineralized skeletal elements are under common developmental control in a wide range of living animals. Although skeletal ultrastructures and materials in different animal phyla are distinct, common features of organic matrices found with skeletons suggest that the process may have a single common ancestry. Homology of bilaterian skeleton formation, if proven, would suggest that the onset of skeletogenesis played a critical role in the Cambrian radiation.

To better understand the relationship between the evolution of bilaterian skeletogenesis and the Cambrian explosion, we propose to: (1) explore the relationships of developmental genes that appear to be associated with skeletogenesis in unstudied animals; (2) compile all available ultrastructural information pertaining to setae, spicules, and similar skeletal precursor organs in order to develop a model of the evolutionary origin of the simplest skeletal elements; and (3) use this model to choose candidate genes for further study.

The *engrailed* gene, best known for its role in bounding the exoskeletal units of arthropod segments, appears to bound skeletal elements in other invertebrate phyla including shells of molluscs, setae of annelid polychaetes and the arm plates of brittle star echinoderms (Jacobs et al. 2000, Seavers et al. 2002). Thus, the *engrailed* gene is one of several clues linking the process of skeletogenesis across a range of bilaterian animals. To assess the breadth of this phenomenon we propose to measure the expression of *engrailed* in related phyla that have mineralized hard parts, including brachiopods, echiurids, sipunculids, and tube dwelling annelids. The *engrailed* gene has already been sequenced from echiurids and sipunculans.

8.3.3 Role of constraints on animal development through morphometric studies of Cambrian trilobites

The magnitude and rate of evolutionary innovation associated with the Cambrian explosion was not sustained in post-Cambrian times. This implies either ecological constraint, where filled niches limited opportunity, or developmental constraint, where evolution of development limited the ability of organisms to evolve new morphology regardless of ecological opportunity.

Cambrian trilobites are the best group to test competing ecological and developmental explanations for the post-Cambrian decrease in evolutionary innovation. Detailed reconstruction of paleoenvironments across significant Cambrian extinction events permit control for ecological factors and assessment of responses to changes of diversity that reduce the degree to which niches are filled. This, in combination with detailed examination of the developmental series through time, permits a formal comparison of the ecological and developmental limits on morphological diversification. The proposed research will use detailed morphometry (Webster et al., 2001; Webster, 2003) to investigate new collections of high-quality, silicified (uncompacted) trilobites made at high stratigraphic resolution both across and between extinction events. These studies will lead to a greater understanding of the unique patterns of metazoan evolution associated with the Cambrian explosion.

8.3.4 Using stem group taxa to order characters important in body plan evolution

Modern mobile animals, technically known as bilaterians, are characterized by complex, functionally and developmentally integrated anatomies. Insight into how such complexity arose can be gained through study of the Cambrian fossil record, which contains a number of "oddball" animals that have unfamiliar morphologies or lack features found in modern groups. Recently it has been recognized that these problematical animals are not members of extinct phyla (Gould 1989). Instead, they are often stem-group representatives of modern groups. When they are placed within a phylogenetic framework, stem-group taxa can help reveal the evolutionary steps through which modern body plans arose (Budd 2002).

Halkieriids are among the most widespread, diverse, and abundant early Cambrian "oddball" animals. These scaly organisms are either stem-group members of the lophotrochozoans or, more likely, of one of its contituent phyla such as the molluscs, brachiopods, or annelids (Annelida, Brachiopoda, Mollusca; Jell 1981; Bengtson 1992; Conway Morris and Peel 1995; Runnegar 2000; Holmer et al. 2002). Porter and Runnegar propose to study the morphology and ontogeny of halkieriids and their relatives in unprecedented detail. First, ultrastructural studies will be conducted to reconstruct the original composition, morphology, and structure of halkieriid sclerites (Porter 2002; in press); variation within the group necessitates comprehensive representation of halkieriid taxa. Second, 3D models of halkieriid scleritomes, constructed from laser scanned images of well-preserved sclerites (Lyons et al. 2002) and guided by data from articulated specimens (Conway Morris and Peel 1995), will be used to test competing hypotheses of halkieriid ontogeny and skeletal growth. These data, combined with those obtained through similar investigations of fossil and modern lophotrochozoan groups, will lead to a detailed cladistic analysis of selected lophotrochozoans and, hopefully, provide some insight into how complex body plans arose. Halkieriid specimens from Australia and Siberia are in existing collections. Funding is requested for scanning electron microscopy and laser scanning of specimens, and for fieldwork in China to obtain new collections from Early Cambrian horizons known for exceptional preservation of halkieriids and other problematical fossils.

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Plan for Strengthening the Astrobiology Community

1 INTRODUCTION

The UCLA Center for Astrobiology is engaged in developing the field of astrobiology through a number of initiatives that play to the strengths of an institution of higher learning such as ours. These include formal courses, public lectures, sponsorship of the UCLA Astrobiology Society, participation in the Minority Astrobiology Collaborative, and convening of international meetings concerned with astrobiology-related research. Numerous activities are planned for the next five years, as outlined below.

In the past several years members of the UCLA lead team have taught a number of courses related to astrobiology. These courses were inspired in part by the existence of the Center for Astrobiology. In addition, the Department of Earth and Space Sciences has hired Edwin Schauble as a new Assistant Professor in Astrobiology. A new course in Astrobiology is planned as part of the CAB five-year plan.

Our series of free public lectures entitled "Astrobiology Superstars" has proven popular and will continue in the next two years. However, the historically-significant venue, the Midnight Special Bookstore in Santa Monica, California, is closing and so we will continue the series in a new location (see below). After the first two years the "Superstars" series is to be replaced by a series of public lectures at the Griffith Observatory, also described below.

The activities of the UCLA Astrobiology Society continue to grow and will be encouraged under CAB sponsorship. New initiatives include a research apprenticeship involving CAB team members and mentoring programs to facilitate the proliferation of similar societies at other institutions of higher learning.

Current CAB Administrative Assistant, Barbara A. Laval, will continue in her roles as University Liaison for the Minority Astrobiology Collaborative (MIAC) and NAI Minority Institution Collaboration lead. As she is taking on more duties, her position will be upgraded from Administrative Assistant to Administrator (UCLA Senior Analyst). Through this contact and future participation in the MIAC faculty sabbatical program, CAB remains committed to the goal of increasing the number of minority students in the sciences by providing opportunities for participation in, and learning about, cutting-edge research.

2 EDUCATION AND PUBLIC OUTREACH (EPO)

2.1 Astrobiology curriculum

Several courses related to astrobiology have been taught over the past several years at UCLA. These include: Origin and Evolution of the Solar System (Earth and Space Sciences 9); Astrobiology (Molecular Biology 298); Life in the Universe (Astronomy 4); and Origin of the Cosmos and Life (Cluster General Education course no. 70). This last course, taught by Morris, McKeegan, and UCLA faculty in the life sciences ran for 3 quarters each of the past several years. The cluster courses in general were designed to provide students with the option of a common intellectual experience during their freshman year (i.e., "freshman studies").

The Department of Earth and Space Sciences is in the midst of designing a new General Education course in Astrobiology that will be taught by our faculty. The first draft of a course description can be found in § 2.4 below.

2.2 Public lectures

The "Astrobiology Superstars" lecture series will continue but at a new venue due to closure of the Midnight Special Bookstore in Santa Monica, California. This lecture series has been free to the public and this policy will continue in the future. Lecturers for 2001 included team members Zuckerman, Moore,

Manning, and Rivera as well as JPL lead team member Kenneth Nealson. Lecturers for 2002 included UCLA lead team members Song, Schubert, Lyons, and Venkatesan, among others.

The new venue for 2003 and 2004 will be the UCLA Faculty Center. A suitably sized hall within the Faculty Center costs approximately $130.00 per three hour session. The Lectures are scheduled to be bimonthly and the total cost of $780.00 will be paid from our budget.

Twelve of the Superstars lectures are on videotape. The UCLA Office of Instructional Media has been contacted about processing the tapes and encoding them for on-demand viewing on the UCLA webcast web site (webcast.ucla.edu). Encoding and steaming each video on the site will cost $203.00 per tape. This includes a title, credits, editing, and 13 weeks of web hosting on webcast.ucla.edu. Additional time on the web site is provided at a rate of $50.00 per academic quarter. We are also considering the possibility of providing the digitized lecture tapes to the NASA Astrobiology web host if the server is capable of handling the band width and disk space. Experiments with the first 12 tapes will begin immediately. The outcome will be used to inform future taping of lectures and to decide how long to run each of the lectures.

In late 2005 we intend to discontinue the Superstars lecture series in favor of a more visible series to be held at Griffith Observatory in Los Angeles. These Griffith Observatory lectures are in the planning stages at present because the Observatory is currently undergoing an $83 million renovation and is due for reopening in late 2005. Until then, the Observatory staff functions out of the Satellite facility near the Los Angeles Zoo. The PI (Young) has contacted Dr. Ed Krupp, Director of the Observatory about prospects for using both the Satellite facility (including the nearby Autry Museum of Western Heritage) and ultimately the newly-renovated Observatory for the new lecture series. Krupp has in turn put us in contact with the Friends of the Observatory group for further planning.

The new lecture series at Griffith is envisioned to be one in which the highest caliber speakers are brought in from around the world to educate the community about astrobiology-related science (the concept is based on the Carnegie Institution of Washington's Capital Science lecture series). Lectures will be held approximately four times per year and will be sponsored jointly by both the UCLA lead team and the JPL-II lead team headed by Victoria Meadows. It is our intention to take advantage of our proximity to a prominent local public radio station in Pasadena (KPCC) to obtain air time for some of the guest speakers on their morning talk show "Air Talk." This show often devotes up to an hour to scientific issues. Firm plans for media coverage will have to wait until closer to the time of the lectures.

2.3 Additional team members related to EPO

The UCLA Center for Astrobiology has facilitated the training of numerous students and postdoctoral researchers. Some of them are listed below. Other team members not listed as collaborators, co-investigators, students, or postdoctoral researchers are staff or collaborators engaged in EPO activities. They are also listed here.

2.3.1 Administration

Todd Gary (team member) is a molecular biologist and Associate Professor in the Department of Geological Science at Tennessee State University (which currently has 850 undergraduate Biology majors that are African-American) and the director of the Institute for Understanding Biological Systems. Gary was one of the first NAI Minority Astrobiology Faculty Fellows. He spent the summer of 2002 in the laboratory of James Lake at UCLA investigating evolutionary genomics and horizontal gene transfer. He is also the co-Director of the Minority Institution Astrobiology Collaboratory (MIAC), which was formed to create a "virtual collaboration" of faculty and students at minority institutions focused on achieving common educational and research goals in astrobiology.

Barbara A. Laval (Manager, Education & Public Outreach Coordinator) manages the Center for Astrobiology and its Education and Public Outreach programs. She acts as liaison to NAI Central, the UCLA Astrobiology Society, and the NAI Minority Institution program (**blaval@ucla.edu**).

2.3.2 Past and present students and postdoctoral researchers (field, advisor, date completed)

Jocelyn Couetdic, undergraduate intern, celestial dynamics, Ference Varadi (advisor), 2003

Andrew Czaja, graduate student, paleobiology, J. William Schopf (advisor), present

Tracy Herrera, graduate student, geochemistry, Craig E. Manning (advisor), present

Andrew Hock, graduate student, planetary science, David A. Paige advisor), present

Cristopher H. House, graduate student, paleobiology, J. William Schopf (advisor), 1999

Ravi Jain, graduate student, molecular biology, James A. Lake (advisor), 2002

Dana N. Kovaric, graduate student, planetary science, David A. Paige (advisor), 2002

Crispin T.C. Little, postdoctoral researcher, geobiology, Bruce Runnegar (advisor), 2001

James R. Lyons, postdoctoral researcher, geochemistry, T. Mark Harrison (advisor), 2001

Caer-Eve McCabe, graduate student, astronomy, Andrea M. Ghez (advisor), 2003

Sabrina S. Mayerberger, graduate student, astrobiology, Bruce Runnegar (advisor), present

Michael A. Mischna, graduate student, planetary science, David A. Paige (advisor), present

Stephen J. Mojzsis, postdoctoral researcher, isotope geochemistry, T. Mark Harrison,(advisor) 2000

Johnathan E. Moore, graduate student, molecular biology, James A. Lake (advisor), 2003

Jeffrey D. Silberman, postdoctoral researcher, microbiology, Patricia J. Johnson (advisor), 2003

AnneSimonson, graduate student, molecular biology, James A. Lake (advisor), present

Richard A. Webb, graduate student, astronomy, Benjamin Zuckerman (advisor), 2000

Alycia J. Weinberger, postdoctoral researcher, astronomy, Ian S. McLean (advisor), 2000

2.4 Description of Astrobiology General Education Course (by William I. Newman, March 2002)

2.4.1 Nature of the Course

The mission of astrobiology is to study the origin, evolution, distribution, and future of life on Earth and in the Universe. NASA statement as well as objective of this course.

> UCLA Catalog Description: 3. **Astrobiology. (5)**
>
> Lecture, three hours; discussion one hour; field trips. Origin, evolution, distribution, and future of life on Earth and in the Universe paralleling NASA's mission. Course material primarily from planetary and earth science, paleontology and biology, as well as some elements from astronomy, chemistry, and physics. Contains relatively little mathematics Letter grading.

Astrobiology shares with other space related science programs a broad range of research interests. Astrobiology encompasses the understanding of biology as a planetary phenomenon. This includes how planetary processes give rise to life, how they sustain or inhibit life, and how life can emerge as an important planetary process; how astrophysical processes give rise to planets elsewhere, what the actual distribution of planets is, and whether there are habitable planets outside of our solar system; a determination of whether life exists elsewhere and how to search for and identify it; what the ultimate environmental limits of life are, whether Earth's biota represent only a subset of the full diversity of life, and the future of Earth's biota in space.

This will be a three hour per week lecture course, as well as have two full-day field trips. (Field trips will include visits to spacecraft assembly facilities at JPL, as well as various UCLA laboratories. In addition we will visit sites of geological/biological interest, since they illustrate processes pertinent to the evolution of diversity in varied terrestrial environments.) Presentations will be based largely on lecture notes, as well as Jakosky, B. 1998. *The Search for Life on Other Planets*, Cambridge: Cambridge University Press and Goldsmith, D. and Owen, T. 1992. *The Search for Life in the Universe* (second edition), Reading, Massachusetts: Addison-Wesley Publishing Company and other existing texts. (The professor proposing this course has had direct discussion with Cambridge University Press regarding developing a textbook for this course.)

The *sine qua non* of astrobiology is its multidisciplinary nature and, as a result, a substantial number of lecture will be given by experts from other disciplines. The course will be given for letter grade, and grades will be assessed on the basis of an extensive research paper, on a topic to be selected by individual students in consultation with their TA/professor, a paragraph/essay-based mid-term examination, and final examination. A preliminary syllabus for the course appears below.

2.4.2 Week 1. Prologue: From philosophical speculation to scientific exploration

Astrobiology, as defined by NASA, is the study of the origins, evolution, distribution, and future of life in the universe. It requires fundamental concepts of life and habitable environments that will help us to recognize biospheres that might be quite different from our own. Interdisciplinary research is needed that combines molecular biology, ecology, planetary science, astronomy, information science, space exploration technologies, and related disciplines.

2.4.3 Week 2. The meaning of "life"—could we recognize life elsewhere?

How do we go about characterizing life? We can design machines that regulate their environment, undergo self-organization, and reproduce themselves, but when do we call a complex system a living organism? E.g., D.E. Koshland, Jr., 2002. "The Seven Pillars of Life," *Science*, **295**, 2215-16. The 1976 Viking I and II Mars spacecraft life-science experiments; the Galileo spacecraft's search for life on Earth; future experiments to be conducted on Mars.

2.4.4 Week 3. Case Study: Earth

Archaeobacteria's unfulfilled destiny. The sedentary life of prokaryotes, and the Cambrian invention of eukaryotic life and the explosion of diversity. Catastrophism, impacts, and extinctions: the case for coevolution of planets and life. Extremophiles and exotic environments: life on sea-floor vents and Antarctica, and implications to extraterrestrial life. Evolutionary divergence and convergence: is intelligence desirable and therefore inevitable? Is there any evidence supporting the evolution of intelligence?

2.4.5 Week 4. Goldilocks and the three terrestrial planets

Goldilocks would have found that Venus was too hot, Mars was too cold, and that Earth was just right. Or would she? The early dim sun paradox and habitable zones. Europa's oceans, Titan's environs, and Jovian chromophores. From terrestrial extremophiles to possible habitats on Mars. NASA's exploration strategy. Expanding perspectives on our

solar system.

2.4.6 Week 5. NASA's Voyages of Discovery and the Future of Space Exploration

The five phases of planetary exploration. History of the space program, and possibilities for its future. Free samples from space: the SNC meteorites, Martian meteorite ALH84001, Pathfinder and Sojourner, and the case for past life on Mars. Search strategies and the problem of forward and backward contamination.

2.4.7 Week 6. Good planets are hard to find: other solar systems and extraterrestrial life.

What can we say about the formation of stable habitats where life could emerge? From where do suitable stars and planets come? How do we look for them, and have we found any, or are likely to observe any? Where does life fit in, and how long can life persist on different worlds? From geoastrobiopoetry to astrobiology—the role of multidisciplinarity in the quest for life. Hallmarks of intelligence—are any observable?

2.4.8 Week 7. Space Travel

Two post-Columbian perspectives: 1492 and European colonization vs. 2003 and the Columbia shuttle disaster. What lessons have we learned? Will we colonize the solar system, solely pursue its exploration by humans and their robotic agents, or enter a state of eternal senescence. Are there lessons learned that we can apply to other extant civilizations?

2.4.9 Week 8. Life Beyond: From physical constraints to design principles.

By blending together elements of physics and chemistry with those of biology and physical anthropology, especially ingredients learned from recent advances ranging from genetics and planetary science, what can we say about life elsewhere? Can scaling principles observed on Earth, e.g., allometric relationships governing animal metabolisms and scaling laws describing the size and mass of mammalian and other brains, tell us anything about life elsewhere?

2.4.10 Week 9. Are we alone?

The Fermi "paradox," Drake equation, and the Heraclitusian dilemma. The media and the popular view of the subject: from UFO sightings and alien abductions to mass hallucinations. How can we communicate with alien life? Emergent technologies: the medium is the message.

2.4.11 Week 10. Societal implications for our "Pale Blue Dot"—planetary stewardship and the future of this planet.

From anthropocentrism to a cosmic perspective. What if we are alone? What if we are not? What have we learned from studying other planets and solar systems? What implications does this have to life on Earth? What lies ahead for humanity in the distant future?

3 THE ASTROBIOLOGY SOCIETY

The UCLA Astrobiology Society is a student-run organization sponsored by the IGPP Center for Astrobiology and the IGPP Center for the Study of Evolution and Origin of Life (CSEOL) at UCLA. The organization was founded in 1999 by two undergraduate students (Jason Finley and Laurel Methot) inspired by what they had learned in the General Education Cluster course (Cluster General Education course no. 70).

The Society is the first student-run organization devoted to fostering the discipline of astrobiology at the university level. Its mission is to “present the studies and goals of astrobiology to science and non-science majors in an integrated fashion that is both interesting and applicable to all fields.” The Society has been lauded by NASA officials, including the former and current directors of the NAI, for its activities directed

towards engaging undergraduate and graduate students in the burgeoning field of astrobiology. Their value to the field is evidenced by the fact that they were awarded recently their own funding from NAI central (administered through UCLA IGPP).

Astrobiology Society activities include regular meetings, a monthly newsletter, a joint project with the Space Frontier Foundation aimed at high school students, participation in the NAI's Pathfinder program, and running a student research program that pairs students seeking research opportunities with faculty and researchers in the Center for Astrobiology. The Society also hosts lectures on a regular basis. Past lecturers include Dr. Juan Perez Mercader from del Centro de Astrobiologia, Madrid, Spain and Dr. Bruce Jakosky of the University of Colorado, Boulder.

Most recently, the Society is hosting a public symposium with an astrobiology theme. The event, to take place 1 June 2003 on the UCLA campus (Ackerman Union Grand Ballroom), will begin with a lecture by Dr. Jill Tarter, Director of the Search for Extraterrestrial Intelligence (SETI) Institute, who will discuss SETI activities. This lecture will be followed by questions and an opportunity to peruse displays with information about astrobiology and hands-on items such as meteorites and fossils. The symposium will end with a lecture by Bill Nye (the "Science Guy") who will provide an overview of the field of astrobiology. The purpose of the event is to enhance public awareness of astrobiology and science in general.

A new long-term goal of the Society and the NAI is proliferation of the concept of the UCLA Astrobiology to other colleges and universities. They have begun this process through presentations at the NASA NAI General Meeting in 2003.

The UCLA lead team regularly supports the Astrobiological Society through financial support of their speakers program and underwriting travel expenses. The high value placed on the Society is manifest by the fact that the two current Co-Presidents, Geoff Robertson and Dan Fingal, are members of the UCLA lead team.

4 MINORITY PROGRAMS

The UCLA lead team is committed to fostering minority participation in the new field of astrobiology. Their commitment manifests in two ways: 1) participation in the NAI Minority Institution Involvement Program's Faculty Sabbatical Program; and 2) participating in the Minority Institution Astrobiology Collaboratory (MIAC).

UCLA lead team member Lake was one of the first hosts in the new Faculty Sabbatical program. Dr. Todd Gary (§ 23.1) spent the summer of 2002 in Lake's laboratory at UCLA working in the area of evolutionary genomics and horizontal gene transfer. In addition to Lake, several other UCLA team members have offered to participate in the Minority Institution Sabbatical program in the future, including Ghez, Jacobs, Jura, and Zuckerman. Team member Barbara Laval (§ 23.1) is the first point of contact for faculty at minority institutions seeking to collaborate with NAI investigators. Laval has established credibility with participants in the program and can take credit for providing the follow through that is necessary to ensure that the program succeeds.

MIAC was formed in 2002 and includes representatives from several minority institutions, including Hampton University inHampton VA and Tennessee State University in Nashville TN. MIAC's goals include increasing minority participation in astrobiology, building research capabilities at the participating minority institutions, and affording minority students with greater opportunities in the sciences. The ultimate goal is that members of MIAC should one day become an NAI lead team. UCLA lead team member Laval is the University Liaison to MIAC. Founding MIAC member Dr. Todd Gary of Tennesee State is to be named a UCLA lead team member in CAN-3.

As MIAC University Liaison, Laval's role is to advise on establishment of student societies analogous to the UCLA Astrobiology Society at other minority institutions of higher education, to coordinate minority institution faculty and student research internships in the Evolutionary Genomics Astrobiology Focus

Group, and to advise MIAC members in establishing virtual meetings and seminars related to the NAI. Inclusion of Gary in the UCLA lead team facilitates his ability to carry out MIAC activities. It provides him a base of operations from within the NAI from which he can seek out research opportunities for faculty from minority institutions and educational opportunities for students from those institutions.

5 RUBEY COLLOQUIA

Rubey Colloquia are held by the Department of Earth and Space Sciences at UCLA in honor of W. W. Rubey (1898-1974). Rubey was a career geologist with the U.S. Geological Survey and Professor of Geology and Geophysics at UCLA. The colloquia, funded by the Division of Physical Sciences, are flexible in their format, but typically consist of a week of lectures addressing a "hot topic" delivered by preeminent scientists from around the world. Students enroll in the colloquia and receive academic credit for their participation.

The last Rubey Colloquium was held in February of 2002 and was organized by UCLA lead team member

Frank Kyte and Peter Ward from the University of Washington (a NAI lead team PI). The Colloquium was entitled "Impacts and the Origin, Evolution and Extinction of Life." About 30 experts (see below) in the field of impacts were brought together to present their latest research and ideas. Topics included planet

formation and early bombardment of Earth, the impact history of Earth and catastrophic causes of mass extinctions, the environmental effects of impacts, and impacts as mechanisms for dispersal of life with the Solar System and beyond.

Team members McKeegan, Morris, and Young propose to hold another Rubey Colloquium in January of 2005. The theme will be the origins of Solar-like planetary systems. The total budget for this event is estimated to be $9,000.

Rubey Colloquium, February 9-10, 2002, UCLA

University of Colorado, Boulder
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EXECUTIVE SUMMARY

The University of Colorado Center for Astrobiology proposes a coherent and integrated plan for research, teaching, and community development in astrobiology. We are located at a major research University, and our approach is to build on the substantial institutional commitments across the entire breadth of astrobiology, to assemble a team of scientists who together span the entire range of disciplines that comprise astrobiology, and to use this group to leverage resources via participation from a larger group of scientists who do research in astrobiology. Our Co-Investigators were included based on their cutting-edge research and their desire to help develop a larger program in astrobiology.

Research. Our research efforts are divided into three distinct themes that provide a structure under which the individual research tasks are organized, spanning the entire range of astrobiology disciplines. In addition, we propose a fourth theme based on the need for astrobiology technology development connected to NASA flight missions.

Theme 1: The origin and evolution of life on Earth. The evolution of life on Earth can be broken down into five qualitatively different processes: (i) Creation of appropriate conditions for a habitable world; (ii) the origin and early development of a genetic code; (iii) the evolution of biochemical pathways; (iv) the evolution of microbial life; and (v) the origins and evolution of multicellular life. We have designed our biology theme to cut across this diverse spectrum in order to examine these fundamental issues in the development of life on Earth. This will provide a more subtle understanding of how life elsewhere in the universe might develop. Our goal is to understand the major processes that governed the development of life on Earth, so that we can provide guidance as to the constraints on environments that will govern our search for habitable planets and life elsewhere in our solar system or on planets outside of our solar system.

The specific research tasks that we are proposing are: (i) Laboratory studies of the origins of an RNA world (led by Co-I M. Yarus). (ii) Evolution of biochemical pathways (led by Co-I S. Copley). (iii) Re-tracing steps towards a habitable world via the biogeochemical evolution of sulfur on the early Earth (led by Co-I S. Mojzsis). (iv) Molecular survey of extremophile microbial diversity in hypersaline ecosystems (led by Co-I N. Pace). (v) Origin of multicellularity and complex land-based ecosystems (led by Co-I W. Friedman).

Theme 2: The origin and evolution of habitable planets. Understanding the potential for life beyond Earth requires applying our knowledge of what allows an environment to be capable of supporting life to our discoveries regarding the various environments that exist within our solar system and beyond. In doing this, we can look at the different environments that exist on planets (and satellites) in our solar system and ask which ones are capable of supporting life, what factors control their habitability, and what processes are responsible for the evolution of suitable characteristics. To understand what makes a planet habitable and what makes a planet non-habitable, we need to look at specific characteristics of individual planets as well as at general characteristics that might apply to all planets. We will approach the problem of understanding planetary habitability by looking in detail at the two objects in our solar system other than the Earth that are most likely to be habitable (Mars and Europa) and by examining more general physical and chemical processes that control habitability. In addition, we will examine the astrophysical processes that govern the formation of planets to begin with.

The specific research tasks that we are proposing are: (i) Biogeochemical cycling and resources on Mars (led by P.I. B. Jakosky). (ii) Geological evolution and habitability of Europa (led by Co-I R. Pappalardo). (iii) The impact of atmospheric aerosols on life (led by Co-I O.B.

Toon). (iv) Geochemical-microbe interactions in chemolithoautotrophic communities on Earth (led by Co-I T. McCollom). (v) Astrophysical constraints on the origins of planetary systems (led by Co-I Bally).

Theme 3: Philosophical and societal issues in astrobiology. Astrobiology is set apart from many other scientific disciplines by its relevance to understanding the ways in which the science interacts with the broader society. Ranging from using astrobiology as a way to better understand the nature of science to exploring the public's fascination with the topic and the societal implications, astrobiology provides a powerful way to better understand these interactions. Our group is one of the few that from the beginning has explored the connections between the sciences and the humanities, and we will continue to do so during the coming five years.

The specific tasks that we will explore are: (i) The nature of historical science and the elucidation of biosignatures from the perspective of philosophy of science (led by Co-I C. Cleland). (ii) Societal issues in astrobiology (led by P.I. B. Jakosky). (iii) The emergence of astrobiological thought from evolution (led by Co-I W. Friedman).

Each of these three research themes will coordinate its activities through a discussion group involving the key research personnel and other interested persons. The purpose of each group will be to bring together the individual research tasks into a coherent program and to provide cross-task interaction that integrates the individual tasks.

Theme 4: Astrobiology technology development. The NASA Astrobiology Institute has little formal connection to the NASA flight missions other than through the activities of its individual members. This is especially problematic with regard to the need to develop technology for instruments and missions that will allow upcoming missions to explore astrobiological problems. We propose to create a technology consortium with nearby Ball Aerospace Corporation and Lockheed Martin Astronautics (LMA) that will bring together scientists and technologists from academia and industry to work together to define the technology needs for astrobiology. This activity will help to create a coherent astrobiology community, will bring the astrobiology science community up to speed on how to design, develop, and implement flight programs, and will serve the goals of the NAI and of NASA by helping to integrate the technology programs into a coherent program. We will hold workshops, run short courses, and write white papers on the technology and science needs, and communicate the results to the community and to NASA Headquarters. Participants who will form the core of a community steering group include scientists from the CU program (led by P.I. Jakosky), senior technologist from the LASP Engineering Division that oversees instrument, spacecraft, and mission development and mission operations (Co-I M. McGrath), senior personnel from Ball (Harold Reitsema and Steve Kilston) and from Lockheed Martin (James Crocker and Ben Clark), and a leader from the biotechnology development area (Collaborator Andrew Steele from Carnegie Institution of Washington).

Education and public outreach. We propose three distinct yet complementary efforts that together comprise a comprehensive program of education and public outreach: (i) We will continue our public symposia in astrobiology at the University of Colorado in order to interact with the local university community and the Boulder-Denver public. These will address diverse aspects of astrobiology, and will bring cutting-edge research to the public along with thoughtful discussion of the broader societal significance. Speakers will be selected from nationally known scientists. (ii) We will take public symposia involving CU astrobiologists to communities and colleges that typically are not able to interact with leading scientists. We will visit 2- and 4-year colleges that do not have

funds to bring in outside speakers, as well as colleges attended predominantly by historically underrepresented groups. These programs, provided at no cost to the receiving institution, will allow students and faculty to interact with leading astrobiologists in both small groups and large venues. We will do three of these per year, with a "warm up" symposium planned for April 2003 at Ft. Lewis College (Durango, Colorado), and have planned tentatively our next symposium for Hampton University in Virginia. (iii) We will educate and inform the public via a series of workshops intended to provide background material to science journalists on issues in astrobiology. The goal is to inform the writers of the deep background of the science issues, so that they can better report on the issues. Our first workshop will be held in December 2003, and will deal with the Mars landings to take place in December and January. Emphasis will be on the missions, the science to be done, and the astrobiological connections.

Astrobiology community development. We are extremely active in helping to develop the astrobiology community. Activities include leadership and participation in the NAI Focus Groups, in senior-level committees that have involvement in and oversight of astrobiology research activities, on the editorial boards of astrobiology-related journals, in NASA flight missions pertinent to astrobiology, and in national and international conferences devoted to astrobiology.

Training. CU has created a number of undergraduate and graduate level classes in astrobiology and its component disciplines. We have instituted a Graduate Certificate in Astrobiology that provides training and recognition for students who are emphasizing one of the component disciplines. We are active in training graduate students and postdoctoral research associates in astrobiology and its component disciplines.

Collaboration and networking. The CU Center for Astrobiology will continue to participate in activities designed to take advantage of the "virtual" aspect of the NAI. We will participate in videoseminars, videoconferencing, and ongoing technology development and implementation activities led by the NAI. We will utilize the videoconferencing equipment for point-to-point discussions and research collaborations with other NAI teams.

Institutional commitment. CU has a commitment to developing the discipline of astrobiology, as seen for example in its hiring of several new faculty in astrobiology during the past four years. In addition, it is committing resources of funding from state funds, matching funds on equipment, Graduate Teaching Assistant support to enhance interactions between astrobiology research and teaching, IT/Tech personnel support, and faculty time to participate in programmatic and scientific issues in astrobiology. Ball and Lockheed Martin are both providing matching support in kind. In total, the dollar and equivalent dollar value of resources contributed by CU, Ball and LMA sum to approximately $1.3 million over five years.

Management. Each Co-I will have authority over funds allocated to them, with oversight provided by the Laboratory for Atmospheric and Space Physics and the University of Colorado. Subcontracts will be made to Ball and LMA to participate in our collaborations. The P.I. has final authority and responsibility over all expenditures, subject to the rules and regulations of the University of Colorado, the State of Colorado, and NASA.

Research and Management Plan

1. INTRODUCTION

1.1. The discipline of astrobiology

Astrobiology as a suite of disciplines has taken on a life of its own (so to speak) in the last few years, driven by major discoveries that point to the potential for life to exist beyond the Earth. On the Earth, these discoveries include the nature of the early environment and the earliest evidence for life, our understanding of diverse life functions, and the wide range of environments that can support life. Within our solar system, we have learned about the occurrence of similar environments on other planets and satellites, the processes responsible for producing the architecture of our solar system, and their implications for formation and evolution of other planetary systems. And discoveries beyond our solar system, of course, include the discovery of planets orbiting other stars and the implications for Earth-size and Earth-like planets.

Astrobiology brings together these otherwise disparate fields into an integrated approach to understanding the boundary conditions surrounding the existence of life. As such, it not only cuts across the components of space science and biological science, but integrates them together in support of addressing a common theme. In this sense, the issues surrounding the possible existence of life elsewhere have become, *de facto*, the intellectual centerpiece of the NASA space science efforts. This can be seen, for example, in the recent National Research Council Decadal Survey on Solar System Exploration, in which understanding the potential and actual distribution of life in our solar system is included as one of the highest level goals of the planetary program, and in the recent NASA Space Science roadmaps produced by Code S, which emphasize the connections of each discipline to astrobiology.

The strength of astrobiology, and of the NASA Astrobiology Institute (NAI), lies in its ability to address the interdisciplinary aspects of the field. In order to make advances in astrobiology, we need to understand not just whether there is life on a particular planet, for example, but also why and how the planet evolved to be able to support life or not, how life interacts with the local and global environment on a planet (including the Earth), and how these same governing processes might play out on other planets and in other planetary systems. This way, we can begin to understand the boundary conditions surrounding life's occurrence, and use them as a guide to determining what the distribution of life throughout the universe is likely to be (e.g.: Drake and Jakosky, 2002). In this context, the major components of astrobiology center on understanding the following (as described, for example, in the recent roadmap for astrobiology):

- The environment on the early Earth and its ability to support life;
- The processes involved in the origin of life on Earth, and the earliest evidence for life on Earth;
- The biological record of early life on Earth, the evolution of life, and the origin of complex life;
- The origin and evolution of our solar system and the nature of the governing processes;
- The environments and potential for life on other planets and satellites in our solar system, and whether life actually exists on any of them;
- The characteristics of extrasolar planetary systems and the implications for understanding the origin and evolution of planetary systems and Earth-like planets; and
- The habitability of extrasolar planets and implications for life beyond our solar system.

Thus, astrobiology requires not only in-depth investigation of problems within each relevant discipline but also interdisciplinary col-

laboration in order to relate each set of issues to the others.

1.2.. University of Colorado approach

As an educational institution, the University of Colorado plays an important role in the broader program. We are in a position to bring together expertise in each of these science areas to focus on the common problem of understanding the nature and distribution of life in the universe, and to create a program of training the next generation of citizens, scientists, and astrobiologists through undergraduate, graduate, and postgraduate programs. Our approach in addressing the science questions in astrobiology is as follows:

- To support research from the breadth of astrobiology, by including as Co-Investigators researchers who, together, span the entire range of disciplines that comprise astrobiology;
- To promote cross-disciplinary interaction, both formally and informally, among the Co-Is and their research groups, and between Colorado participants and other researchers both within the NAI and outside of it, in order to address interdisciplinary and multidisciplinary questions; and
- To use the Co-Is and the program as a means of connecting to a larger group of faculty and researchers both locally and nationally who are involved in astrobiology-related research, in order to leverage the NAI support into a much larger program.

Using these concepts as broad guidelines, we have developed a research program to take best advantage of the university setting and expertise. Our astrobiology program was initially built five years ago on the cornerstones of the existing University strengths that also are cornerstones in astrobiology — RNA chemistry and evolution, planetary science, and astrophysics. We added expertise (including new faculty hires) in biochemistry of metabolism, microbial ecology and life in extreme environments, the geological record of ancient terrestrial life, and the humanities. In addition, for this proposal we are adding expertise in the complementary areas of the outer solar system and terrestrial and planetary biogeochemistry. Our approach of breadth rather than focusing our resources on addressing a single problem takes advantage of the strength of being in a university environment, and is complementary to the approach taken by individual research groups elsewhere or at national laboratories.

Our approach to developing our proposed research is as follows:

- To focus research on distinct science themes in order to maximize cohesiveness and collaboration;
- To “evolve” our program, rather than automatically continuing the same activities, by including new Co-Is and new research tasks (e.g., five of our eleven science Co-Is have been added since we began our efforts five years ago); and
- To include an additional theme, one that deals with astrobiology technology development in support of upcoming space flight missions, in order to help the NAI and the astrobiology community as a whole develop a stronger connection to the flight programs.

Through this approach, the University of Colorado will continue to build a substantial program in astrobiology that recognizes the unique, coherent, and integrating nature of astrobiology while at the same time fostering the connections to a much broader research program than can be funded through the NAI. We expect that we will continue to contribute in significant ways to the overall efforts within the NAI and to the broader national effort in astrobiology.

1.3. Science and technology themes

We are proposing tasks that take advantage of the expertise of each individual Co-Investigator and that fit together into distinct science themes. These themes link closely related tasks, allow each group to address complex problems that span a range of topics, and provide the guiding structure to our research program. In addition, we propose a fourth theme involving technology development that allows us and the NAI to address the central issue of the connections between astrobiology and the flight programs. These themes are described briefly here and then expanded on in the remaining research portion of the proposal.

The four themes are:

(i) The origin and evolution of life. Life on Earth is the only example we have of life in the universe, and we use it to understand the nature of life, its history, its interactions with its local environment that drive ecosystem evolution, and how it can inform our view of what makes a planet habitable or non-habitable. We propose five distinct tasks that fall under this theme and make substantial contributions to our overall understanding. These tasks address the behavior of RNA as a catalytic agent and the nature of the RNA World that likely preceded our present DNA world; microbial diversity and ecology in a complex ecosystem (the Guerrero Negro hypersaline ecosystem in Baja California) in order to understand the general character of interactions between organisms and their environment and the processes of ecosystem evolution; the nature of evolution at the biochemical level and, in particular, gene duplication and protein functions in archaea; evolution at the macroscopic level and implications for the development of complex ecosystems either here on Earth or around other stars; and the characteristics of the Earth's early environment and biosphere and of their mutual interplay.

(ii) The origin and evolution of habitable planets. With an understanding of the characteristics that an environment must have in order to be able to support life, we can ask where in our solar system these conditions might be met and how the processes that control planetary formation and evolution operate, both in our own solar system and in other planetary systems. From the more specific to the more general, the tasks within this theme include determining the extent to which Mars has had habitable environments and the geochemical constraints that we can place on possible martian life and ecosystems; the extent to which Europa has evolved over time, and implications for the habitability of its (probable) global subsurface oceans; studying the physical, chemical, and radiative effects of atmospheric aerosols and their ability to control the evolution of climate on terrestrial planets (including the early Earth); exploring the biogeochemistry of environments on Earth that can support chemolithoautotrophs, with implications for the potential for life to exist on Mars, Europa, or elsewhere; and determining the ability of planetary systems to form in "hazardous" stellar environments, using direct observations of forming systems.

It is not surprising that some of the tasks proposed do not separate cleanly into either the "biology" or the "planetary" theme, but overlap both. This is apparent in the tasks dealing specifically with interactions between terrestrial organisms and their environments. It results from the fact that the Earth is a planet, too, and that we need to understand terrestrial environments and their interactions with microbes in order to understand how planetary environments might be capable of supporting life.

(iii) Philosophical and societal issues. One aspect of astrobiology that separates it from many other science disciplines is the tremendous public interest in it, and this interest allows us to use astrobiology to explore the connections between science and society. This includes various philosophical and societal issues, and our group is one of the few groups that has been

exploring these themes by including participation from the humanities as an integrated component of our program. We are proposing three tasks that explore these issues: the philosophy of science issues in astrobiology, the nature of defining life, and the implications for life-detection strategies; the societal impact of astrobiology, including in particular the reasons behind the tremendous public interest in life elsewhere; and the history of evolutionary thought ("the evolution of evolution") and the connections between early evolutionists and astrobiological thought.

(iv) Astrobiology technology development initiative. The NAI exists in part to "provid[e] scientific and technical leadership on astrobiology investigations for current and future space missions and for ongoing research programs" [quoted out of the NAI mission statement]. However, there has been minimal connection between the NAI and advance planning for flight programs except through the separate efforts of individual members. We are proposing an astrobiology technology development initiative, in which we will involve scientists and technologists from academia and industry to provide leadership to the astrobiology community in this area. We propose to create an oversight group on astrobiology technology development within the structure of the NAI focus groups, and to use this as a vehicle by which to organize community workshops on technology, to offer short courses for astrobiologists and technologists, and to work with NASA to galvanize the community and provide direction to technological development. In order to reach beyond the existing players in astrobiology technology and to increase "P.I. savvy", we are involving members of industry as key participants in our efforts, and bringing together within a single forum the necessary science, instrument, spacecraft, mission, and biotechnology leadership.

2. THEME 1: ORIGIN AND EVOLUTION OF LIFE

The evolution of life on Earth can be broken into discrete and qualitatively distinct processes: (i) Creation of appropriate conditions for a habitable world; (ii) the origin and early development of a genetic code; (iii) the evolution of biochemical pathways; (iv) the evolution of microbial life; and (v) the origins and evolution of multicellular life. We have designed our biology theme to cut across this diverse spectrum in order to examine these fundamental issues in the development of life on Earth. This will allow us to gain a more nuanced understanding of how life elsewhere in the universe might develop. Our explicit goal is to understand the major processes that governed the development of life on Earth, so that we can provide guidance as to the constraints on environments that will govern our search for habitable planets and life elsewhere in our solar system or on planets outside of our solar system. We will focus on broad aspects of the problems faced by life on Earth at a variety of different times in its development. This will provide the greatest potential to elucidate key hurdles that life had to "overcome" as well as to provide details on how these key evolutionary transitions might have taken place on Earth or elsewhere in the universe.

Our specific tasks are as follows, with each being described in detail in the remainder of this section.

(i) Laboratory studies of the origins of an RNA world (led by Co-I Yarus). We will experimentally explore the properties of the RNA molecule that might have allowed it to develop the ability to catalyze chemical reactions and thereby led to an RNA world. To do this, we will determine the utility of short sequences of RNA that might have allowed much smaller numbers of molecules to serve as the original source for chemically reactive molecules, explore the nature of the active region of a particular ribozyme, and examine the interactions of RNA molecules with mineral surfaces that

might have acted as efficient concentrators to increase reactivity.

(ii) Divergence of protein families to provide novel functions (task led by Co-I Copley). A key process that enabled the evolution of the complex biochemistry typical of modern microbes was gene duplication, followed by mutations that allowed development of a new function in the protein encoded by one copy. Thioredoxins are ancient proteins that are found in all forms of life and therefore probably existed in the last common ancestor. They provide an excellent system for studying the recruitment of ancient proteins to serve new functions via gene duplication and evolution. We will examine thioredoxins in a hyperthermophilic, sulfate-reducing member of the domain Archaea, in order to understand the evolution of multiple proteins from a single ancestral protein.

(iii) Re-tracing steps towards a habitable world: The biogeochemical evolution of sulfur on the Early Earth (task led by Co-I Mojzsis). We use the geological record of the Earth to understand the evolution of planetary habitability, focusing in particular on the information that can be derived from sulfur isotopes as a constraint on biogeochemical processes. Sulfur isotopic measurements provide detailed information on mass-independent fractionation processes involving anoxic atmospheres. We will continue our analysis of sulfur from key locations in Precambrian terranes, in order to provide key details on the abundance of oxygen at the time that the sulfur-bearing minerals were deposited. This history of oxygen is important for understanding the evolution of planetary climates and habitability.

(iv) Molecular survey of extremophile microbial diversity in hypersaline ecosystems (task led by Co-I Pace). Because of their relative stability and comparative simplicity, hypersaline mats have become models for studies of chemical and energetic factors that support photosynthesis-driven communities. The principles derived from such studies are pertinent to planetary ecology, which is the summation of many such systems, and to the nature of life in extreme environments. We will continue our detailed study of the Guerrero Negro hypersaline mats as a model extreme ecosystem, surveying the microbial makeup and organization of selected hypersaline mats using culture-independent molecular methods based on cloning and sequence analysis of ribosomal RNA (rRNA) genes.

(v) Origin of multicellularity and complex land-based ecosystems (task led by Co-I Friedman). We plan to significantly enlarge the scope of our molecular investigations to investigate the role of symbioses associated with the establishment of photosynthetic multicellular life on land. Recent paleontological evidence suggests that the one-time colonization of land-based environments by plants may have required a key symbiosis between plants and fungi (in essence a cooperative relationship between photoautotrophs and heterotrophs). Our goal is to sample the fungal symbionts of representative members of all living plant lineages whose origins can be traced to the first one hundred million years of multicellular plant life on land. The results will provide a better understanding of the establishment of complex ecosystems as they have evolved here on Earth and that might populate Earth-like planets around other stars (and thereby be suitable for detection from Earth).

2.1. Laboratory Studies of the Origins of an RNA World (led by Co-I M. Yarus)

2.1.1. Introduction and premise.

There is only one current idea for the succession from an earlier Earth biota that commands significant experimental evidence. That idea is the RNA world — the notion that the present kind of life on Earth was preceded by simpler creatures that used only RNA (ribonucleic acid or some molecule that resembles it)

for everything that modern cells do using protein, DNA, and RNA. While there is much data supporting these biological events (despite the fact that they probably occurred ca. 4 x 10^9 years ago), data that would apply to the beginnings of life in other settings also, the main difficulty lies in seeing how RNA molecules for the RNA cells (ribocytes) could themselves have originated. Below, we summarize several areas we will pursue during the period of this proposal which bear on the origins of the primordial ribocytes and its RNAs. A successful conclusion to these experiments would define what sorts of RNA molecules must have been present in an early-life setting, how the catalysts needed for RNA cells could have been found among them, and in particular how the RNAs needed for the first translation system might have arisen.

2.1.2. Studies of the minimal evolutionarily useful RNA populations.

RNAs are the simplest molecules currently known to catalyze reactions and replicate themselves. This makes them logical candidates for catalysis and replication in early cells. However, RNAs are large and their synthesis is difficult, particularly under prebiotic conditions. The relevant question then is: how much of this rare species is actually needed? In other words, what is the smallest RNA population that can serve for the isolation of new RNA activities? Clearly the smaller the magnitude of the answer, the earlier during life's history on Earth and the more likely it is that RNA organisms could have become possible. Our first potentially realistic calculations of the amount of RNA (the number of molecules of arbitrary sequence) needed to evolve particular ribozymes like those that would have been required by the early RNA cells (ribocytes) suggested that the number of random-sequence RNA molecules required is anti-intuitively small (Yarus and Knight, 2002; Knight and Yarus, 2003). Zeptomoles of RNA molecules (from 1 zmol = 602 molecules to 1000 zmol [1 amol], 602,000 molecules), less than in a modern bacterium, might suffice to begin accumulating RNA activities (the "zeptomole world" hypothesis). This range begins 12 orders below the nanomole levels used in modern selection experiments. Thus, an RNA world could be up to 12 orders more accessible than would have been thought from modern selection experiments. Alternatively, we might express the result by saying that the problem of prebiotic synthesis of nucleic acids could be up to 12 orders less severe than might have been thought from a survey of modern experiments.

Our proposed work will extend these findings, which bear significantly on the initiation of an RNA world. First, we will extend our mathematical and statistical studies by performing experiments intended to confirm or correct them. We necessarily predict that the methods of selection-amplification, in which RNA active sites are isolated from experimental populations of molecules of randomized sequence, can be used to isolate RNA catalysts from zmol of independent sequences instead of the nmol usually used. This would confirm the existing analytical calculations (two independent methods come to similar numerical conclusions; see Yarus and Knight, 2002, and Knight and Yarus, 2003) as well as the computer simulations (searching *in silico* through short computer-generated randomized sequences) that agreed with the theory.

The experiments are selection-amplifications or SELEX protocols, using decreasing numbers of independent sequences to isolate a given RNA structure. We propose to isolate the isoleucine-binding RNA we have called the UAUU motif (Majerfeld and Yarus, 1998), which is simple, well characterized, and has an interesting relationship to the code for isoleucine (reviewed in Yarus, 2002). The goal of the experiment is to show whether the isolation of the isoleucine binding site continues down to zmol of RNA molecules. One thousand zmol however, is so few molecules (6.02 x 10^5)

that it is difficult to handle such pools of individual molecules without having them disappear, say by irreversible adsorption onto the walls of the plastic tubes used for experiments. Therefore, the pools will contain normal total numbers of RNA molecules (e.g., 10^{14}) but with each sequence replicated 10^8 times; thus one can have an initial amount of RNA that is practical to handle though it only contains zmol of different sequences.

Successful experiments would make an RNA world a much more plausible event in the Earth's history and in the possible history of life elsewhere. These experiments might require the full attention of a postdoc and graduate student for 2 years.

2.1.3. Poisoning.

How is it possible that no one has noticed that modern selections employ 12 orders of magnitude too many RNA molecules? There may be an unappreciated 'poisoning' phenomenon that has hidden the true nature of selection. The essence of this is that molecules with too many randomized positions actually inhibit the folding of potential active sites within themselves. Imagine a small sequence (the potential active site) in the midst of a much larger arbitrary nucleotide sequence (the rest of the randomized region). The small active site can be poisoned by the accidental ability of the surrounding nucleotides to pair with it and thereby alter the folding of the potentially active sequence. Thus, many possible active RNAs are not detected, and the larger the randomized sequence, the worse the problem. Accordingly, an immense number of molecules must be used to survey many that have a potential active sequence, in order to find the rare example that has an active site that will fold unmolested. The result is that experiments have greatly overestimated the numbers of molecules required in a selection, and thereby greatly underestimated the capabilities of RNA. This effect was noticed by Sabeti et al. (1997), but we believe was greatly underestimated.

We therefore propose computational experiments in which RNAs containing the subsequences required for an active isoleucine binding site are mostly composed of arbitrary sequences to see how often folding of the isoleucine site is preserved. Some such sequences can be synthesized by transcription and experimentally tested for isoleucine binding by isoleucine-Sepharose affinity chromatography, in order to confirm the validity of the conformational calculations. However, if the calculations are shown to be relatively accurate, cases can be computed much faster than experiments can be done, and a more refined understanding of the magnitude and conditions of poisoning can be reached. Active sequences become more probable if there is a bigger sequence neighborhood to search for them; however, bigger random sequences are more likely to poison their contents. Thus, it is likely that there is an optimal size for the randomized molecule used to search for a new activity. Therefore, these experiments should illuminate the size of the randomized sequence required for selection of primordial RNAs as well as the modern practice of SELEX.

These computations and related experiments are the work of about 6 months to one year for two persons, postdoctoral or graduate student.

2.1.4. New transition state analogues for the peptidyl transferase.

During the previous funding cycle, our lab helped Nissen et al. (2002) to establish that the ribosome is a ribozyme, making peptide bonds using an RNA catalyst that probably dates to the RNA world itself (Nissen et al., 2000). Our contribution was in devising a transition state analogue, CCdApPuromycin, which bound specifically to the ribosome's active site. CCdApPuro thereby identified the ribosomal elements involved in making peptide bonds. To better understand this RNA patrimony from the Earth's

early history, new transition state analogues will be tested and supplied to crystallographers studying the structure of ribosomes and, particularly, peptidyl transferase. In particular, there is evidence that the dA of CCdApPuro, which is an unnatural deoxyribose sugar right at the point of reaction, would be better replaced by a ribose in CCApPuro.

We therefore propose the synthesis and characterization of CCApPuro, particularly with regard to whether it fits the peptidyl transferase active site better than does the original transition state analogue. These experiments would involve studies of binding to the peptidyl transferase center, as well as crystallography to define the position of the compound within the ribosome. Synthesis and biochemical characterization of CCApPuro will require one to two work years of effort by a postdoctoral level bioorganic chemist.

2.1.5. RNA interactions with mineral surfaces.

Having reduced the likely magnitude of the RNA abundance problem by up to 12 orders, we propose to virtually eliminate it as a salient concern. Our hypothesis is that the final step of the solution to RNA abundance is chromatography on natural minerals. That is, we expect that certain plausible Archean minerals (obtained and manipulated in collaboration with our geochemical colleague Steve Mojzsis) will adsorb and release RNA under different likely early Earth conditions. This will produce the well-known effects of RNA chromatography — concentration, stabilization and purification.

Concentration — RNA can be adsorbed from a large volume and released in a small volume, thereby harvesting the RNA synthetic efforts of a bigger natural reactor. This is the primary effect of adsorption of RNA, that it can be concentrated from a large volume and made more available. However, this primary effect is probably supported by other consequences of chromatographic adsorption. For example, Stabilization — adsorbed RNA can be much more stable than RNA free in solution because internucleotide bonds of adsorbed RNA cannot rotate into conformations favorable to hydrolysis, again boosting the concentration of available RNAs. RNA could therefore have appeared over a long period and been preserved on a mineral 'sink'. Purification — Some RNAs should adsorb better or elute better or both, thereby partially purifying the necessarily heterogeneous products of prebiotic chemical nucleic acid synthesis. Clearly, a potential combination of these three chromatographic effects could deliver unexpectedly concentrated and somewhat purified populations of RNA to hypothetical early ribocytes.

Therefore we propose the laboratory synthesis of pure samples of the following common cationic clays:

Kaolinite, $Al_4[Si_4O_{10}]\ (OH)_8$
Illite, $K_{1\text{-}1.5}\ Al_4[Si_{6.5\text{-}7}Al_{1\text{-}1.5}O_{20}]\ (OH)_4$
Smectite, $Ca_{0.5}\ Na_{0.7}\ (Al, Mg, Fe)_4\ [(Si, Al)_8\ O_{20}]\ (OH)_4.nH_2O$

and the study of the adsorption and elution of RNA molecules therefrom, and of the stability of bound RNA. Methods for elution should be simple and potentially prebiotic, such as temperature, high ionic strength (e.g., via evaporation), low ionic strength (e.g., via rainwater) or chelation (e.g., by oligophosphates). An elaboration of this project would be to use selection-amplification to isolate particular RNA molecules that bind and/or elute from the synthetic clays to see if particular sequence classes or structural types might have been accumulated in this way.

These studies have an inevitable uncertainty because of the exploratory element, in that synthesis of the materials must be optimized and a list of clays and chemical possibilities for adsorption and elution must be explored. Therefore we are estimating that this may require 3

person-years to reach an interesting point, with a postdoctoral and a graduate student as possible experimentalists. A clear success would illustrate how infrequent, dilute natural synthesis may have nevertheless yielded concentrated RNA populations that would have supported the emergence of a ribocyte. In addition, such experiments might help design an effective search for biological or protobiological materials in other planetary environments; e.g., on Mars.

2.2. Divergence of Function in an Ancient Protein Family: Thioredoxin-like Proteins in *Archaeoglobus fulgidis* (led by Co-I S. Copley)

2.2.1 Introduction

The diversification of microbial life on the early Earth involved an extraordinary expansion of the rudimentary capabilities of the first living organisms. Catabolic pathways evolved that allowed organisms to exploit a variety of sources of carbon and other nutrients. Biosynthetic pathways evolved that allowed synthesis of building blocks for cellular macromolecules. Elaborate control mechanisms evolved to allow rapid responses to changing environmental conditions. A key process that enabled the evolution of the complex biochemistry typical of modern microbes was gene duplication, followed by mutations that allowed development of a new function in the protein encoded by one copy. The extent of this phenomenon is vast. While there are over 18 million gene sequences in GenBank, the estimated number of distinct protein folds is only 10,000 (Koonin, 2002). Clearly, the adaptation of existing protein folds to serve new functions has been a dominant theme in the evolution of life.

Thioredoxins (Trxs) are ancient proteins that are found in all forms of life and therefore probably existed in the last common ancestor. The Trx fold is very versatile and has been adapted for many purposes as life has become more complex. Extant organisms contain multiple Trxs and Trx-like proteins. Although some of these proteins have very specific functions, others have retained overlapping functions. Thus, the divergence of Trxs has provided new functions that contribute to the metabolic and regulatory complexity of the organism, while maintaining some functional redundancy that protects organisms from deleterious mutations in critical genes.

The functions of Trxs have been studied primarily in bacteria and eukaryotes. Trxs serve as reductants for several enzymes involved in metabolic pathways and in defense against oxidative stress. These include ribonucleotide reductase, arsenate reductase, 3'-phosphoadenylyl-sulfate reductase, methionine sulfoxide reductase, and peroxiredoxin (Follman and Häberlein, 1995/1996). Eukaryotes have recruited Trxs to serve regulatory and signaling functions, as well (Follman and Häberlein, 1995/1996; Nishiyama et al., 2001). In chloroplasts, Trxs activate enzymes involved in the Calvin cycle (which incorporates CO_2 into carbohydrates) in response to light. Trx regulates several transcriptional regulators that control gene expression in response to changes in redox conditions. Trx also interacts with apoptosis-regulating kinase, which is involved in the signaling cascade that initiates programmed cell death. In multicellular organisms, Trxs have been recruited to serve as cytokines (chemical messengers that allow cells to communicate with each other) in the extracellular milieu.

Trxs provide an excellent system for studying the recruitment of ancient proteins to serve new functions as life became more complex and organisms diversified to occupy different ecological niches. An enormous piece of the puzzle is missing, however, because we know almost nothing about the functions of Trxs in Archaea. Studies of Trxs in Archaea will help us identify functions that are shared with bacteria and eukaryotes, and thus may have derived from the last common ancestor, and will also identify functions that have developed within the Ar-

chaeal domain in response to selective pressures experienced by Archaea in their particular environmental niches, which include some of the most extreme environments on the planet. We propose to study the functions of Trxs in *Archaeoglobus fulgidus,* a hyperthermophilic, sulfate-reducing Archaeon that is capable of growth on a variety of carbon and energy sources, as well as autotrophic growth on hydrogen, thiosulphate, and carbon dioxide (Klenk, et al., 1997). Homologues of several enzymes that are reduced by Trxs exist in *A. fulgidus*. However, we expect that Archaea in general and *A. fulgidus* in particular may have recruited Trxs to serve functions that have not been observed in bacteria or eukaryotes.

The aims of the proposed work are:

1. To identify protein targets that are reduced by the six Trx-like proteins in *A. fulgidus*;

2. To use bioinformatics coupled with an understanding of the physiology and metabolism of *A. fulgidus* to suggest functions for these target proteins; and

3. To purify the target proteins, test their functions *in vitro*, and confirm that the Trx-like proteins can reduce the target proteins.

2.2.2. Experimental Plan

Aim 1. Trxs use two active site cysteine (Cys) residues to transfer reducing equivalents to a variety of targets. These include a) enzymes in which a disulfide bond formed between two Cys residues during catalysis must be reduced in order to complete the catalytic cycle, b) proteins that are activated by reduction of a disulfide bond, and c) disulfides that are formed as a consequence of oxidative damage. The mechanism of these reactions is shown in Figure 2.2-1. A clever approach to identifying the targets of Trxs has recently been described (Motohashi et al, 2001). This approach uses immobilized mutant proteins that lack the second Cys required for completion of the thiol-disulfide exchange reaction to trap target proteins (see Figure 2.2-2). The target proteins become covalently attached to the Trx because the second Cys is not available to release them. The target proteins can be released by treatment with a small thiol reagent. We will use this procedure to separate target proteins from cell extracts of *A. fulgidus*. The released target proteins will be separated by gel electrophoresis. The bands containing each target protein will be cut out and the proteins will be digested with a protease in the gel slice (Rosenfeld et al., 1992). The masses of the resulting peptide fragments will be determined using MALDI-TOF (matrix-assisted laser desorption ionization – time-of-flight) mass spectrometry. Comparison of the fragments produced with those expected from proteins in the *A. fulgidus* genome will allow us to identify the genes encoding each of the target proteins. We will carry out these studies for each of the six Trx-like proteins in *A. fulgidus*.

Aim 2. The next step in our analysis will be prediction of function for the target proteins identified in Aim 1. *A. fulgidus* contains 2420 protein-encoding genes; functions have been predicted for about 45% (Klenk et al., 1997). Thus, we are likely to find target proteins for which no function has yet been predicted. For these proteins, we will attempt to predict function using a combination of clues. Information available at the National Center for Biotechnology Information (NCBI) includes a) assignments to groups of orthologous proteins in the COGS (clusters of orthologous groups) database (1930 proteins); b) identification of conserved domains (1531 proteins); and c) sequence similarity to proteins of known structure (778 proteins). We will supplement this information with searches for distant relatives with known functions using the Shotgun algorithm (Pegg and Babbitt, 1999) and motif analyses for identification of conserved regions using the MEME algorithm (Bailey and Elkan, 1994). We will incorporate knowledge of the genomic context of the gene, since genes involved in similar functions are often clustered in *A. fulgidus*. Fi-

nally, we will use Northern analysis (Ausubel et al., 1995) to measure mRNA levels observed in cells grown under various conditions to reveal the circumstances under which the gene is expressed. We have previously predicted and verified the function

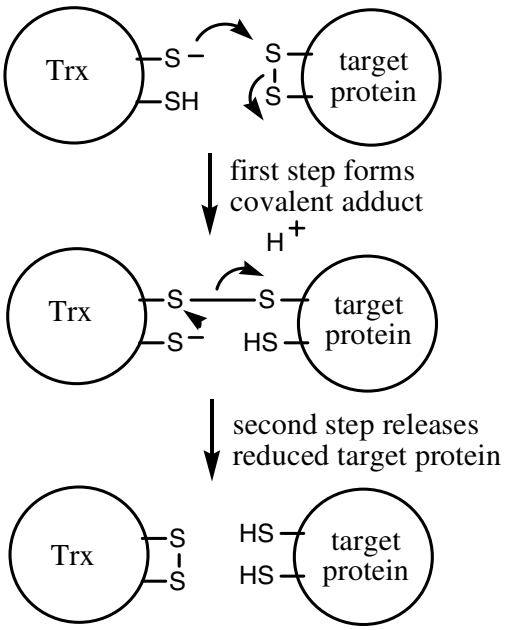


Figure 2.2-1. Transfer of reducing equivalents from the sulfhydryls (-SH) of the active site cysteines of thioredoxin (Trx) to oxidized target proteins. The oxidized Trx is subsequently reduced by Trx reductase.

of several bacterial genes, so this effort is within our area of expertise. We expect to be able to predict functions for approximately 80% of the target proteins identified in Aim 1.

Aim 3. Our next step will be to purify target proteins and verify both their functions and the nature of their interactions with Trx-like proteins. Each target protein will be over-expressed in *E. coli* using standard techniques (Ausubel et al., 1995). Proteins from hyperthermophiles can often be purified from *E. coli* cell extracts simply by heating the cell extract, causing the *E. coli* proteins to denature and precipitate. After removal of the denatured proteins by centrifugation, the desired *A. fulgidus* protein can be further purified if necessary by standard procedures such as ion exchange chromatography and size exclusion chromatography. (If necessary, we will use affinity-tagged proteins. However, af-

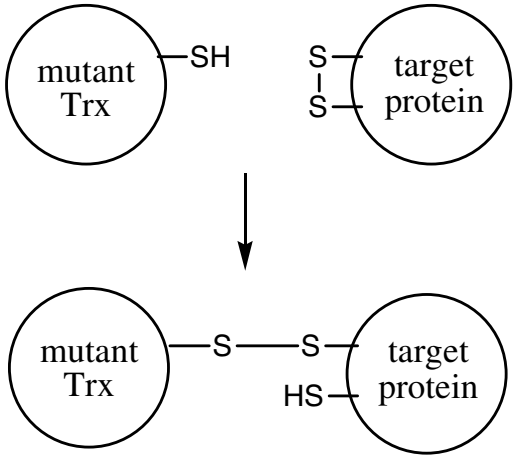


Figure 2.2-2. Trapping of target proteins by mutant Trxs that lack the second Cys at the active site.

finity tags sometimes interfere with catalytic activity and protein-protein interactions, so we prefer to avoid them.)

For each target protein, we will set up an assay to quantify activity and the effect of Trxs. We will use literature procedures when available, and devise our own procedures when necessary. Dr. Copley has 20 years of experience in designing and executing enzyme assays, so we do not expect this step to be a problem.

Trxs are expected to play a role in either activation of the target proteins (by a one-time reduction of a disulfide that converts the protein from an inactive to an active form), or in substrate turnover (by providing reducing equivalents during every catalytic cycle). We will differentiate between these possibilities by carrying out assays in the presence of a) stoichiometric amounts of reduced Trxs, and b) catalytic amounts of Trxs and a regenerating system consisting of Trx reductase and NADPH (which will convert oxidized Trx to reduced Trx capa-

ble of interacting again with the target protein). If time permits, we will compare the abilities of different Trx proteins to interact with the target proteins so that we can assess both the specialization of certain proteins and the degree of functional redundancy in the system.

2.2.3. Relevance to Astrobiology

These studies are relevant to astrobiology for several reasons. First, they will flesh out the emerging picture of how Nature has modified the simple and ancient Trx-fold to serve a variety of metabolic and regulatory purposes as life became more complex. Second, greater understanding of the functions of Trxs in Archaea will supplement existing knowledge of their functions in bacteria and eukaryotes and consequently provide insights into the biochemical and physiological properties of the last common ancestor. Finally, these studies will enhance our understanding of metabolic pathways, gene regulation, and response to oxidative stress in *A. fulgidus*, a thermophilic sulfate-reducing Archaeon that is a valuable model for life in extreme environments.

2.2.4. Plan of Work

Experimental work will be carried out by a graduate student and a post-doctoral associate. Dr. Copley will supervise and train the graduate student and post-doc and carry out the bioinformatic tasks.

Year 1 – Clone the 6 Trx-like proteins from *A. fulgidus*; use site-directed mutagenesis to change the second active site Cys to Ser; express, purify, and immobilize each mutant protein

Year 2 – Isolate target proteins from *A. fulgidus* and characterize by mass spectrometry; use bioinformatics and transcriptional profiling to predict their functions

Year 3 – Clone and express genes for target proteins in *E. coli* and purify target proteins

Year 4 – Develop assays for approximately 5 target proteins; quantify activity and define role of Trx-like proteins

Year 5 – Continue Year 4 activities for an additional 5 proteins; if time permits, examine abilities of all 6 Trx-like proteins to interact with target proteins to identify overlapping target ranges

2.3. Re-tracing Steps Towards a Habitable World: The Biogeochemical Evolution of Sulfur on the Early Earth. (Research task led by S.J. Mojzsis)

2.3.1. Introduction.

A key element in the further success of astrobiology will be to use the terrestrial geologic record as a baseline for understanding the origin and evolution of a habitable world. Indeed, this knowledge is critical in developing plans for astrobiology-focused space missions such as the search for Earth-like planets elsewhere in the universe. Towards this aim, our research group seeks to constrain the duration and magnitude of the switchover from an essentially anoxic ($pO_2<10^{-5}$ present atmospheric level; PAL) to oxygen-rich (>0.1% PAL) planet between ~2.4 and 1.9 billion years ago (2.4–1.9 Ga) using the mass-independent behavior of sulfur isotopes. This pivotal era in Earth history is termed the "Great Oxidation Event" (GOE; Holland, 1984). What were the biogeochemical mechanisms for its advent? How did life respond to such a marked rise in free O_2? The rise in free oxygen on Earth led to the rise of a biosphere dominated by aerobic multicellular life. This life ultimately developed the ability to investigate its own origins. How long does it take an Earth-like world to reach this state?

To explore the record of the GOE, we exploit the fact that sulfur isotopes can exhibit mass-independent (as opposed to mass-dependent) fractionation behavior, thereby revealing unique information about past atmospheric oxygen levels and past metabolic styles of sulfur-utilizing organisms. We will undertake an extensive program of high-resolution

multiple sulfur isotope measurements of sulfides preserved in rocks from the GOE; the new sulfur isotope technique described herein has been developed specifically for this study (Mojzsis et al., 2003).

2.3.2. Sulfur isotope variability in nature.

Sulfur isotopes (^{32}S, ^{33}S, ^{34}S and ^{36}S) have long been recognized as a powerful tool for deciphering geobiological processes on Earth (Thode et al., 1949). Sulfur isotopes can be used to follow the evolution of sulfur metabolisms (Monster et al., 1979; Canfield and Raiswell, 1999; Shen et al., 2001) and to explore fundamental interactions between chemical reservoirs from the dawn of the rock record, such as the rise of free oxygen in the atmosphere (Berner and Petsch, 1998; Farquhar et al., 2000, 2001).

Fractionation of sulfur isotopes ($^{34}S/^{32}S$) and ($^{33}S/^{32}S$) is expressed in the conventional δ notation (per mil; ‰) as:

$$\delta^{34}S_{CDT}\ (‰) = [(R^{34}_{sample} / R^{34}_{CDT}) - 1] \times 1000$$

$$\delta^{33}S_{CDT}\ (‰) = [(R^{33}_{sample} / R^{33}_{CDT}) - 1] \times 1000$$

where $R^{34} = {}^{34}S/^{32}S$, $R^{33} = {}^{33}S/^{32}S$, and the standard is Cañon Diablo troilite (CDT). On Earth, these values exhibit a range near 0‰ for igneous (0±5‰) and hydrothermal (0±10‰) rocks and ore bodies (Ohmoto and Rye, 1979). In contrast, biological effects have been invoked to account for the >150‰ range in $\delta^{34}S$ observed in sediments (Nielsen, 1979).

Plots of $\delta^{33}S/\delta^{34}S$ yield a highly correlated and mass-dependent array defined by the relationship $\delta^{33}S = 0.515\ \delta^{34}S$ (Bigeleisen et al., 1947; Hulston and Thode, 1965). The slope (0.515) in $\delta^{33}S$ vs. $\delta^{34}S$ arises from the mass dependence of vibrational frequencies, velocity terms, kinetic effects, diffusion, evaporation/condensation and metabolic fractionations. These processes fractionate according to the relative mass differences of the isotopomers such that $\delta^{33}S/\delta^{34}S$ variations define a slope of ~0.5 because of the ratio of the mass difference between ^{33}S and ^{32}S (1 amu) and ^{34}S and ^{32}S (2 amu) or about 1/2.

2.3.3. Mass-independent sulfur isotope effects – atmospheric and biological implications.

Some mass-*independent* fractionation (MIF) processes exist that result in anomalous 3-isotope values that do not describe the slope $\delta^{33}S/\delta^{34}S = 0.515$. MIF in sulfur is expressed as: $\Delta^{33}S = \delta^{33}S - 0.515\ \delta^{34}S$ when $\Delta^{33}S \neq 0$. Only certain gas phase reactions in the atmosphere, or nuclear processes as recorded in meteorites, produce MIF (Thiemens, 1999). UV SO_2 photolysis experiments in the gas phase result in both anomalous $\Delta^{33}S$ values and fractionations in $\delta^{34}S$ rivaling bacterial sulfate reduction (Farquhar et al., 2000; Farquhar et al., 2001), but only under *very low* pO_2 conditions.

2.3.4. Hypothesis – MIF can be used to constrain ancient terrestrial atmospheric oxygen levels.

Quantitative constraints on Archean pO_2 levels have long been sought. The current paradigm is that the free oxygen levels were much lower ($pO_2 << 10^{-2}$ PAL) before *ca*. 2.3 Ga (Cloud, 1972; Walker, 1977; Walker et al., 1983; Holland, 1984, 1994, 1999; Kasting, 1992, 1993, 2001; Rye et al., 1997; Rye and Holland, 1998; Farquhar et al., 2000; Pavlov and Kasting, 2002). An alternative view argues that the atmosphere was relatively oxygen-rich throughout the Archean (Dimroth and Kimberley, 1976; Towe, 1990; Ohmoto, 1996, 2001; Watanabe et al., 1997). A major review of the geological record of atmospheric evolution (Holland, 1984) noted several pieces of evidence as important for establishing the case for an anoxic atmosphere before ca. 2.3 Ga, highlighting in particular the study of detrital gold, uraninite and pyrite grains in ancient sediments (cf. Barnicoat et al., 1997). Other geochemical

indicators such as (Fe^{2+} vs. Fe^{3+}) in paleosols (Holland, 1994; Rye and Holland, 1998; Rasmussen and Buick, 1999), arguments regarding the origin and possible mode of deposition of banded irons (Cloud, 1968), of isotopically light carbon isotope signatures in Precambrian organic matter (DesMarais, 1997) and isotopically heavy carbon in carbonates (Karhu and Holland, 1996) have been used to support models for an early anoxic atmosphere (cf. Ohmoto, 1999).

MIF behavior of sulfur provides three independent constraints on past pO_2.

(1) Separate mass-dependent arrays (*slope* ≈ 0.5) within Archean MIF $\delta^{33}S/\delta^{34}S$ data argue against effective pyrite oxidation and homogenization of both reduced sulfur and (oxidized) sulfate reservoirs either by biological cycling or inorganic processes. This supports conclusions based on paleosol and detrital mineral data cited above that pO_2 was $\leq 10^{-4}$ PAL and marine sulfate was of limited availability prior to ~2.3 Ga.

(2) Experiments delimiting the range of conditions for 190- to 220-nm (UV) SO_2 photolysis in the lower atmosphere and the lifetime of this reaction under various parameters succeed in producing large MIF signatures (Farquhar et al., 2001) mimicking those observed in ancient sulfides. This poses a problem for interpreting large sulfur isotope fractionations as exclusively due to biology. Experiments imply that atmosphere column abundances of O_3 and O_2 were so low that 190–220 nm UV could penetrate deep into the pre-2.3 Ga atmosphere. Anomalous $\Delta^{33}S$ can thus be used to place firmly quantitative upper limits to Archean pO_2 of ~ 10^{-1}–10^{-2} PAL.

(3) The only fractionation mechanism that appears to be consistent with the pre-2.3 Ga MIF sulfur data involves atmospheric reactions such as SO_2 photolysis by hard UV. Hence, chemical models must be developed to provide mechanisms for transferring MIF to the surface in early anoxic atmospheres. Theoretical work (Pavlov and Kasting, 2002) shows that MIF in sulfur aerosols are preserved from homogenization in oceanic sulfate reservoirs only in atmospheres with pO_2 well below 10^{-5} PAL. Models suggest it is not possible to create anomalous $\delta^{33}S/\delta^{34}S$ in high pO_2 atmospheres; the cessation of MIF signatures is complete before the $pO_2 = 10^{-5}$ PAL threshold is reached.

In both the experimental (1, 2) and theoretical (3) studies cited above, magnitudes of $\Delta^{33}S$ anomalies may be proportional to the residence time of reactive sulfur species (e.g., SO_2) in the atmosphere, balanced with the surface deposition of reaction products, including elemental sulfur (Pavlov and Kasting, 2002). The signature of this unusual $\Delta^{33}S$ sulfur chemistry can be transferred to and preserved in the geologic record, probably via sulfate and elemental sulfur (*e.g.* S^8) aerosols from the atmosphere into surface minerals, sediments, waters and ices (Farquhar et al., 2000; Thiemens et al., 2001).

2.3.5. Techniques: Measuring MIF in Precambrian sedimentary sulfides.

A new ion microprobe technique developed by our group and collaborator K. D. McKeegan (UCLA) can quickly resolve $\Delta^{33}S$ anomalies *in situ* at the intra-grain scale (<30 μm) of individual sulfides (pyrite, pyrrhotite, etc.) in rocks, facilitating exploration of the magnitude and distribution of MIF sulfur in a wide range of natural samples.

In a recent feasibility study (Mojzsis et al., 2003), we identified $\Delta^{33}S$ in several Precambrian sulfides and compared our results with $\Delta^{33}S$ data obtained by conventional bulk rock or mineral separate methods (Farquhar et al., 2000). More than 150 separate analyses were made on standards interposed with a suite of sulfide samples collected from high- to low-grade metamorphic sedimentary rocks spanning a large range of ages (Figure 2.3-1).

It is apparent from Figure 2.3-1 that data track a major change in sulfur geochemistry sometime between 1.9–2.4 Ga related to the

demise of the $\Delta^{33}S$ effect. We ascribe this change to the rise of oxygen: $\delta^{33}S/\delta^{34}S$ compositions of samples older than ~2.4 Ga display MIF with a total range between $\Delta^{33}S$ = -1.72±0.34‰ (2σ) and +2.02±0.08‰ (2σ).

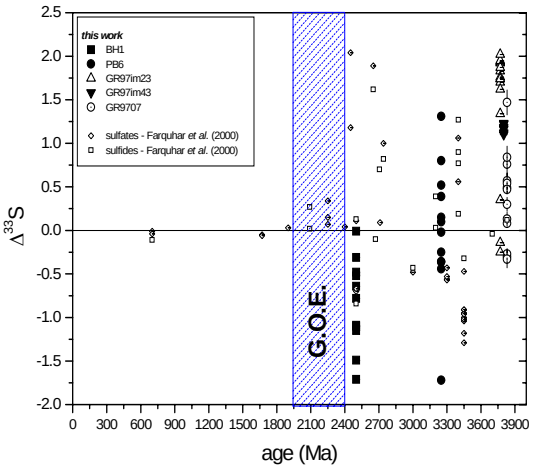


Figure 2.3-1

These values can only be explained by the presence of a global anoxic atmosphere facilitating the mass-independent fractionation of sulfur isotopes by UV-driven photolytic reactions in the gas phase. Samples younger than ~1.9 Ga fall within a relatively narrow range about $\Delta^{33}S \approx 0‰$ indicating that MIF sulfur was absent just as it is in the present O_2-rich atmosphere.

2.3.6. Hypothesis – Ancient sulfur metabolisms can be investigated by MIF.

Sulfur metabolism is probably an ancient biological phenomenon (Stetter, 1996). Many hyperthermophilic organisms in Bacteria (Thermotogales, Proteobacteria) and in Archaea (sub-Domain Crenarcheota, *Pyrococcus* and *Thermoplasma* in sub-Domain Euryarcheota) perform elemental sulfur (S^0) reduction (ESR) to H_2S using both H_2 and organic compounds as an electron donor (**bold** in Figure 2.3-2). Bacterial and Archaeal lineages share this metabolic style (Stetter and Gaag, 1983; Stetter, 1996; Canfield and Raiswell, 1999; Pace, 2001) underscoring the antiquity of this metabolic pathway and ESR may even be an inherited trait from the last common ancestor(s) of all life. Rapid cooling of volcanogenic sulfurous gases where $2SO_2 + 2H_2S \leftrightarrow 4S^0 + 2H_2O$, shifts the equilibrium in favor of S^0 (Grinenko and Thode, 1970) which is metabolically available for reduction to H_2S.

A consequence of anomalous $\Delta^{33}S$ sulfur aerosol formation is that sedimentary pyrite forming via ESR to H_2S would retain <u>positive</u> $\Delta^{33}S$ signatures, while pyrite forming from sulfate reduction would preserve <u>negative</u> $\Delta^{33}S$ values (Farquhar et al., 2001). The latter case may be seen in our data for *BH1*, a ~2.5 Ga BIF (Banded Iron Formation), where $\Delta^{33}S$ in sulfides is consistently negative (Figure 2.3-1). The pres-

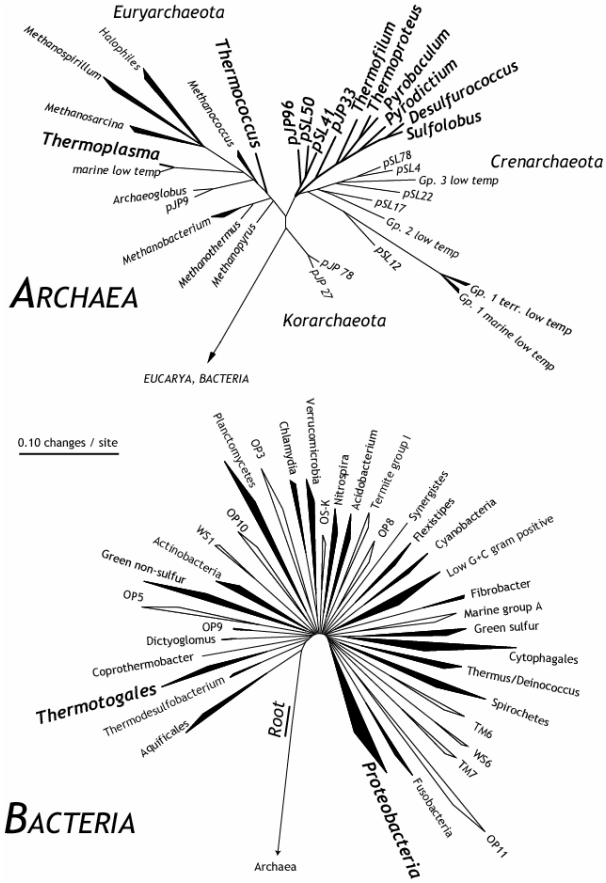


Figure 2.3-2

ence of positive $\Delta^{33}S$ in pre-2.4 Ga rocks and dominantly positive $\Delta^{33}S$ in pre-3.6 Ga rocks makes a compelling case that sulfur in these systems was cycled through the atmosphere and introduced to the oceans in the Archean/early Proterozoic. That MIF is preserved in the sediments clearly means that the sulfur was isolated from rehomogenization by volcanic and hydrothermal (non-anomalous) sources of sulfur. Moreover, sulfur aerosols derived from atmospheric reactions and deposited into the oceans would provide a ready source of sulfur to a plethora of microbial environments at the global scale.

To explore the significance of these results for the co-evolution of the atmosphere and metabolic styles requires us to obtain much more data on a variety of ancient sediments through an intensive project of fine-scale sampling of sediments spanning 1.9–2.4 Ga to track the disappearance of $\Delta^{33}S$.

2.3.7. Work Plan – MIF in sulfur traces the rate and duration of the oxidation of Earth.

Current data (Figure 2.3-1) are consistent with low pO_2 levels ($<10^{-5}$ PAL) and a photochemically active atmosphere before the mid- to early Proterozoic. However, data that we seek to generate in this project specifically aims to fill in the time period 1.9–2.4 Ga when the Earth went from anoxic to oxic (Figure 2.3-3) using samples collected from fieldwork with collaborator J.A. Karhu (University of Helsinki). How might the GOE correlate with the demise of a resolvable $\Delta^{33}S$ signal? Existing MIF data for this time period as plotted in Figure 2.3-3 underscore the urgent need for detailed sampling of rocks across this time interval. We will undertake collecting trips in northern Finland (Rovaniemi district) for samples containing pyrite that have been well characterized by geochronology (Perttunen and Vaasjoki, 2001) and stable isotope (carbon and oxygen) studies (Karhu, 1993). Pyrite (FeS_2) for MIF measurements will be selected for this study on the basis of textural and chemical evidence indicative of a syngenetic origin and analyzed by the ion microprobe methods cited here (see Greenwood et al., 2000; Mojzsis et al., 2003). All sample preparation, archival, microscopy and trace element analyses will be done at the University of Colorado.

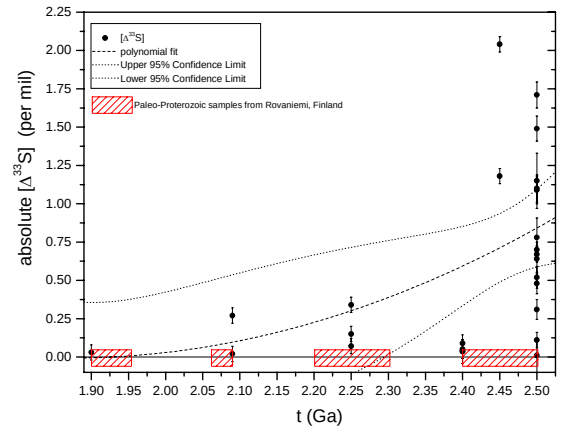


Figure 2.3-3

Collaborator J.A. Karhu (Univ. Helsinki) will provide samples for these studies in Yrs. 1-3. Cores stored in the Geological Survey of Finland and in open pit quarries of the Peräpohja schist belt will be sampled and sent to University of Colorado for petrographic, chemical and isotopic analyses on a regular basis. Mojzsis' lab will concentrate efforts on the sulfides from all field areas and will carry out MIF measurements; the isotope measurements will be made at UCLA and funded by subcontract from CU as described in the budget. In years 1-3 of the proposed research, team members will concentrate on preparing samples and gathering MIF data. Years 3-5 will be focused on finishing the analytical work and expanding the MIF search to other localities that potentially provide significantly improved or enhanced temporal resolution of the GOE. For instance, there is much potential in the Paleoproterozoic (1.9–2.4

Ga) sediments of the nearby Nash Fork Fm. in southern Wyoming (e.g., Bekker et al., 2003).

Budget justification of proposed work – The project requests support of a Ph.D. graduate student for their dissertation, as well as support of analytical expenses for Years 1-5. Collaborator K.D. McKeegan (UCLA) will assist with data collection by ion microprobe during the project. Collaborator J.A. Karhu (Helsinki) will undertake fieldwork and sample collection in Finland (Yrs. 1-3). Data analysis and interpretation will be shared by all project members and published in peer-reviewed journals. Exploration of unusual sulfur isotope chemistries in the Precambrian is an intensive analytical task on many hundreds of samples and will require the full 5 years of support requested in this proposal.

2.4. Molecular Survey of Microbial Diversity in Hypersaline Ecosystems (led by Co-I Norman R. Pace)

2.4.1. Overview.

Hypersaline microbial mats are complex, integrated ecosystems that occur worldwide, wherever evaporation results in a stable, high-salinity setting. Because of their relative stability and comparative simplicity, hypersaline mats have become models for studies of chemical and energetic factors that support photosynthesis-driven communities (Oren, 1999, for reviews). The principles derived from such studies are pertinent to planetary ecology, which is the summation of many such systems, and to the nature of life in extreme environments. The NAI Ecogenomics Focus Group (Sogin, DesMarais, co-Chairs) has undertaken study of selected Guerrero Negro hypersaline mats as a model extreme ecosystem. The combined goal of the Group is to define the relationships between microbial diversity, complex gene expression patterns, and biogeochemical gradients in shaping ecosystems and, ultimately, to predict the behavior of planetary systems.

The role of the Pace laboratory in this effort is to survey the microbial makeup and organization of selected hypersaline mats using culture-independent molecular methods based on cloning and sequence analysis of ribosomal RNA (rRNA) genes. This molecular census of microbial diversity is a critical component of the Ecogenomics Group effort, but also stands alone as an independent task. The results will provide a basis for relating microbial activities to the chemical properties of the system. The survey also is a substantial venture in microbial discovery. Remarkably little is known about the makeup of microbial communities in general and we have already encountered a wide breadth of novel diversity. Some of the results call into question past assumptions about the driving organisms in these systems.

2.4.2. Introduction.

Studies of the biological and geochemical properties of hypersaline ecosystems have been conducted worldwide, notably at Guerrero Negro, Mexico, in the context of the NAI Ecogenomics Group effort. Detailed measurements with microelectrodes of gas and sulfate fluxes (e.g., Jorgensen et al., 1989; Revsbech et al., 1989; Canfield and DesMarais, 1993) have painted many details of mat metabolism. Some groups of microbes have been studied, for instance cyanobacteria (D'Amelio et al., 1989) and sulfate reducing bacteria (Risatti et al., 1994). The mats are metabolically dynamic on the daily scale, driven by photosynthesis and oxygen metabolism during the daytime, and by sulfate metabolism at all times. Mats also are thought to be structurally dynamic, as organisms migrate in the search for favorable chemical zones created during the photocycle. Seasonal and ephemeral variations in local conditions undoubtedly influence the composition of mats, as well.

The outlines of the metabolic themes that drive these ecosystems are emerging, but our

understanding of the organisms responsible for the chemical themes is rudimentary. The communities are expected to be highly complex. The main energy input to hypersaline mats is photosynthesis. Beyond photosynthesis, the general occurrence of high concentrations of molecular hydrogen (presumably a metabolic product) and sulfate fuel lithotrophic cycles; and, of course, organisms with heterotrophic metabolisms are supported by the overall primary productivity in the system. Hypersaline mats are highly stratified on the sub-millimeter scale. Oxic metabolism at the surface (ca. 1 mm) grades into anaerobic metabolism to depths of several cm. Organisms representing those various physiologies have been observed by culture or microscopy, but their actual contribution to the overall biomass could not be determined until recently. The actual makeup of the community could not be assessed with the traditional culture-based methods because most (>>99%) naturally occurring microbes can not be cultured using the usual techniques. Thus, any characterization of the microbiota of Guerrero Negro hypersaline mats that is relevant to large-scale chemical fluxes must use culture-independent techniques.

DNA sequence-based methods provide ways to identify and study organisms without the requirement for culture (Pace, 1997, for review). The phylogenetic types of organisms (phylotypes) that make up these communities are identified by ribosomal RNA (rRNA) gene sequences. The sequences also are the basis of molecular methods with which to study *in situ* the properties of organisms detected by the sequences. rRNA genes most often are obtained by cloning amplified products of the polymerase chain reaction (PCR). The rRNA sequences are specific identifiers of organisms independently of morphological or physiological variation. Thus, comparison of the sequences with known rRNA sequences reveals phylogenetic relationships of organisms in the community to known organisms. Furthermore, fluorescently labeled oligonucleotide hybridization probes that bind selectively to rRNA can be used with confocal microscopy for study of the physical distributions of particular organisms in environmental settings.

It is possible to infer some properties of an unknown organism based on the properties of its relatives because the organism, as a member of the particular phylogenetic group, is expected to have the properties that are common to the group. Beyond focus on particular organisms, the phylogenetic results identify novel biodiversity, previously unknown organisms, and potential sources of interesting or useful genes. Molecular studies of environmental diversity have expanded our view of microbial life dramatically, in bacterial, archaeal and eucaryotic lineages. Results have shown that our knowledge of the types and distributions of microorganisms that occur in the environment is rudimentary. There is a genuine need for aggressive sequence-based surveys of microbial community-composition in general. Our ongoing molecular study of the Guerrero Negro hypersaline mats is a leading example of how the methods (and philosophy) of culture-independent analysis are extending and revising our perception of microbial ecosystems.

2.4.3. Preliminary results.

The Pace laboratory has been involved in the analysis of hypersaline mats for about two years, in the context of the Ecogenomics Group. Our results in microbial diversity stand alone, however, independently of the success of other members of the Group. Based on extensive previous studies, efforts focus on a specific site, “Pond 4 near 5” at the Guerrero Negro salt works, Baja California, Mexico. We have made three collecting trips in concert with other members of the Group and have sufficient materials under various modes of storage for the introductory surveys. DNAs have been purified

from different locations within the study site, and from different strata in mat cores. Our effort currently is on the analysis of rRNA sequences, which will constitute a phylogenetic census of the specific site under study by the Group. This work currently is conducted by Collaborators Drs. Ruth Ley and John Spear, postdoctoral fellows in the laboratory, with a few undergraduates. (Ley has been supported in part by an NAI fellowship. This will end 09/03 and further support will be from the proposed budget. Spear has been supported in part by an NSF fellowship, which ends 07/03. His further participation in the project will be as an Agouron Institute fellow. As these postdoctoral workers obtain academic positions, they will be replaced with other postdoctoral or graduate students.) We have so far accumulated a few thousand rRNA sequences from various strata in the mat, and analyses are ongoing. We have just obtained through a collaborative effort with the Sogin sequencing facility (MBL) another several thousand sequences. Preliminary analyses of the sequences indicate important and sometimes unexpected findings. A few highlights include:

1. The rRNA sequences document a vast extent of novel microbial diversity, including new candidate divisions (main phyla) of Bacteria. Surprisingly, the main contributors to the rRNA libraries from the photic portion of the mats, and presumably the main biomass of that portion of the mat, are not those of the classically expected, morphologically conspicuous cyanobacteria. Rather, the dominant bacterial rRNA sequences in clone libraries, even if made with different sets of PCR primers, are those of "green nonsulfur" (GNS) bacteria. GNS sequences outnumber those of cyanobacteria 6-7:1. If this result is validated (below), it will change fundamentally the conception of primary productivity (carbon flow) in these photic ecosystems. For instance, carbon incorporation currently is thought due mainly to the cyanobacterial Calvin Cycle, which posits the use of the enzyme Rubisco for carbon fixation. Only a few GNS bacteria are known, and they do not use Rubisco, but rather, conduct carbon fixation by "reductive carboxylation," a relatively little-known pathway. This finding potentially has impact on the interpretation of biogeochemical studies, for instance measurements of isotope fractionation in the mats.

2. We (John Spear with an undergraduate) have completed analysis of the microbial makeup of a laminated endolithic community in brine-saturated gypsum precipitates associated with the Guerrero Negro salt works. Such communities have been reported, but the microbiology had not been studied. We have documented several hundred novel species (rRNA sequences) and currently are microscopically evaluating the distribution of different organisms in the laminated structure.

3. The archaea and microbial eucaryotes of hypersaline mats have not been studied and essentially are unknown. We have so far characterized a few thousand archaeal rRNA gene clones from selected strata of the Guerrero Negro mat and find, again surprisingly, that crenarchaeota outnumber classic halophilic archaea by 10- to 100-fold. No hypersaline crenarchaeota previously were known, so the finding raises substantial future research opportunities with these kinds of organisms. Similarly, studies of microbial eucaryotes that are underway are revealing a wealth of novel diversity, including organisms that are not related to known microbial eucaryotes at the kingdom level.

2.4.4. Proposed Studies.

We propose to continue the community analysis of the Guerrero Negro mats. This ecosystem is proving to be a spectacular source of novel biodiversity and results so far pose many questions. The complexity of the system, while not unexpected, is daunting. Specific first-order efforts proposed for the immediate future are the following:

1. Sequence-survey of current samples will be continued, to analyze the bacterial, archaeal, and eucaryal constituents of these mats, and to obtain diurnal and annual comparative information. (We have June and October, day and night samples; and a February sample needs to be acquired.) Some sequence analyses will be conducted at different sites with similar chemistry in order to test the uniformity of the mats. The complexity of the communities demands higher sequencing capacity than our current facility. Consequently, we have recently acquired a capillary sequencer that will expand our capacity >10x. We anticipate the determination of 20-30,000 rRNA sequences during the course of the study. This analysis of the Guerrero Negro mats will be a landmark study in microbial discovery.

2. An early goal is to validate the PCR-based results that indicate the major role (abundance) of the GNS bacteria in the system. This was an unexpected result and shakes a number of microbiological shibboleths. Validation of the results will come from analysis of selected samples with alternative methods to PCR. These will include use of fluorescence *in situ* hybridization (FISH) with specific nucleic acid probes and direct microscopic enumeration; bulk hybridization (slot blot) analysis of *in situ* rRNA concentrations, a measure of biosynthetic capacity; and selected pigment analyses. Additionally, we are developing real-time PCR approaches for quantification of particular phylotypes that are deemed important by group-abundance (e.g., GNS bacteria) or unusual character (Crenarchaeota). These methods will be used to map the distribution of selected phylotypes in the mats, for instance across and along gradients of salinity, more efficiently and less expensively than direct sequence analysis.

3. The microscopic distribution of selected organisms in mat samples will be determined using FISH with confocal microscopy. The distributions of different phylotypes will lend clues as to their roles and the workings of the community, and are likely to indicate novel symbioses (intermixed co-occurrence of particular phylotypes). One potential such symbiosis under study involves "trichomes" (sheath-enclosed bundles) of *Microcoleus*, a conspicuous cyanobacterium in the mats. Microscopy shows, however, that the sheaths enveloping the *Microcoleus* cells are crowded with other morphotypes besides *Microcoleus* filaments (possibly the enigmatic GNS bacteria?). These and other apparent consorts will be studied by harvesting (micromanipulation) morphological units for PCR-based molecular identification and FISH analysis to understand the fine-scale structure of the units.

4. As a high priority, representatives of the apparently abundant GNS bacteria will be cultured for further study. Only a few examples of GNS bacteria (one of about 40 main relatedness groups of bacteria) have been cultured. The rRNA sequences will be useful tools with which to track the organisms into culture. The abundance of the GNS sequences in clone libraries indicates that they engage in primary productivity, so presumably derive energy from photosynthesis. Culture conditions, therefore, will focus on photoautotrophic organisms under both oxic and anoxic states. Long-term goals will include physiological characterization and determination of the genome sequences of cultivars that represent the dominant Guerrero Negro microbiota.

5. Comparative studies in hypersaline sites other than Guerrero Negro will be important to generalize the currently developing molecular survey results. Therefore, analysis of other photosynthetic hypersaline communities will be continued in concert with efforts of the EcoGenomics group. Potential comparative ecosystems for study include Great Salt Lake, Utah, and selected tidal sediments. We propose to begin during the later portion of the program (yrs. 3-5) to conduct selected sequence analyses in these environments. The Great Salt Lake set-

ting, although inland, is generally similar to marine hypersaline settings, including the presence of relatively high concentrations of sulfate (Spencer et al., 1985). Consequently, we predict that similar suites of organisms and physiologies will be encountered at Great Salt Lake as at Guerrero Negro. The general makeup of Great Salt Lake hypersaline mats is unknown at this time. Tidal sediments occupy a substantial fraction of the terrestrial surface and must significantly influence global gas fluxes. It is likely that the same phylogenetic kinds of organisms encountered in the Guerrero Negro mats also occur in the outer centimeters of photic sediments. We propose to begin during the late stages of the program, with lowest priority, analysis of selected tidal sediments (e.g., Bolinas, CA).

2.4.5. Astrobiological relevance.

There are two particular ways in which the program fits into the larger scope of Astrobiology. One area of contribution by the program is pertinent to the charge to the NAI to understand the course of evolution on Earth. Through the discovery of new major lines of descent, the survey expands our knowledge of biodiversity. The new sequence results of the study also enhance the accuracy with which we can infer the deepest branches in the universal Tree of Life. As a theoretical side, the Co-I (Pace) has long been engaged in inference and interpretation of large-scale phylogenetic trees. Those efforts will continue into the proposed period, and are expected to refine and extend our view of the evolutionary topology of life.

The program additionally contributes to our growing understanding of what it takes to comprise an ecosystem. The spectroscopic search for life outside our Solar System is not for particular organisms, but for the products of ecosystems. The places to search for life in our own planetary system are dictated by the chemical niches occupied by terrestrial life. For what do we look? The terrestrial examples of life are our only models. Microbial ecosystems offer the best chance for understanding the interplay between organisms and biochemistry that results in ecosystems, be they small or large. The proposed research will provide rich new perspective on an extreme ecosystem dependent on metabolisms that are likely to be encountered any place there is life. The results of studies such as these provide the experimental fodder and intellectual basis for speculation on potential ecosystems elsewhere than on Earth, past and future.

2.5. Origin of Complex Land-based Ecosystems. (Led by Co-I W. Friedman.)

The most significant evolutionary events in the history of life (on Earth) are the development of a genetic code (RNA/DNA), the origin of unicellular life, the 30 or 40 separate origins of multicellularity, and the migration of multicellular life forms from water-based environments to land. This last event began with the migration of aquatic green algae onto land some 475 million years ago (Shear, 1991; Edwards and Selden, 1993; Raven, 1997) and resulted in a veritable explosion of evolutionary innovation and consequent diversification of terrestrial ecosystems. Thus, after nine-tenths of the history of life on Earth to date had transpired, land-based environments were finally colonized by diverse multicellular life forms - a radical change from pre-Ordovician microbial crusts (Raven and Edwards, 2001). The centrality of photosynthetic life to the diversification of ecosystems with large multicellular heterotrophic organisms (such as animals) is related to the obvious need for energy (from light) to be channeled (via plants) into the organisms that comprise an ecosystem.

Current molecular and paleontological data agree that evolution onto land by complex multicellular algae occurred just once in the 4 billion year history of life on Earth (Gray, 1985; Kenrick and Crane, 1997; Karol et al., 2001).

This suggests that this one-time transition (from an aqueous environment to a terrestrial one) may not have been simple for photosynthetic organisms and that the precise evolutionary innovations required for life out of water were (and are) not easily acquired.

Prior to the first significant weathering of surface rocks and subsequent buildup of soils in the late Silurian and Devonian (a consequence of the evolution of land plants and their rooting structures; Raven and Edwards, 2001), land surfaces on Earth were comparatively nutrient-poor with respect to the diverse mineral nutrients required for plant growth. Thus, the transition from a water-based environment (in which the organism is continuously bathed in a nutrient-rich medium) to a nutrient-poor land environment may have been constrained by the inability of multicellular photoautotrophs to gain sufficient access to scarce inorganic nutrients that are required for basic biological activity (Pirozynski and Malloch, 1975; Smith and Read, 1997).

Currently, over 70% of land plants develop a critical symbiosis with fungi (termed a "mycorrhizal association") that is essential for normal plant growth. The fungus, which lives within the plant body and whose filaments (hyphae) ramify into the soil, receives organic carbon from the plant, while the plant acquires increased access to various "limiting" mineral nutrients through the fungus' significant surface area contact with the soil (Smith and Read, 1997).

It has long been hypothesized that a mycorrhizal symbiosis with fungi enabled the first terrestrial photoautotrophs to gain sufficient access to scarce micronutrients in the environment (Pirozynski and Malloch, 1975; Remy et al., 1994; Redecker et al., 2000; Brundrett, 2002); and that this association was the key to the one time transition from aqueous environments to land. Remarkably, despite a century of (largely ecological and physiological) study of this important and widespread (mycorrhizal) symbiosis, virtually nothing is known of the specific identity of the fungi that were involved in the one-time transition of multicellular photosynthetic life forms from water to land, and the subsequent evolutionary radiation of these plants into diverse environments.

During years four and five of the currently funded astrobiology effort, we began to identify (in essence, discover) some of the fungal species that grow symbiotically within the bodies of ancient lineages of plants. Until now, molecular identification of the fungal symbiotic partners of land plants had been restricted to groups of plants with extremely recent origins (less than 100 million years ago).

Using molecular identification techniques, we are beginning to piece together the story of a long and complex symbiotic relationship between two major types of life on Earth: plants and fungi. Identification of fungal symbionts in plant tissues can be accomplished with standard and newly emerging DNA amplification and sequencing protocols (Bridge, 2002) using carefully targeted fungal specific primers (Abbas et al., 1996; Redecker et al., 1997; Schüssler et al., 2001). BLAST analysis (against sequences in GenBank) of amplified and sequenced fungal DNA from living representatives of ancient plant lineages can then be used to determine the phylogenetic identity of the specific fungi (Strumer and Morton, 1997; Morton and Redecker, 2001).

We have exploited genomic small subunit ribosomal DNA (18S rDNA) and genomic ribosomal internal transcribed spacers (ITS) sequences to identify the biotypes of the fungal symbionts of early land plant lineages. Preliminary identification and phylogenetic analysis of these fungal symbionts has yielded exciting results. Based on comparisons to databased sequences, all of the sequenced mycorrhizal fungi in ancient lineages of land plants we have studied to date (Psilotales and Ophioglossales) belong to the genus *Glomus*, the same group of

fungi that form symbiotic associations in most other (and recently evolved) lineages of land plants. This is the first time that mycorrhizal symbioses in early lineages of land plants have been documented on the basis of the DNA sequence identity of the fungal symbiont. Our preliminary data provide some of the first concrete molecular evidence that the extremely widespread mycorrhizal symbiosis found in extant plants may have arisen during (and potentially enabled) the earliest phases of the colonization of land by plants (Figure 2.5-1).

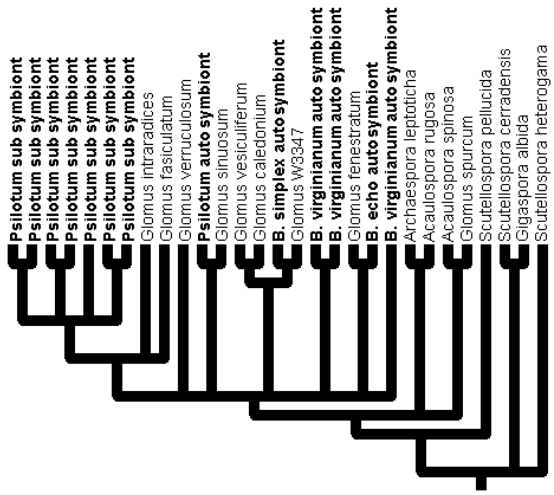


Figure 2.5-1: Molecular identification of fungal partners of ancient land plant lineages. Neighbor-Joining bootstrap phylogenetic tree using 560bp of 18S rDNA . All fungal symbionts of Psilotum nudum subterranean phases ("sub") are found in one well supported monophyletic group. This clade may represent one biotype or several different species. Fungal symbionts in the photosynthetic phases ("auto") of Psilotum and Botrychium are found in many different fungal lineages and represent many potential biotypes/species. All taxa in bold are newly discovered in our lab; sequence identities of taxa not in bold are from known fungal species listed in GenBank.

Recently, we have detected strong evidence (unpublished data – see Figure 2.5-2) that approximately 75 million years after the original colonization of land by plants, and concurrent with the first increase in land plant stature from centimeters to meters, part of the life cycle of land plants became entirely subterranean (and strictly heterotrophic). This extreme alteration in the ecology of early plants appears to have been accomplished through an alteration of the symbiotic relationship of plants to their fungal partners. In essence, the flow of organic carbon from the plant to the fungus was reversed, and the plant became a parasite upon the fungus and its carbon (ultimately derived from fungal connections with other plants that are photosynthesizing).

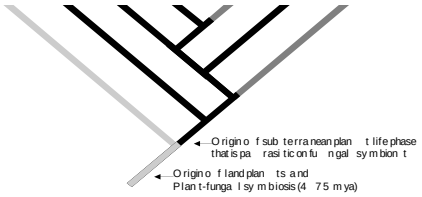


Figure 2.5-2: Evolutionary tree (phylogeny) of land plants with grey bars indicating a "typical" mycorrhizal symbiosis (photosynthetic plant obtains scarce mineral nutrients from fungus and fungus receives organic carbon from plant). Black bars indicate lineages of plants with a subterranean phase that is parasitic on the fungal symbiont for both its mineral nutrients and its organic carbon.

This type of subterranean habitat during a major portion of the life cycle of plants still exists today and is widely found in ancient lineages of plants: lycopods, ferns, and psilophytes (see Figure 2.5-3). In these lineages of extant plants, the entirely subterranean heterotrophic phase of the life cycle may be as long as 15 years. A major unanswered (and until now, unasked) question is what kinds of fungi were involved in bringing plants onto land, and then shortly thereafter, "driving" them underground?

We plan to significantly enlarge the scope of our molecular investigations to discover the identities of the fungal partners of ancient lineages of plants. The goal is to sample the fungal symbionts of representative members of all living plant lineages whose origins can be traced (based on the fossil record) to the first one hundred million years of multicellular plant life on land (Table 2.5-1). During years one through three of the proposed effort, we will specifically extract fungal DNA from diverse species of ferns and lycopods whose current life cycles include an extended subterranean phase and subsequent above-ground photosynthetic phase. These plants will serve as a proxy for examining the key symbiotic relationships of land-based photoautotrophs during the first major radiation and significant increase in plant stature during the Silurian and Devonian.

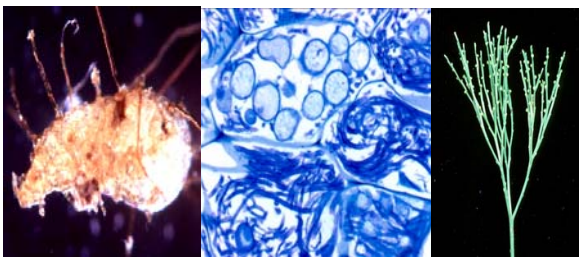


Figure 2.5-3. A. Subterranean phase of extant Psilotum plant. B. Microscope section of subterranean phase of Psilotum showing massive invasion of fungal partner in each cell of the body. We have discovered the DNA-based sequence identity of these fungi. C. The above-ground photosynthetic phase of extant Psilotum.

During years three through five of the proposed grant activities, our research will focus on the evolution of the mycorrhizal symbiosis in the most ancient lineage of land plants, the liverworts. These extremely primitive plants (still living) have long been known to associate with fungi, potentially in a symbiotic relationship. To date, the fungal partners of liverworts have not been identified (Duckett et al. 1991; Read et al. 2000). In light of the fact that liverworts are the most ancient lineage of land plants, and appear to have evolved shortly after the colonization of land by aquatic green algae, these organisms will serve as a direct proxy for investigating the role of one of the most critical symbioses in the history of life on Earth, that of ancient land plants and their fungal partners.

Table 2.5-1

Target Plant Taxa Years 1-3	Collection Sites
Psilotum (Psilotales)	Australia*, Georgia, Hawaii, Florida
Tmesipteris (Psilotales)	Australia*
Botrychium (Ophioglossales)	South Carolina*, Missouri*, Idaho*
Ophioglossum (Ophioglossales)	Australia*, Florida
Lycopodium (Lycopsida)	Colorado, Florida,
Huperzia (Lycopsida)	Florida, Washington
Target Plant Taxa Years 4-5	
Marchantia (Liverworts)	England, U.S.
Frullania (Liverworts)	Hawaii, Georgia,

* denotes where taxa have already been collected

The relevance of this proposed research to the NASA Astrobiology Program derives from the prospect of gaining a far more nuanced understanding of the evolution of complex ecosystems that might populate planets around other stars. For example, while life throughout the universe may be common, the issue of the relative organismic structural and ecosystem level complexity (i.e., unicellular vs. multicellular life forms; water-based ecosystems vs. land-based ecosystems) of life that might be expected to evolve elsewhere in the universe remains largely unaddressed. This, in turn has important implications for choices that will be made vis à vis detection of extrasolar life. What kinds of life and ecosystem signatures will be looked for? In land-based environments, should subterranean complex ecosystems be searched for? Finally,

the proposed research will have a major impact on understanding how major evolutionary and ecosystem transitions have occurred during the history of life on Earth.

3. THEME 2: ORIGIN AND EVOLUTION OF HABITABLE PLANETS

Understanding the potential for life beyond the Earth requires applying our knowledge of what allows an environment to be capable of supporting life to our discoveries regarding the various environments that exist within our solar system and beyond. The environmental requirements for being able to support life come directly from our knowledge of terrestrial life, as we have no overriding theoretical understanding independent of our one example of life. In doing this, we can look at the different environments that exist on planets (and satellites) in our solar system and ask which ones are capable of supporting life or not, what factors control their habitability, and what processes are responsible for the planet having evolved so as to have suitable characteristics. In order to generate an understanding of what makes a planet habitable and what makes a planet non-habitable, we need to look at specific characteristics of individual planets as well as at general characteristics that might apply to all planets.

To do this, we are taking a multi-faceted approach. No single planetary object and no single suite of processes that occur on an individual planet can provide the information we need to understand habitability. Instead, we will look across the range of the most interesting objects and the most interesting processes, in order to bring multiple approaches to the problem.

We will approach the problem of understanding planetary habitability by looking in detail at the two objects in our solar system other than the Earth that are most likely to be habitable (Mars and Europa) and by examining more general physical and chemical processes that control habitability (radiative transfer and chemistry of atmospheric aerosols, and geochemistry of aqueous geological systems that might support life). In addition, we will look at the astrophysical issues evident in planetary formation by trying to understand what processes govern the formation of planets to begin with. By looking at a range of objects and processes, we can avoid getting locked into thinking that will lead us down blind alleys. And by using a group of tasks that address a common problem from multiple perspectives, each task will benefit from the results obtained by the others.

The tasks that we propose are as follows, with each one being discussed in more detail in the remainder of this section.

(i) Biogeochemical cycling and resources on Mars (task led by Co-I Jakosky). We will constrain the habitability of Mars by estimating the energy available from geochemical sources to support metabolism in hydrothermal and aqueous systems and by using measurements from a variety of sources combined with theoretical models to better understand the history of martian volatiles and climate as a boundary condition to the existence of life.

(ii) Geological evolution and habitability of Europa (task led by Co-I Pappalardo). The habitability of Europa depends largely on whether there has been and still is a large quantity of liquid water (i.e., an ocean) beneath the surface covering of water ice. We will use digital mapping and modeling of surface stress fields to determine whether the patterns recorded by Europa's numerous lineaments are indicative of a steady-state level of activity, or whether they indicate a decrease in activity level through time. This will strongly constrain the geological evolution of the surface, and thereby the history of liquid water.

(iii) The impact of atmospheric particles on life (task led by Co-I Toon). Atmospheric aerosols affect planetary climate and habitability by their radiative effects, which depend on their composition and structure. We will model the

effects of aerosols on climate using theoretical models, and we will use an aerosol mass spectrometer to measure the composition and physical properties of aerosols formed under various conditions that might have obtained on the early Earth.

(iv) Geochemical-microbe interactions in chemolithoautotrophic communities on Earth (task led by Co-I McCollom). We will examine the habitability of terrestrial environments that are likely to be significant for life but have been all but ignored—moderate- and low-temperature environments that occur on the margins of hydrothermal systems and in aqueous systems involving ophiolite sequences that contain very reducing environments. We will determine the nature of geochemical weathering and alteration in these environments and the implications for their potential to support microbial communities.

(v) Origins of planetary systems (task led by Co-I Bally). We wish to understand the formation of planetary systems themselves, in light of observations of astrophysical processes that can destroy protoplanetary disks very effectively before they can form planets. We will combine theoretical models with observations at many wavelengths to understand the destructive processes in disks, with the corollary of understanding what combinations of processes allow planetary systems to form successfully.

3.1 Biogeochemical Cycling and Resources on Mars (task led by Co-I Jakosky)

3.1.1. Introduction

The potential for life on Mars depends on the availability of geochemical resources. Key factors include the presence of liquid water, access to the biogenic elements, and the availability of energy to support metabolism (Jakosky, 1998, and references therein). Abundant geological and geochemical evidence suggests the availability of liquid water. Geochemical reactions involving water and rock and capable of releasing energy that could support metabolism likely have occurred on Mars. This may have been either in high-temperature environments such as hydrothermal systems or low-temperature (ambient) conditions at or near the surface. The spatial and temporal availability of geochemical energy from these sources is not well understood, however. The amounts of usable energy have been estimated in broad terms, but those results are too general to assess particular environments that can be explored by spacecraft.

Further, even the limited data available indicates that there is complex coupling between the atmosphere and crust. Volatile exchange affects the composition of the atmosphere with implications for greenhouse warming, the nature of the climate, and the availability of volatiles within the subsurface that can drive geochemical weathering and alteration. Our ability to define and later to interpret measurements that can be made *in situ* or on returned samples depends on understanding these complexities.

We propose to investigate the potential for biogeochemical cycling on Mars and the implications for evolution of the biogenic elements and availability of geochemical energy to support metabolism. We will examine the geochemical energy available from alteration of martian surface and near-surface materials, the coupling to atmospheric and subsurface volatiles, and the implications for volatile cycling and evolution of the atmosphere-and-crust system throughout time. We will combine measurements made by spacecraft and using the martian meteorites with numerical models to determine the implications for the system as a whole.

Specific tasks will address:

(i) The geochemical energy that can support metabolism via mineral alteration and fluid-rock interactions in martian hydrothermal systems;

(ii) The geochemical energy that can support metabolism via chemical weathering at low temperatures in aqueous environments; and

(iii) The exchange of volatiles between the martian atmosphere, surface, and subsurface, as a constraint on martian volatile and climate evolution and possible biogeochemical cycling.

The results will tell us about the availability and history of resources that can support life and the overall biological potential for Mars. Although they will be specific to Mars, they also will allow us to understand the general behavior of terrestrial planets, with implications both for our solar system (including the early Earth) and for planets around other stars. In addition, they will be key to defining measurements that can be made by upcoming Mars missions to better understand the potential for life and its geochemical context.

3.1.2. Energetics of martian hydrothermal systems.

Hydrothermal systems probably are present on Mars, based both on the geological evidence for water and volcanism and on the widespread occurrence of impacts that have intensely heated the subsurface (Walter and Des Marais, 1993; Carr, 1987; Newsom, 1980; Brakenridge et al., 1985). The martian meteorites also contain minerals indicative of alteration by water at elevated temperatures (Romanek et al., 1994; Treiman et al., 1993). Energy derived from water-rock reactions in similar systems supports metabolism of some microorganisms on Earth and may have supported the earliest life (Brock et al., 1991; Baross and Hoffman, 1985; Shock, 1990); martian systems may support similar

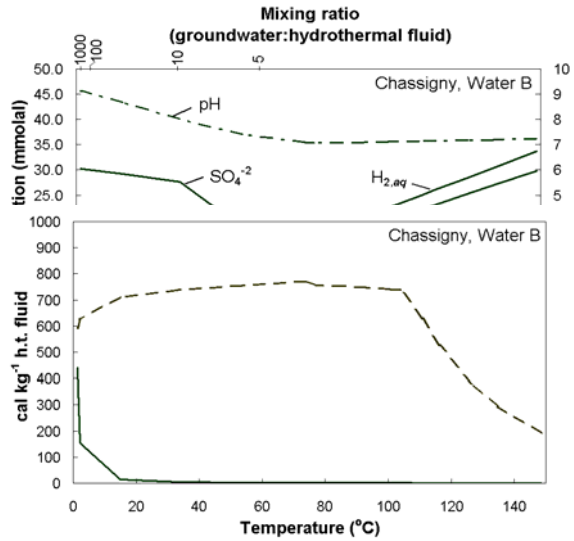


Figure 3.1-1. Energy yields and chemical species concentrations for mixing of fresh "martian" groundwater with hydrothermal fluid derived from the interaction of a Chassigny-composition rock with groundwater. Methanogenesis shown with a solid line, sulfate reduction with a dashed line.

thermophilic and hyperthermophilic life. In earlier work, we examined the alteration of volcanic rocks under martian conditions and estimated the energy available and the amounts of biota that it could support (Jakosky and Shock, 1998). We also used the known mineralogy of martian meteorites and the inferred composition of martian groundwater to refine these energy estimates (Varnes et al., 2003; see Fig. 3.1-1).

A more realistic calculation of the energetics would incorporate the evolution of hydrothermal systems and the resulting spatial and temporal variations of the alteration reactions. We will construct coupled models of the physical and chemical evolution of martian hydrothermal systems, in order to model the alteration within a single system. In this way, we can determine the alteration and the energy available to support metabolism at a single point, the regional behavior of a single hydrothermal system, and the total energetics of the martian crust through time.

The physical and chemical histories of a hydrothermal system can be separated to a large extent. The physical behavior involves the two- or three-dimensional history of temperature and fluid flow through the system subsequent to volcanic intrusion or crater formation. Calculations of these have been carried out for both terrestrial (e.g., Norton, 1984; Norton and Taylor, 1979) and martian systems (Gulick, 1998), and can be easily modeled or parameterized.

Chemical alteration is driven by the rock and fluid compositions, the temperature, and the prior history of the water and rock. We will calculate the 2- or 3-D history of alteration by tracking individual parcels of water and rock

separately and calculating the resulting chemical interactions. The chemical models will use the standard EQ3/6 geochemical modeling software that we used in our earlier analyses. The EQ3/6 software package includes an equilibrium aqueous speciation code, EQ3NR, and a reaction path code, EQ6 (Wolery, 1992; Wolery and Daveler, 1992), which we use in conjunction with a customized database of thermodynamic values (McCollom, 1999). These programs are used to determine the distribution, activities, and saturation states of species in solution and the fugacities of gases, allowing us to model processes such as fluid mixing, mineral dissolution and precipitation, and heating or cooling of fluid-rock systems. Reaction path codes model a succession of equilibrium states over a progress variable that corresponds with relative time, and will depend here, for example, on the spatial and temporal variation of water-rock ratio (see Griffith and Shock, 1995, 1997). Rock composition will be based on the mineralogy inferred from remote-sensing observations and derived from the martian meteorites; initial groundwater composition will be constrained by the composition of the martian crust and atmosphere (see Varnes et al., 2003).

The results will provide detailed information on the extent of alteration and the availability of energy, resolved both temporally and spatially, and will represent a significant advance over the representative calculations done previously. We will be able to determine the energy available, for example, at locations where temperatures are low enough for life to exist. We anticipate that our results will substantially modify our earlier analyses and will provide strong constraints on the ability of martian hydrothermal systems to support life.

3.1.3. Energetics of martian ambient-temperature aqueous systems.

Recent spacecraft results also suggest the possible occurrence of near-surface liquid water at or near $0^{o}C$ on Mars. Near-surface aquifers or surface deposits could be the sources of water for carving small-scale gullies (Malin and Edgett, 2000; Mellon and Phillips, 2001; Christensen, 2003); thin films or outright melting of polar and high-latitude ground ice may occur at moderate or high obliquities (Jakosky et al., 2003); outflows may debouch from beneath the polar ice deposits (Payne and Farmer, 2002); or transient lakes may exist within craters (Cabrol and Grin, 1999). We wish to understand the resulting geochemical environment, in which biota might exist. We will determine the weathering products that will result, using numerical geochemical modeling methods similar to those used at high temperatures and using terrestrial systems as analogs, and we will quantify the chemical energy potentially available to organisms. Although addressed in our preliminary model (Jakosky and Shock, 1998), we have not applied this approach systematically.

Geochemical modeling of low-temperature aqueous environments will be carried out using the same EQ3/6 software package used for geothermal systems. These systems can include both subsurface and surface water, such that systematic models of these environments could include weathering at various temperatures and water and rock compositions, formation and evolution of brines, and evaporation of water (Ridley et al., 1997; Ridley and Plumlee, 1995; Marion, 2000). As described by them, weathering products of basalt in a freezing or sub-zero environment depend upon the composition of the fluid altering it, which in turn depends on the composition of materials with which the fluid has been in contact as well as the prior history of reaction, evaporation, or freezing. Terrestrial systems can be used as analogs, based on the observed composition of weathering products. For example, the weathering of basalt near $0^{o}C$, buffered by the present martian atmosphere, can produce a range of minerals including hematite, goethite or ferrihydrite, smec-

tites clays, Mg-silicates, carbonates and sulfates (Ridley and Plumlee, 1997). Most of the thermodynamic data for aqueous species are available at relevant temperatures, and databases exist to deal with brines and freezing solutions (see Wolery, 1992). These will allow calculation of the energy available from these weathering reactions.

We will explore a range of water and rock compositions, determine the assemblages of minerals that will result by calculation and by analogy with Earth systems, and quantify energy available from weathering reactions for use by microorganisms. We will combine our results with current estimates of the distribution of surface and subsurface liquid water on Mars based on interpretation of morphology in order to derive an estimate of the potential ambient-condition biota both in specific locales and globally. We anticipate that the results will be of direct relevance to estimating the biological potential of the martian surface and subsurface in non-hydrothermal regions that can support liquid water.

3.1.4. Biogeochemical cycling of volatiles.

Exchange of volatiles can occur between the martian crust and atmosphere. Exchange of water depends on the climate, ongoing geological processes, and mineral reactions; CO_2 can dissolve in and be carried by the water, with carbonates forming by mineral reactions or releasing CO_2 again as a result of heating (e.g., Kahn, 1985; Pollack et al., 1987). Evidence for exchange comes from the martian meteorites, with the isotopic composition of C, H, and O in mineral weathering products reflecting fractionation that most likely resulted from atmospheric escape processes (Watson et al., 1994; Romanek et al., 1994; Jakosky and Jones, 1997). The history of the partitioning of water and CO_2 between crustal reservoirs and the atmosphere has important implications for Mars' climate history, volatile inventory, and crustal evolution, and will provide important constraints on Mars' biological potential. Understanding this exchange will help define martian geochemical cycles, which in turn allows us to understand the roles that biota might play in possible biogeochemical cycling and to identify key measurements that can be made from spacecraft that will constrain this role.

To address these issues, we wish to track current reservoirs where volatiles might be stored, exchange rates between reservoirs (including rates of loss to space), and how these vary with time. We will address these issues as follows:

(i) We will assemble a current understanding of martian cycles for C, O, and H. We will track CO_2 and H_2O in atmospheric, polar, and crustal inventories, including loss to space, exchange between the atmosphere and crust, and supply of juvenile gases from the deep interior. We will include exchange of O between CO_2 and H_2O and with crustal minerals. We also will track nitrogen; however, N_2 is not a climatically active gas, and mineral forms such as nitrate or nitrite have not been identified in martian surface materials or meteorites.

(ii) We will describe and, where possible, quantify the nature and sizes of non-atmospheric reservoirs of gases and their rates of exchange. Constraints will come from models of the related processes and measurements of the modern system. These will incorporate escape of atmosphere to space (Luhmann et al., 1992; Yung et al., 1988; Krasnopolsky, 2000), isotopic measurements within different reservoirs (Jakosky, 1991; Jakosky et al., 1994; Jakosky and Jones, 1997), the geological history inferred from images (Greeley and Schneid, 1991; Tanaka et al., 1988) and the martian meteorites (McSween, 1994), and from models of volatile-related processes (Pollack et al., 1987).

(iii) We will utilize our current understanding of martian geology, geophysics, volatiles, and history, as based on measurements from spacecraft and of the martian meteorites. In particular, we will pay attention to the history of

water at the surface (Carr, 1996; Phillips et al., 2000; Jakosky and Phillips, 2001) and the constraints that it imposes on both the crustal inventory of water and the history of its release, exchange, and availability to the surface.

We will include the nature of the weathering reactions that can occur at both ambient and elevated temperatures to understand the ability of volatiles to exchange with the atmosphere. The output of our analysis will be a "box model" of the martian volatile system, showing reservoirs, exchange between reservoirs, and rates, of the type that has been so useful in understanding terrestrial biogeochemical cycles. The result will be stronger constraints on the nature of the martian biogeochemical system and the movement of volatiles within it. This will have important implications for understanding the history of the atmosphere (and thereby of climate), the availability of volatiles within the regolith and crust, and the possible coupling between biological processes and geochemical processes.

3.1.5. Work plan and personnel

The three tasks are distinct and will be carried out in parallel. Results from each will be integrated with the others as appropriate. Work will be done by a graduate student (not yet identified) in collaboration with and under the supervision of Co-I Jakosky. Co-I Tom McCollom will consult on geochemical issues.

Collaborator Raymond Arvidson (Washington Univ. St. Louis) will provide input into the geochemical cycles task based on geological and mineralogical analysis from the MER rover missions to be launched in 2003, on which he is Deputy Principal Investigator. Collaborator David Des Marais (NASA/Ames Res. Ctr.) will consult on the geochemical cycles task based on his experience with analogous terrestrial processes.

3.2. Geological Evolution and Habitability of Europa (task led by Co-I R. Pappalardo)

3.2.1. Background and relevance

A subsurface ocean may be able to support life within Europa today. Understanding the astrobiological potential of Europa requires knowledge of its geological history, including the origin of its surface features and possible changes in the satellite's level of geological activity over time. If Europa's internal activity is robust today, its potential to harbor life is much greater than if the satellite's activity and heat sources have dwindled through time. We propose to use digital mapping techniques and modeling of candidate stress fields to determine whether the patterns recorded by Europa's numerous lineaments are indicative of a steady-state level of activity, or whether they indicate a change in activity level through time.

Imaging data from the Galileo spacecraft provide strong evidence for a subsurface ocean within Europa today (Pappalardo et al., 1999; Hoppa et al., 1999; Kivelson et al., 2000). Moreover, models of impactor dynamics and the observed paucity of craters suggest a surface age of only ~10 to 250 Myr (Zahnle et al., 1998; Pappalardo et al., 1999), implying that Europa is probably still geologically active today. However, it is unknown whether Europa's activity level has remained constant through time or dwindled in the recent past.

Possible energy sources for life on Europa are hydrothermal vents at its deep rock/water interface (Jakosky and Shock, 1998; McCollom, 1999), or oxidants created by charged particle bombardment of the surface and carried into the ocean by geological activity (Chyba and Phillips, 2001). A fundamental issue in understanding the current habitability of Europa is whether there is a great enough supply of chemical energy to the ocean to sustain a significant biosphere today (McCollom, 1999; Chyba and Phillips, 2001). Thus, understanding whether the satellite's level of internal activity has changed

through time is critical to understanding the satellite's potential habitability.

It has been suggested that Europa may be in a steady state, with the rate of formation of its geological features constant through time (Greenberg et al., 1999, 2002; Hoppa et al., 2001a, 2001b). On the other hand, geological mapping suggests that there may have been a change in Europa's geological style from ridge- and-band-dominated to chaos-dominated over time (Prockter et al., 1999; Greeley et al., 2000; Figueredo and Greeley, 2000; Spaun et al., 2003). A change in geological style could be indicative of cooling and thickening of Europa's ice shell, potentially in response to episodic or secular changes in orbital eccentricity and the corresponding rate of tidal heating (Ojakangas and Stevenson, 1986; Showman and Malhotra, 1997). The record of Europa's geology, specifically its ridges and bands, is the key to unraveling this evolution.

Deciphering the geological evolution of Europa's surface is a task that has confounded researchers to date because of the spaghetti-like intricacy of the satellite's lineaments and the complexity of the several stressing mechanisms that may have operated through time. We will undertake a study that combines digital mapping, computer-based stratigraphic analysis, and sophisticated stress modeling to determine the formational history of Europa's structures. Our integrated, state-of-the-art approach will be able to unravel the satellite's geological evolution through time.

3.2.2. Stressing mechanisms

The complexly lineated pattern of Europa's surface may have been shaped over time by as many as three different types of tidal distortion, each of which induces a characteristic stress pattern. First, "diurnal" stresses result from the raising and lowering of tides as Europa orbits, including resultant libration of the ice shell, on the rapid time scale of Europa's 3.55 day orbit about Jupiter (Greenberg et al., 1998). Diurnal stresses are probably responsible for Europa's unique cycloidal shaped features and may also produce other cracks (Hoppa et al., 1999).

Second, Europa's ice shell probably rotates slightly faster than the tidally locked interior on a very long time scale (>10^4 yr period). This "nonsynchronous rotation" sets up a stress pattern that sweeps easterly across the surface over time, leaving a distinctive structural pattern in its wake (Helfenstein and Parmentier, 1985; McEwen, 1986; Leith and McKinnon, 1996; Geissler et al., 1998). Early studies suggested that the surface records a total of ~60° of nonsynchronous rotation (McEwen, 1986; Leith and McKinnon, 1996; Geissler et al., 1998). However, more recent studies suggest that the surface may record 1-2 full rotations of the ice shell (Figueredo and Greeley, 2000; Kattenhorn, 2002) and perhaps hundreds or thousands of rotations (Hoppa et al., 2001a).

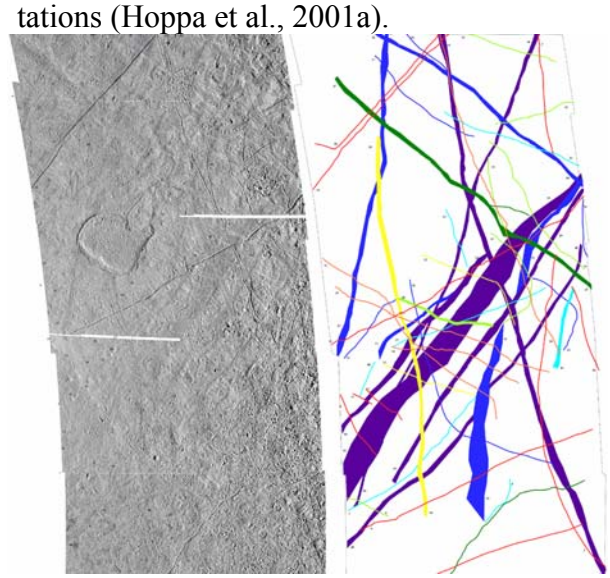


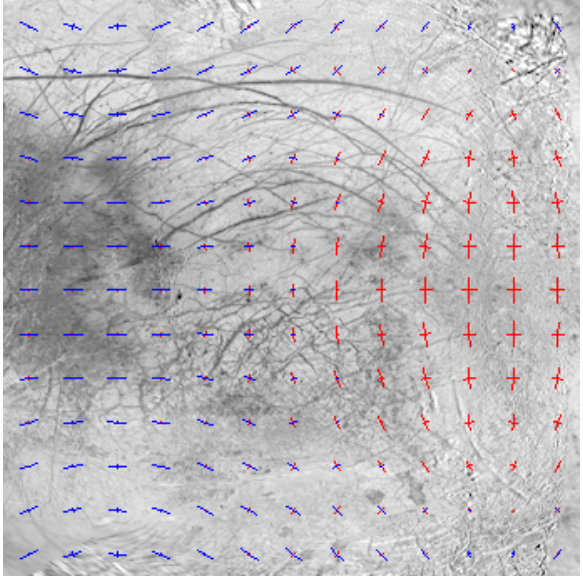
Figure 3.2-1. Image and corresponding map of ridges and bands near the leading point of Europa's orbital motion (0°, 90°). Approximate stratigraphy is mapped through the spectrum from violet (oldest) through red (youngest) (Stempel and Pappalardo, 2002). Preferred NE and NW trends are consistent with lineament formation in a zone of compressive stress pre-

dicted along Europa's equator due to nonsynchronous rotation. The lack of E-W structures suggests that the visible surface records <90° of nonsynchronous rotation. Reconciling these observations with the locations of cycloidal ridges (cf. Hoppa et al., 2001a) could indicate whether Europa's geological activity is in a steady state or has waned over time.

Third, Europa may have undergone polar wander, in which the rotation axis of the ice shell shifts with respect to the axis of orbital motion (Ojakangas and Stevenson, 1989). This would also set up a distinctive stress and structural pattern as a record of the event (Leith and McKinnon, 1996). There is plausible though limited geological evidence that polar wander has taken place (Leith and McKinnon, 1996; Sarid et al., 2002). These three candidate stress mechanisms (diurnal, nonsynchronous, and polar wander) can combine. To date only the combination of short-term diurnal stresses and secular nonsynchronous stress has been studied (Greenberg et al., 1998).

Figure 3.2-2. Superimposed on a map of Europa (135-265° lon., ±65° lat.) are the direction and magnitude of tensile (red) and compressive (blue) stresses at one point in the daily tidal cycle (45° before apojove). Integration of these stresses through the diurnal cycle, and addition to nonsynchronous and polar wander stresses, will allow for quantitative comparison to global lineament patterns, and inference of Europa's evolutionary history.

If Europa has experienced a steady-state level of activity, then combinations of diurnal and nonsynchronous stresses and perhaps polar wander stress are expected to have produced geological structures that overlap like a ball of string, with some roughly constant rate of structure formation



as the ice shell rotates nonsynchronously. This has been the assumption (implicit or explicit) of several geological studies (Figueredo and Greeley, 2000; Kattenhorn, 2002; Greenberg et al., 1999; Hoppa et al., 2001a, 2001b). On the other hand, if Europa's level of activity has declined though time, then the surface should record a change in the level of activity as reflected in the number and/or style of geological structures formed during each nonsynchronous rotation, and the surface might record few (perhaps <1) complete nonsynchronous rotation(s) of the ice shell (Stempel and Pappalardo, 2002).

3.2.3. Approach

Existing analyses of Europa's structural history adopt three simplifying assumptions regarding Europa's stratigraphy that are probably incorrect. All of these problems are remedied in our approach.

First, existing stratigraphic analyses (Prockter et al., 1999; Figueredo and Greeley, 2000; Kattenhorn, 2002; Spaun et al., 2003) group Europa's structures into discrete stratigraphic intervals in a subjective manner. That is, individual lineaments deemed to have formed in a generally similar time interval are grouped together without objective criteria for determining the precise relative age of individual structures. This approach is necessary in manual stratigraphic mapping of Europa because the cross-cutting relationships are so numerous and intricate. This can lead to oversimplification of the region's stratigraphy and misinterpretation of the stress that formed the structures.

We will employ Geographic Information Systems (GIS) techniques to map Europa's structures. For each intersection of two lineaments, we will record which lineament overlays which. Like a child's game of Pickup Sticks, our software will be able to peel back and decipher a *lineament-by-lineament* stratigraphy of each mapped area. Any ambiguous intersections will be keyed and flagged as such in the output, potentially indicating simultaneously active structures. The resulting objective and precise stratigraphy can then be compared to calculated stress models, making it clear whether or not there are systematic variations in lineament orientation (for example, as predicted by the sweeping of nonsynchronous rotation stresses across the surface), or more complex patterns (as discussed next).

Second, with the exception of the earliest such analysis (Helfenstein and Parmentier, 1980), published studies have assumed that all Europa's structures are fractures that formed perpendicular to the local least compressive stress direction. This assumption does not consider the possibility that shear failure has occurred in portions of Europa's equatorial region, where stress modeling suggests that it could occur (Helfenstein and Parmentier, 1985; Leith and McKinnon, 1996). In contrast to previous assumptions, shear failure can create simultaneous structures of two conjugate orientations oblique to the principal stress directions (as is inferred in the region mapped in Figure 3.2-1) (Stempel and Pappalardo, 2002; Spaun et al., 2003).

We will account for the possibility of shear failure and near-simultaneous formation of conjugate structures in Europa's equatorial regions. Unlike past analyses that assume lineament orientation is perpendicular to the least compressive stress direction, our lineament-by-lineament stratigraphy will allow us to decipher any back-and-forth swinging of lineament orientation, with potential implications of near-simultaneous formation of conjugate structures.

Third, existing studies have compared structural orientations to either the nonsynchronous or polar wander stress patterns alone, or to a combination of nonsynchronous and diurnal stresses at a single specific point in Europa's orbit. Existing studies have considered neither combinations of these stresses with polar wander stresses, nor the integration of diurnal stress over an entire orbit.

Our study will calculate stress at each point on Europa's surface based on any combination of diurnal, nonsynchronous, and polar wander stresses, comparing the results to observed structures to find most reasonable fits. Moreover, we will examine the pattern of combined nonsynchronous and diurnal stresses where the relevant least compressive stress is integrated over Europa's entire orbit. In this way, we determine the least compressive failure stress that is most likely to occur at each surface location during the course of the Europan orbit. This is the most appropriate pattern to compare lineament orientations, rather than any arbitrary moment in the diurnal stress cycle.

Our study will calculate surface stresses in a manner that is mathematically rigorous yet easily adaptable. Previous investigators have assumed deformation of a thin ice shell and have calculated stresses as arising from a discrete distortion of the ice shell (e.g., Leith and McKinnon, 1996). Some other researchers have not provided the mathematical foundation for their stress predictions (Greenberg et al., 1998; Hoppa et al., 1999). Working with Collaborator John Wahr, we will calculate stress by determining the vector components of displacement at Europa's surface as functions of the degree 2 Love numbers for any ice shell thickness. We calculate the stress tensor components, then diagonalize to obtain its eigenvectors and eigenvalues, which are the directions and magnitudes of the principal surface stresses. An example of the diurnal stresses derived from this method is shown in Figure 3.2-2. The resultant Matlab

software that calculates and outputs stress vectors across Europa will be distributed freely to interested researchers.

3.2.4. Implications for geology, convective transport, and astrobiology

Researchers currently disagree as to whether stratigraphic relationships suggest that Europa's activity level is in steady state or has decreased over time. The proposed study will unravel the history of Europa's complexly lineated surface through improved stress calculations and GIS-based stratigraphic mapping, techniques that will define a new state of the art. In this way we will determine whether Europa is in a steady state or if its level of geological activity has changed through time, while determining the sources of stress that have affected the surface and thus the satellite's geological evolution through time. These results are fundamental to assessing Europa's potential to harbor life.

This study will be complementary to (but will not overlap with) two other studies currently underway by Dr. Pappalardo at the University of Colorado. One study funded through NASA's Planetary Geology and Geophysics Program examines the morphology and nature of tectonic structures on Europa, with comparisons to its neighbor satellite Ganymede. There is fundamental synergism between that study and new modeling of the stress regimes in which structures may have formed.

Another synergistic study is funded through NASA's Exobiology Program. That study examines the nature and style of solid-state convection on Europa, concentrating on the implications of this vertical transport mechanism for movement of nutrients and perhaps organisms within Europa's ice, and for planetary protection. The stress models developed in the proposed study have direct bearing on modeling of convection within Europa. To date we have provided an initial assessment of the effects of non-linear ice rheology on the thermal structure and deformation style of Europa's ice if it is convecting (Barr and Pappalardo, 2003). However, in order to best consider the effects of non-linear ice rheology in a geophysically self-consistent manner, one must consider the tidal strain on Europa's ice shell as a driver of convection. This is because the thermal structure within the shell due to tidal heating and convection are linked to the applied tidal force through the ice viscosity. The convective and tidal effects must be treated simultaneously, and modeling the stresses that act on Europa will permit this. Collaborator Shijie Zhong will assist with this integration.

Understanding the processes that have operated on Europa and the manner in which they have changed through time is fundamental to understanding the satellite's present-day habitability. This study develops innovative techniques to achieve that goal.

The proposed work will be carried out by Dr. Pappalardo and a postdoctoral researcher, emphasizing the GIS-based analysis of Galileo images. Collaborator John Wahr (CU Physics Dept.) will carry out stress calculations using models that he will develop for ice shells of varying thickness and properties. Collaborator Shijie Zhong (CU Physics Dept.) will develop analytical and numerical models of coupled convective and tidal stress fields. Wahr and Zhong are funded under other grants, and no funding for their time is requested here.

3.3. The Impact of Atmospheric Particles on Life (task led by Co-I O. Toon).

3.3.1. Introduction

Two outstanding problems in astrobiology are to understand the environments in which life originated, and to determine how to detect places where life did or could arise. While much work has been done to understand the gases that compose atmospheres conducive to the origin of life and their spectroscopic signatures, relatively little work has been done to un-

derstand the clouds and aerosols in such atmospheres. This is an especially important gap since aerosol particles and cloud droplets provide unique physico-chemical environments that may provide a path to some critical biochemical reactions. For example, atmospheric aerosols have been recently postulated as playing an essential role in the origin of life on Earth (Dobson et al., 2000; Donaldson et al., 2002).

In the oxygen poor Archean atmosphere (Pavlov and Kasting, 2002), reduced atmospheric gases (such as methane and elemental sulfur) could have been polymerized (Pavlov et al., 2001) as happens in the present Venus and Titan atmospheres. The presence of a haze layer, or smog, would have affected the paleoenvironment in several ways. Both organic and sulfur aerosols are effective UV absorbers and could have provided necessary UV shielding for ancient biota. On the other hand, aerosols would have absorbed solar radiation affecting ancient climate. Whether aerosol forcing was important is a strong function of aerosol optical properties, which in turns depends on aerosol chemical composition and size/number density distribution.

We propose a combined theoretical and laboratory investigation to shed further light on these issues. We discuss our planned theoretical studies and then the laboratory work. We finish with our work plan. Our goal is to understand the aerosols and clouds in the atmosphere of early Earth, how these particulates influenced the UV radiation reaching the surface and the climate, and how they may have served as food for the biota.

3.3.2. Theoretical studies of aerosols

We plan to investigate three coupled issues. First we will simulate the formation of organic aerosols and sulfur aerosols in the early methane rich atmosphere of Earth. Second we will explore the dependence of haze formation on the changing atmospheric chemistry. Third we will investigate the role of these particulates in the climate and chemistry of the atmosphere.

We have adapted the NASA Ames/University of Colorado Community Aerosol and Radiation Model for Atmospheres (CARMA, Toon et al., 1988) to the problem of haze "evolution" in the atmosphere. Once haze has been formed at the top of the atmosphere (through photochemical reactions), it is subject to coagulation, gravitational settling, rainout etc. These processes change the particle size/number density distribution with height, and therefore affect both UV-shielding and the "antigreenhouse" effect of haze.

Pavlov et al. (2001) predicted that the rate of production of organic aerosols was a strong function of the CH_4/CO_2 ratio in the Archean (this result has been confirmed experimentally by our group as discussed below). A major constraint for the abundance of hydrocarbon aerosols comes from climate. Hydrocarbon aerosols absorb visible radiation and are relatively transparent to outgoing IR radiation creating an "antigreenhouse" effect. Therefore, if haze abundance is high, the surface temperature drops below the freezing point of water most likely shutting down methane production.

Organic haze is not the only haze that would have been generated in the anoxic Archean environment. Elemental sulfur would also polymerize, forming S_8 particles and other sulfur allotropes (as in the present Venus atmosphere). We have also developed a one-dimensional isotopic photochemical model to simulate isotopic composition of sulfur particles. Pavlov (in Ono et al., 2003) proposed that anomalously high mass independent fractionation in sulfur isotopes in the Archean rocks is direct evidence for sulfur aerosols, as opposed to sulfates which now occur in the atmosphere.

We will perform the following tasks:

First, we will include the hydrocarbon chemistry up to C_8 in a 1-D photochemical model (Pavlov et al., 2001) to get an initial pro-

file of hydrocarbon and S_8 chemical production under different CH_4/CO_2 atmospheric ratios and different SO_2 volcanic outgassing rates. We will then input those production rates to the 1-D aerosol transport model (CARMA), which will allow the calculation of particle size distributions at all atmospheric levels. We will then perform simulations of hydrocarbon and sulfur aerosol abundance with modified atmospheric vertical transport, because if the haze layer was present it would stratify the atmosphere and dramatically decrease the tropopause height (little rainout).

Second, we will conduct radiative transfer (climate) simulations using "realistic" optical properties of the Archean haze. (Optical properties of the haze have not been measured yet and this is an essential part of the lab work we will discuss below.) We will also concentrate on the magnitude of the UV shield from haze particles for ancient biota (since there was no ozone UV shield).

We will use the preceeding two steps to build up a conceptual model of an Archean biosphere without photosynthetic primary production. Effective production of high-altitude organic haze from the atmospheric methane could provide enough food for the methanogenic community so they would have kept resupplying atmospheric methane.

S_8 aerosols form relatively low in the atmosphere (6-10 km) so their removal time is short (rainout) and S_8 could not build up in the atmosphere to substantial levels. However, if organic haze was also present in the Archean, the Archean atmosphere would have been stratified down to the ground level (Pavlov et al., 2001). Therefore, S_8 aerosols (potentially) could have accumulated in the atmosphere to substantial levels and affected climate and photochemistry.

The presence of a haze layer, or smog, would have affected the paleoenvironment in many important ways (photochemistry, climate, "food" source, etc.). We hope to get quantitative answers to the following "key" questions for the Archean paleoenvironment: 1) What were the rates of haze production? 2) How much UV-shielding was produced? 3) How strong was the climatic impact of the haze layer and what limited its thickness in the atmosphere? 4) How digestible were haze particles for the ancient biosphere and was it possible to maintain the primary production without photosynthesis?

While the work we have just described is theoretical in nature, further progress is dependent on lab studies to determine several critical parameters. These include not only the optical properties of aerosols, but also their rates of production in various atmospheres, and their chemical properties.

3.3.3. AMS studies of particles on early Earth

While the Toon research group is primarily theoretical, we recognized that further progress would be limited without an adequate laboratory program to measure the parameters needed by the theory. We therefore developed a collaboration with Professor Margaret Tolbert and Professor Jose Jimenez in the CU Chemistry Department. So far this collaboration has produced several important new results, and led to the involvement of several chemistry graduate students in astrobiology research.

To make progress on understanding clouds and aerosols in planetary and early Earth atmospheres, new laboratory methods must be developed and employed. Recently, a new technique has been developed that has the capability of quantitatively analyzing the size and chemical composition of freely floating aerosol particles in real-time (down to 10 ms time resolution). This aerosol mass spectrometer (AMS) is sparking a revolution in understanding particulate matter in Earth's current atmosphere, with 19 research groups starting to use it in the US, Canada, Germany, the UK, and Japan (Jimenez, 2002). While not yet exploited, this technique

also has the potential to make major, new contributions relevant to laboratory studies of astrobiology

Current research in the Tolbert group represents the first attempt to utilize an AMS to perform detailed studies of particles that may have been present on early Earth. Future work in the Jimenez research group will expand these studies to longer timescales to follow particle evolution under realistic atmospheric conditions. Prof. Jimenez was heavily involved in the development of the AMS and thus has a high level of expertise in its use.

We are performing laboratory experiments to probe the formation and composition of Titan-like particles that might have formed on the early Earth (Sagan and Chyba, 1997). Previous laboratory investigations of this nature have involved either collection of aerosol samples for later analysis or study of gas phase products. Both of these methods have limitations in the ability to accurately determine aerosol composition and particle characteristics at formation. In order to improve on those studies, we are using a novel analysis technique based on detection of particles using an AMS. Using the AMS, we are able to determine the number, size and chemical composition of the particles in real time without first collecting and concentrating them, a procedure known to be riddled with artifacts.

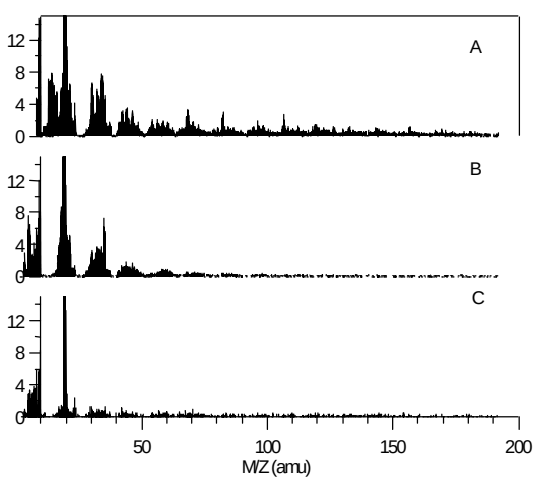
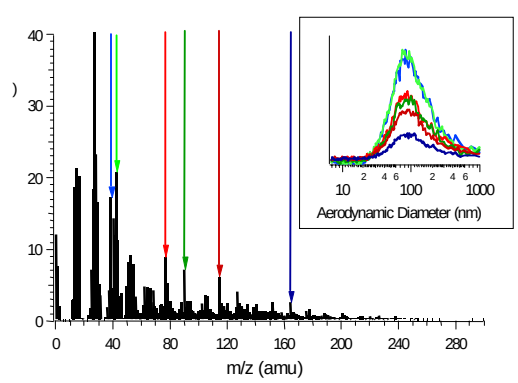


Figure 3.3-1. *(A) 10% CH_4 in N_2 (B) 1% CH_4 (C) 1000 ppmv CH_4*

Our studies focus on characterizing the particles as a function of input trace gas composition, and to date we have looked at methane (CH_4) and carbon dioxide (CO_2) in a background of nitrogen. We have varied CH_4 concentrations from 10% CH_4 in N_2 to less than 1000 ppmv CH_4, a relevant mixing ratio for the early Earth (Kasting et al., 2001a). The mass spectra resulting from these experiments are shown in Figure 3.3-1., and indicate the definite presence of long hydrocarbon chains, as well as aromatics and other hydrocarbon structures previously described (Sagan and Khare, 1979). The spectra also show how the particle composition changes with a decrease in CH_4 concentration; as less methane is added to the reactant mixture the size of the hydrocarbon chains decreases and the structure is altered.

The AMS allows us to measure aerodynamic diameters of aerosols of a particular molecular composition. Since each mass spectrum represents an average composition of the aerosol population, it is not obvious whether the particles in question are internally or externally mixed. Figure 3.3-2. shows the results from an experiment performed to determine whether ions in the spectra were produced from molecules in the same particles. We can show that the aerosols that produce each ion in the spectra have the identical size distributions, as seen in the inset graph. This is consistent with the haze aerosols being internal mixtures of a complex mixture of organic molecules. The size distributions shown in Figure 3.3-2. also indicate that the haze aerosols produced in our experiments are unimodal and are approximately 90 nm in diameter, which is in the size range seen in previous studies of such particles.

An important factor in whether or not these organic haze aerosols could have formed on the



.Figure 3.3-2 Size distributions for several ions in spectrum

early Earth is the C/O ratio of the atmosphere (Kasting et al., 1997). The inclusion of oxygen atoms in the gas mixture is expected to oxidize organics and terminate the synthesis of long chain hydrocarbon molecules, thus altering both the composition of the hazes as well as particle size and concentration. We have studied the effects of adding CO_2 to the N_2/CH_4 mixture and the resulting mass spectra are shown in Figure 3.3-3. The CH_4 concentration in N2 is held constant at 1%.

As more CO_2 is added to the starting gas mixture, the appearance of long hydrocarbon chains diminishes, and ion peaks that are indicative of oxidized products, such as m/z 44 and 58, begin to appear. Once the C/O ratio begins to approach its lowest value, it appears that the aerosol production is greatly reduced, which is consistent with the expected behavior. We are now in the process of determining if the new products formed could be sources of food for the Earth's earliest life.

There are several important lines of research that we will pursue using the continuing access to an AMS that we are proposing. Our first priority is to alter our photolysis source from discharge excitation to UV irradiation to better simulate conditions on early Earth. The particle characteristics such as size and chemical composition may change dramatically with energy source, so our first goal would be to repeat the above experiments with the UV source. Our next goal is to add other trace gas species that might have been available on early Earth. Specifically, we would like to add trace sulfur gases such as H_2S and SO_2 to see how the particle properties were affected.

In addition to determining particle size and composition, climate models require accurate knowledge of the optical properties of the resulting particles. We propose to make optical constant measurements of the particles and correlate those properties with the chemical composition of the particles determined using the AMS. We have previously measured the optical constants of a number of materials over a wide range of temperatures and have considerable expertise in this area.

Finally, there will be new data from the Aerosol Collector and Pyrolyser (ACP) on the Huygens probe of Cassini on the chemical composition of the tholin particles on Titan. The

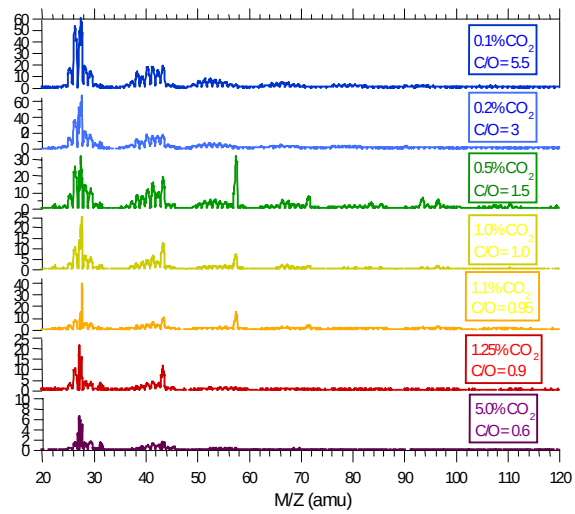


Figure 3.3-3 *Mass Spectra for Haze Aerosols Produced in Varying C/O Mixtures*

ACP uses pyrolysis GC/MS characterization of the molecules making up the particles, a technique that should yield mass spectra very similar to those of the AMS. We will compare the Titan data with AMS data we obtain in the laboratory. We will then vary conditions in the laboratory to unravel the formation mechanism of the Titan particles and learn more about how polymerization and growth processes control the particle characteristics. The AMS is ideal for these studies because it provides a quantitative measure of the various components in the particle in a short period of time. An AMS can do studies quickly and economically that might take years of effort, and cost vast sums of money to analyze by electron microscopy or other chemical techniques.

3.3.4. Studies of early Earth in a "bag"

A new environmental chamber facility being constructed in the Jimenez research group will

be used to study the photochemical evolution of laboratory-generated particles over long time periods (several hours). We will use this facility to study the formation, growth and aging of early Earth tholin particles. These studies complement those described above, but utilize longer periods of time with more complex processes possible. We will use the AMS to probe the chemical composition and size of the particles over time. In addition, the large size of the chamber (30 m^3) will allow us to optically probe the particles to determine optical properties of the aerosols in-situ. The Jimenez and Tolbert groups are co-located so time-sharing the AMS will be readily accomplished.

3.3.5. Work plan

The theory and lab work proposed will be done in parallel. As we learn more from lab studies, for instance the optical constants of particles, the information will be added to climate models and calculations done.

Brian Toon will be responsible for the overall conduct of the research. With his students and postdocs, he will conduct the theoretical studies, and provide guidance to the laboratory work. Professor Tolbert will be responsible for the AMS and optical constants studies. Professor Jimenez will provide access to the aerosol chamber, and provide advice on operating the AMS.

Analysis to date has been done using an Aerosol Mars Spectrometer essentially borrowed from another research group. The proposed funding will allow us to obtain one that will be used jointly by the Toon, Tolbert, and Jimenez groups and which will be much more readily available for the research proposed here. As described in detail in the equipment portion of the proposal (vol. 2), Professor Jimenez will cost share buying the AMS, which he will use separately for experiments in the aerosol chamber.

3.4. Geochemical-Microbe Interactions in Chemolithoautotrophic Communities on Earth (led by Co-I T. McCollom)

3.4.1. Introduction

Our current understanding of the early evolution of life on Earth suggests that the complex machinery of photosynthesis evolved well after life had become established, implying that the first biological communities probably relied on inorganic chemical energy sources rather than sunlight for biomass synthesis (a process known as chemolithoautotrophy). The hostile surfaces of Mars and Europa also suggest that any biological communities that currently exist there probably inhabit subsurface environments where chemical sources would supply the energy for metabolism. There are several environments on the modern Earth where geological processes supply the chemical energy to support chemolithoautotrophic microbial communities with little or no input of photosynthetically derived organic matter. Examples include deep-sea hydrothermal systems, subsurface aquifers in terrestrial and oceanic basalt, and acid mine drainages.

These kinds of chemolithoautotrophic environments may provide the best analogs for biologic habitats on the early Earth and for habitats that might now support life, or have supported life in the past, on other planetary bodies. The more we know about how microbial communities thrive in these environments on the contemporary Earth, the better equipped we will be to interpret biological processes on the early Earth and to evaluate potential biological habitats on extraterrestrial bodies such as Mars and Europa. Therefore, we propose to conduct research that will increase our understanding of the structure and function of biological communities that thrive on geologically-derived sources of chemical energy.

3.4.2 Proposed Research

The unique character of the biological communities that occupy geologically-based chemo-

lithoautotrophic habitats has led to extensive scientific study of these systems in recent years. However, most of this research has focused on isolation and characterization of the novel microbial populations that inhabit these environments and in elucidating their internal metabolic pathways. Largely missing from previous efforts has been corresponding research to understand the underlying geological processes that allow these communities to exist and to evaluate how geochemical processes influence the abundance and distribution of microbial activities. Information of this sort will be required, however, to fully describe the microbial ecology of these habitats. Furthermore, this type of information will be required to extrapolate from modern ecosystems in order to infer the biological potential of analogous habitats on the early Earth and on other planetary bodies where geochemical conditions differ substantially from those on the current Earth.

Accordingly, we propose to conduct research that will focus on the connections between geochemical processes and microbial activities in chemolithoautotrophic environments. Among the issues we will address are: Where and how do sources of chemical energy arise from geological processes? What is the abundance and diversity of chemical energy sources in these environments? To what extent do these energy sources control the composition and spatial distribution of the microbial population? How do variations in geological processes, fluid chemistry, etc., influence the metabolic diversity and abundance of microbial populations? How do chemical energy sources in a particular environment evolve over time? How do microbes alter the chemistry and mineralogy of the environment, and are characteristic chemical and mineral biomarkers produced in the process?

Our effort to address these issues will primarily involve the development of quantitative geochemical models of chemolithoautotrophic environments. Since chemolithoautotrophic microbes gain their metabolic energy by exploiting disequilibria in oxidation/reduction reactions, a primary objective of the models is to identify and quantify sources of redox disequilibria that develop in geological systems as a consequence of ongoing physical and chemical processes (McCollom and Shock, 1997; McCollom, 1999, 2000). This information about chemical disequilibria can then be used to infer the abundance, spatial distribution, and metabolic characteristics of microbes that can live in different habitats within the geological environment (e.g., Fig. 3.4-1).

Initially, the research will concentrate on mid-ocean ridge habitats and ophiolite-hosted alkaline springs, as described below:

Mid-ocean ridge hydrothermal and subsurface habitats – Nearly all efforts to study the

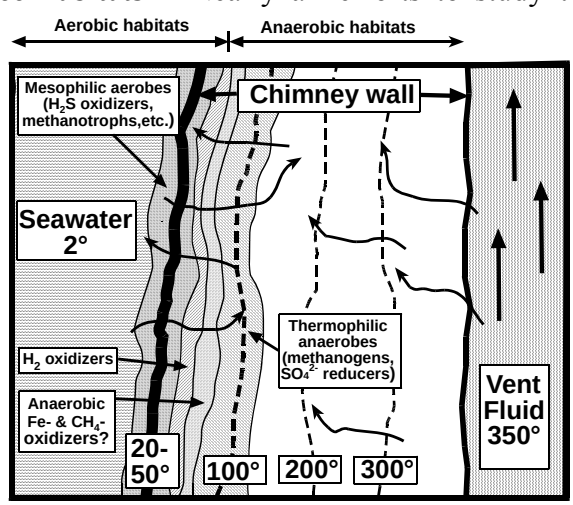


Figure 3.4-1. Distribution of chemolithoautotrophic habitats across a deep-sea hydrothermal chimney wall as inferred from geochemical modeling (McCollom and Shock, 1997). The model indicates that thermophilic microbes should represent only ~1% of the total primary biomass production in this environment. The model results agree with what is known of the microbial population in chimney environments, providing a framework for understanding the distribution of microbes (Karl, 1995; Kelley et al., 2002).

microbial community of deep-sea hydrothermal systems to date have focused on symbiotic and free-living microbes living on dissolved compounds in the direct vicinity of high-temperature, "black smoker" vents. Yet, the high temperature vents and chimneys represent only one of many potential habitats for chemolithoautotrophic microbes that are created by the formation of ocean crust at mid-ocean ridges. Other habitats with a large potential to support chemolithoautotrophic microbes include widespread diffuse subsurface mixing zones where hydrothermal fluids mix with seawater beneath the seafloor, downflow zones on the flanks of ridges where relatively oxidized seawater circulates into the crust on the downward limb of hydrothermal circulation cells, ultramafic-hosted hydrothermal systems where seawater interacts with tectonically-displaced rocks from the upper mantle, and off-axis sites where low-temperature fluids (<~50°C) interact with aging basaltic crust. The proposed models will explore microbial activities in these other habitats. Topics of particular emphasis will be the utilization of solid substrates (basalt, metal sulfide minerals, etc.) as energy sources by chemolithoautotrophs, and the microbial role in mineral alteration and biogeochemical cycling of carbon, iron, and sulfur.

Ophiolite-hosted alkaline springs – Ophiolites represent sections of the ocean crust and upper mantle that have been tectonically transported onto a continent. Several ophiolites occur along the western coast of the U. S. Peridotite, an ultramafic rock composed almost entirely of the mineral olivine, is a predominant rock type in ophiolites. Alteration of peridotite produces strongly reducing conditions through the "serpentinization" reaction: Olivine + H_2O $\rightarrow$ Serpentine + Magnetite + Brucite + H_2, and dissolution of brucite [$Mg(OH)_2$] generates alkalinity. As a result, fluids emanating from ophiolites are frequently alkaline and highly enriched in reduced gases such as H_2 and CH_4. These reduced compounds could support populations of methanogens, hydrogen oxidizers, and other chemolithoautrophs, but the microbiology of the springs has received very little attention to date. Since rocks of similar composition are widespread on the seafloor and in Archean terranes, and are also represented in martian meteorites (e.g., LEW88516), these systems may have direct parallels to environments on the early Earth and Mars.

We will investigate the chemolithoautotrophic potential of the springs through geochemical models, with particular attention on understanding the source of reduced compounds in the subsurface and describing environments for chemolithoautotrophs within the springs. This work will be performed in collaboration with scientists at NASA Ames (David Blake and Mitchell Schulte) who are presently initiating a comprehensive field and laboratory study of these springs. Their study will collect data on the petrology, fluid chemistry, and microbiology of the springs, and these data will be provided to us as constraints on the models. In turn, the models will provide a framework for interpreting the field and laboratory data.

Models will be constructed by combining thermodynamic and kinetic constraints on chemical reactions with information on geological systems (mineral and fluid compositions, fluid flow, etc.) to develop an understanding of geochemical conditions throughout the system. Construction of the models will be facilitated through the use of available computer packages including EQ3/6 (Wolery, 1992) and PHREEQC (Parkhurst, 1995) that model geochemical processes such as fluid mixing, heating and cooling, fluid-rock interactions, and reactive transport. These programs can be modified to account for kinetically inhibited redox reactions (e.g., McCollom and Shock, 1997; McCollom, 1999, 2000). Input parameters such as rock and fluid compositions, water/rock ratios, fluid fluxes, etc., will be derived from published descriptions or obtained from collaboration with

researchers working on the environments under study. Thermodynamic and kinetic parameters will be compiled from published sources as in previous studies. Additional constraints will be derived from ongoing laboratory measurements of geochemical reaction rates conducted by Co-I McCollom, including studies of redox reactions like the reduction of CO_2 to formate and methane (e.g., McCollom and Seewald, 2001, 2003).

It is anticipated that the models will both improve current interpretations of the distribution of the microbial habitats within chemolithoautotrophic ecosystems, and provide new directions for future investigations. For instance, geochemical models of fluid mixing in deep-sea hydrothermal systems have provided a framework for interpreting the distribution of microbes across the walls of hydrothermal vent chimneys (Fig. 3.4-1). In addition, results from previous models indicating that as much as 40% of the chemical energy generated in hydrothermal vents is available in the form of solid substrates rather than as dissolved compounds provided the impetus for new research that led to the discovery of several novel species of chemolithoautotrophic microbes capable of living on sulfide minerals as their source of metabolic energy (Fig. 3.4-2).

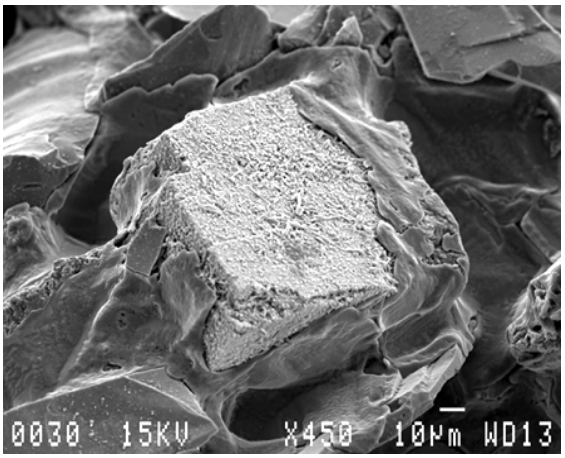


Figure 3.4-2. Axenic culture of an Fe-oxidizing microbe growing chemolithoautotrophically on a pyrite crystal. Unlike previously cultured microbes from deep-sea hydrothermal habitats that are only known to live on dissolved compounds, this species derives metabolic energy from solid substrates such as basalt and metal sulfides. Note thick biofilm and the stick-like Fe-oxide precipitates formed by the microbe where the surface is exposed. This strain was isolated by Co-I McCollom and collaborators K. Edwards and D. Rogers at Woods Hole Oceanographic Institution from samples collected near a deep-sea hydrothermal system (image courtesy of K. Edwards).

The results of this research are expected to have several direct benefits to astrobiology. The models can be readily modified to reflect conditions in analogous habitats on other planetary bodies or on the early Earth (e. g., McCollom, 1999; Varnes et al., 2003). In this respect, this effort will complement studies of potential biological habitats on Mars described elsewhere in this proposal. By providing a rigorous means of evaluating potential microbial habitats on Mars and Europa, the models will be useful in planning future exploration. For instance, the models can be used to define extraterrestrial habitats with the greatest likelihood for supporting life, help evaluate mineralogical and chemical signatures of life in rock samples, and to estimate the likely abundance of biomass in particular environments. In addition, extrapolation of the models to conditions on the early Earth will provide a basis for evaluating the potential for biological activity and resulting biosignatures in ancient rock samples, as well as to provide quantitative estimates of microbial processes for studies of carbon cycling and atmospheric evolution.

Work on this task will be led by Co-I McCollom, who will be responsible for defining model inputs, overseeing model development and execution, and interpretation of the results. Dr. McCollom will be aided by undergraduate student research assistants who will construct computer databases, operate programs, plot results, etc., under his supervision. Collaborators

David Blake and Mitchell Schulte (NASA Ames) will share results from their field studies for use in the alkaline springs models. Co-I McCollom will also assist in the collection and chemical analysis of the field samples. Work on this task will be initiated in year 2, with modeling of mid-ocean ridge and alkaline spring environments performed concurrently.

3.5. Origins of Planetary Systems (task led by Co-I J. Bally)

3.5.1 Introduction

How common are planets beyond the Solar System? How common are terrestrial planets? How diverse are planetary system architectures? How are the materials needed for life processed and accumulated? How do planetary systems form? What hazards do forming and mature planetary systems face? These are key questions in the *"astro"* side of astrobiology. Investigations within our own solar system, studies of nascent planetary systems, searches for and characterization of extra-Solar planets, and *investigation of the environment in which planetary systems form* will eventually provide answers. Our astrobiology research during the last five-years, and the work proposed for the next five, is focused on the planet-forming environment.

3.5.2 Background and highlights of recent research

Planet formation is a byproduct of star formation. Most stars form from the gravitational collapse of *giant molecular clouds* (GMCs) that typically spawn thousands of stars on a time-scale of about 10 million years within regions having dimension of one to tens of parsecs (see reviews in Mannings et al., 2000).

There are many hazards to planet formation. Most low-mass Sun-like stars form in the vicinity of more-massive stars that produce torrents of ultraviolet radiation, powerful winds, and explosions; the typical environment in which most stars are thought to form is violent. The Orion Nebula region, which contains several thousand low mass stars younger than one million years, is the nearest site of ongoing star formation in which both low- and high-mass stars are forming (O'Dell, 2001). The Hubble Space Telescope (HST) has shown that hundreds of these stars are surrounded by protoplanetary disks (the so-called "*proplyds*" – externally illuminated protoplanetary disks). Recently, we found a large population of proplyds in the even harsher environment of the Carina nebula (Smith et al., 2003). These proplyds are being destroyed by the intense UV light emitted by nearby massive stars. Mass-loss rates from disks in the Orion nebula range from 10^{-8} to 10^{-6} Solar masses per year (e.g., Henney and O'Dell, 1999; Bally et al., 2001), implying that a "minimum solar nebula" disk survives only for 10^4 to 10^6 years (Johnstone et al., 1998; Bally et. al., 1998a, b; Bally et al., 2000). If planets form from such disks, they must do so before the disk is destroyed.

Recent observations indicated that most stars are born in groups in which low-mass members can be ejected (e.g., Reipurth, 2000) or cannibalized by their companions (Bonnell et al., 1998). Thus, the star-forming environment may also pose *dynamical* hazards to protoplanetary disks. The jets and outflows produced by young stars during birth provide fossil records of violent dynamical histories (e.g., Reipurth and Bally, 2001). Infrared and radio wavelength observations indicate that over 90% of stars are born in multiple star systems (Reipurth et al., 2001; Reipurth et al., 2002). Thus, the outer parts of most proto-planetary disks surrounding low-mass stars may be destroyed either by UV radiation or by encounters with sibling stars.

Planets *may* form in such hostile environments, if they can do so rapidly by gravitational condensation from the disk (Boss, 2002), or sufficiently close to their parent stars to avoid the

radiation or dynamical hazards of the star-formation environment. Indeed, we found evidence for large dust grains at the translucent outer edge of a large protoplanetary disk seen in silhouette against the Orion Nebula (Throop et al., 2001). The attenuation of background light was found to be "gray" below a wavelength of 2 μm, indicating that most dust grains responsible for attenuating background light are an order of magnitude larger than typical interstellar particles (Shuping et al., 2003). These results are consistent with the predictions of grain evolution models in protoplanetary disks (Throop et al., 2003) and may signal the first steps of planet building. Protoplanetary disks in Orion-like environments may form gas giants rapidly by gravitational collapse (e.g., Boss, 2002). However, there may not be sufficient time for the standard core accretion scenario (e.g., Lissauer et al., 1995). Furthermore, terrestrial planets can form if solids are sequestered into centimeter to meter-sized "gravel" by the time disk gas is removed.

3.5.3 Proposed Research

During the next five years, we will quantify the hazards faced by nascent planetary systems and probe proto-planetary disk structure and compositions at infrared (IR) and radio wavelengths. We will take advantage of new facilities and emerging technologies such as NASA's Space Infra-Red Telescope Facility (SIRTF), adaptive optics on the 8- to 10-meter telescopes, infrared cameras and spectrometers operating at wavelengths from 1 to 30 μm, and emerging new radio wavelength capabilities. These tools will enable us to probe the physical and chemical properties of protoplanetary systems throughout the spectrum with an angular resolution which in some cases will be better than that provided by the HST.

Quantifying hazards to planet formation: Is the Orion nebula region typical of the type of environment in which most stars form? What fraction of proto-planetary disks evolve through a proplyd phase during which they are exposed to harsh and destructive UV radiation fields? What fraction of stars form in multiple systems or larger groups in which protoplanetary disks are truncated or destroyed by star-star encounters? Our own data, and public-domain data sets such as 2MASS (2 micron all-sky survey) and the SIRTF Legacy Science programs, will enable us to study the numbers and distributions of young stars in a representative sample of nearby molecular clouds including Taurus, Ophiuchus, Cepheus, Perseus, Serpens, and Orion (e.g., Ungerechts and Thaddeus, 1987; Dame et al., 2001). We have been investigating these clouds for two decades, first at millimeter wavelengths using carbon monoxide (CO) as a tracer of molecular gas and proto-stellar outflows (e.g., Bally and Lada, 1983; Bally et al., 1987, 1991; Miesch and Bally, 1994; Miesch et al., 1999), and more recently at visual wavelengths using the large-format CCD cameras at the National Optical Astronomy Observatories (NOAO). With our NSF funded "Survey of Nearby Star Forming Clouds" (Walawender et al., 2003; Bally et al., 2003), we obtained narrow-band images in Hα and [SII] to identify young Hα emission line stars and proto-stellar outflows (e.g., Reipurth and Bally, 2001). We propose to combine this optical data with public-domain infrared data (2MASS and SIRTF Legacy) to first identify young stars, and then to study their distribution in a representative sample of nearby clouds.

The NASA 2MASS survey covers the whole sky in three near-infrared bands (wavelength of 1.2, 1.6, and 2.2 μm) to a limiting magnitude of about 14 to 15. Since Sun-like stars at the 460 pc distance of Orion have magnitudes slightly brighter than the 2MASS limits, and most young stars are considerably brighter than "main-sequence" stars, this data-base can be used to identify young (0.1 to a few million year old) Sun-like stars to beyond the distance of the Orion star forming region. NASA's SIRTF

Legacy science program, which will produce a rich harvest of 5 to 240 μm infrared data on many nearby star forming clouds, will enter the public domain in early 2004. Specific data sets which will become accessible include: The Perseus molecular cloud and parts of Orion (Evans et al., 2001), star-forming clouds along the Galactic plane (Churchwell et al., 2002), and several hundred nearby stars with ages ranging from 10 to over 500 million years (Meyers, 2001).

Young stars will be identified using well established criteria including excess near-infrared emission originating from the warm inner part of protoplanetary disks (2MASS, SIRTF), the presence of the 0.656 μm Hα emission line of hydrogen, and the association with an outflow (determined from visual wavelength images, narrow-band infrared data, CO radio maps). This effort will lead to a quantitative estimates of the fraction of stars born in environments where UV radiation destroys disks, and the fraction likely to be affected by dynamical interactions.

Tracing Disk History with Near-IR Observations: Our recent research (e.g., Reipurth and Bally, 2001) has shown that outflows and their symmetries provide an indirect means to probe the recent accretion and dynamical history of forming stars and their disks. We will collaborate with Jon Morse (U. Colorado) to make extensive use of a new instrument being built for the 3.5 meter Apache Point Observatory telescope, the Near Infrared Camera / Fabry Perot Spectrometer (NICFPS; Jon Morse, P.I.). NICFPS, to be commissioned in early 2004, will have field-of-view of 5' and a velocity resolution of 10 km/s, and will acquire three-dimensional data cubes that simultaneously probe both the spatial distribution and velocity field of targets. We will study the structures and velocities of outflows from young stars. However, NICFPS does not have the narrow-band filters needed to carry out our research. Thus, we are requesting funding from this grant to purchase the narrow-band filters required to isolate the spectral lines of H_2, CO, and several bright lines of ions (e.g. [FeII]). (Details on the filters are described in the equipment section in vol. 2.) We will measure the radial velocities of bright emission lines produced by proplyds (such as the 2.12 μm line of H_2) to trace motions of disks and surrounding gas. Disk velocity measurements will enable us to determine the velocity dispersion of the ensemble of young stars to a precision of a few km/s. Combining this with the cluster density constrains the rate of interactions with sibling stars. Are some stars ejected as runaways from multiple star systems? Have such systems retained their disks? NICFPS will be used to study outflow symmetries that trace recent dynamical interactions in clustered star forming environments. "S-shaped" outflows indicate changes in disk orientation; "C-shaped" flows indicate motion of the source through a medium, or a side-wind which deflects the flow.

Measuring Disk Properties with Adaptive Optics Observations: Radiation-induced mass loss rates from proto-planetary disks have been measured only for a few proplyds lying very close to Orion's most massive stars (Henney and O'Dell, 2000; Shuping et al., 2003). Furthermore, these measurements were obtained at visual wavelengths where resolution is limited to about 1 arcsecond by atmospheric turbulence. We will obtain new near-infrared (λ = 1 to 2.4 μm) measurements using adaptive optics (AO) to compensate for this turbulence. AO-assisted 8- to 10-meter telescopes can deliver images limited in resolution only by diffraction, making them as sharp or sharper than those produced by HST.

Last year, Mark Morris, Ralph Shuping (UCLA), and I used the AO system at the 10-meter Keck telescope to demonstrate these techniques on several proplyds in Orion (Shuping et al., 2003). We will continue collaborating in the acquisition of AO assisted images and spectra (with a spectral resolution of about 50,000)

of many more proplyds, extending measurements to the outer parts of the Orion nebula where radiation fields are weaker and more typical. We will measure flow velocities, densities, ionization state, and disk rotation speeds using the many spectral features accessible in this wavelength range such as hydrogen and helium recombination lines, lines of H_2, and the vibrationally excited "overtone" bands of CO (e.g., Greene and Lada, 2000) with ~0.1 arcsecond resolution. These measurements will lead to estimates of central star masses, disk mass loss rates, and survival timescales. AO assisted images of disks seen in silhouette against nebular background light ("silhouette proplyds"; e.g., McCaughrean and O'Dell, 1996; Bally et al., 2000; Throop et al., 2001; Shuping et al., 2003) will establish the wavelength dependence of the attenuation through their translucent parts. In some disks, we will be able to measure the wavelength dependence of reflected (scattered) light from the central star. These measurements will constrain particle size distributions and may provide further evidence of grain growth in protoplanetary disks.

Probing Disk Composition in the Thermal IR: Morris, Shuping, and I have also used a λ= 5 to 24 μm camera at Keck to obtain new images of the core of the Orion nebula and the proplyds located close its bright and massive stars. We were also awarded time to use a new camera on Gemini South for similar measurements in the other parts of Orion. Unfortunately, the Thermal-Region Camera/Spectrograph (T-ReCS) was not ready in time for our observations. We will re-propose to use this facility instrument to extend our studies of Orion's and Carinas's proplyds to a wavelength of at least λ= 24 μm. Thermal spectroscopy will provide data on disk composition as prominent bands of solid silicon, graphite, polycyclic aromatic hydrocarbons (PAHs), various ices (water, CO, CO_2, etc.), and gases such as CO_2 occur in this spectral range. Perhaps these thermal observations may detect warm accreting giant proto-planets forming in the nearest disks.

Probing "Invisible" Disk Components at Radio Wavelengths: We propose to conduct exploratory studies of proplyds using the Very Large Array (VLA) radio telescope in New Mexico to measure of the mass of cold dust, characterize grain sizes in the opaque centers of disks, to search for trace molecules such as SiO and HC_3N, and 21 cm atomic hydrogen. Prior attempts at millimeter wavelength mass determination of Orion's proplyds have only provided limits (e.g., the λ= 1.3 mm study of Bally et al., 1998). However, the new λ = 7 mm system at the VLA will provide an order-of-magnitude improvement in sensitivity. If grain growth models are correct (e.g., Throop et al., 2003), and disks contain a substantial reservoirs of mm-sized (or larger) particles, the detection of λ = 7 mm continuum radiation at the VLA should be easy. While making these continuum measurements, we will also use the VLA's spectral line system to search for molecules whose transitions fall within the observed band such as SiO and HC_3N. Disk photo-evaporation models also indicate the presence of substantial coronae of atomic hydrogen above and below the disk. We will attempt to use the VLA to detect neutral atomic hydrogen in either emission or absorption (against the background nebula) at λ = 21 cm. The detection of strong radio-wavelength spectral lines would open a new window for the future study of proplyds with NRAO's Very-Long Baseline Array (VLBA) which delivers sub-milli-arcsecond resolution (corresponding to a fraction of the Earth-Sun distance for disks in Orion) – an orders of magnitude improvement over present methods.

Personnel and Resources: The work described above consists of two tasks: archival data mining, and the acquisition, reduction, and analysis of data obtained at various observatories. Successful execution will require that we manage between 2 and 4 observing runs per

year at several private (Apache Point and Keck) and public (Gemini, and VLA) observatories for the next five years. I have supervised a program having a similar scope during the past 5 years in which we used over a half-dozen facilities, typically, with between 3 to 6 separate observing runs per year. I managed this program with one or two collaborators and one to two students. Our publication record during this period provides concrete evidence that we have successfully reduced, analyzed, and published the resulting data.

Astronomers must propose year by year for telescope time, and time allocations on ground-based facilities come with no funding. I have a strong history of winning telescope time for my research. But, to conduct a coherent program on planet formation in addition to star formation processes, I need funding from astrobiology to support travel, salaries, and data acquisition, reduction, and analysis.

The astrobiology component of this research will be leveraged by support from NASA and NSF via complementary funding which supported research with HST and the investigation of the large-scale aspects of star forming regions. I continue to seek support for ongoing studies with HST, investigations of the large-scale structure of star-forming clouds, and outflows from young stars through NSF and NASA. Funding from astrobiology is needed to support the high-resolution multi-wavelength investigation of proto-planetary disks and planet formation. Funding from this program will be used to support part of my summer salary, a full-time graduate student, travel to various observatories, and the purchase of near-infrared filters essential for our research.

The work proposed here can be conducted by myself, one supported student, and collaborators. Other students, hired on NSF and NASA grants, will be trained on the same instruments and techniques. However, astrobiology funding is essential for the application of these methods to the investigation or proplyds and other proto-planetary systems.

4. THEME 3: PHILOSOPHICAL AND SOCIETAL ISSUES

One thing that sets astrobiology apart from many other scientific disciplines is the ways in which the science interacts with the broader society. Ranging from using astrobiology as a way to better understand the nature of science to exploring the public's fascination with the topic and the societal implications, astrobiology provides a powerful way to better understand these interactions. Our group at Colorado is one of the few groups in the country that from the beginning has exploited the connections between the sciences and the humanities, and we will continue to explore these connections during the coming five years. In particular, we wish to better understand the powerful insights that philosophy of science can provide for our science, to examine the societal interest in astrobiology and the influence on culture, and to explore the long history of the relationship between astrobiology and evolution.

A key goal of the analysis, and of including topics from the humanities along with research tasks in the sciences, is to better understand the connections between science and philosophy of science. We are doing this by approaching many of the same questions from the perspective of the scientist and from the perspective of the philosopher of science. Only by approaching the boundary between the two disciplines from both sides can we better understand the nature of this boundary and of the different disciplines, and thereby promote useful dialog between the two fields.

The specific tasks that we will carry out are as follows, and they are described in more detail in the following text.

(i) The nature of historical science and the elucidation of biosignatures (task led by Co-I Cleland). We will use astrobiology as a means

by which to explore the nature of historical sciences from the perspective of the philosophy of science. We will use case studies of well-known examples (such as ALH84001 or the recent controversy over the fossil microbes studied by Schopf) to explore the nature of science, and will develop a full-blown theory of historical science. Also, we will use our previous work on the difficulty of defining life to better understand the proper interpretation of biosignatures in measurements from other planets, especially with the potentiality of coming across really weird life.

(ii) Societal issues in astrobiology (task led by Co-I Jakosky). We will explore the connections between science and society by improving scientists' understanding of the nature of science, examining the societal significance of astrobiology, and discussing ways in which the science community can enhance the connections between scientists and both philosophers of science and the public.

(iii) The evolution of astrobiological thought (task led by Co-I Friedman). Well known evolutionists explored the origin of life and the potential for life elsewhere and, in fact, anticipated many of the questions now seen as central to astrobiology. We wish to understand the historical context of our field, and will examine the interface between biology and evolution as played out over the last two centuries.

(Note that tasks ii and iii are a formal part of our proposal but do not utilize any funding from the NAI. They are being done at no cost to NASA.)

4.1. The Historical Character of Astrobiology and Circumventing the Problem of Defining "Life" (task led by Co-I C. Cleland)

My proposed research program for the next five years is two-pronged. First, I plan to develop a philosophical theory of historical science with special emphasis on issues pertaining to astrobiology. I will focus on the pivotal role played by (i) "signatures" (faint, indirect traces) as evidence and (ii) computer simulations as surrogate experiments. This is particularly important to astrobiology because (as discussed below) much of the research is historical in character and extensively utilizes both resources. Second, I plan to investigate general strategies for searching for extraterrestrial life in the absence of a definition of "life". This issue is crucial to astrobiology given the insurmountable problems associated with defining "life" (Cleland and Chyba, 2002) and the tendency of astrobiologists to design instrument packages for searching for extraterrestrial life in light of a favored definition of "life".

There are fundamental differences between historical research and experimental research. Historical hypotheses are concerned with particular past events vs. timeless regularities. The pivotal evidence for historical work comes from fieldwork—from searching the messy, uncontrollable world of nature for traces of long past events—as opposed to performing controlled experiments in the contrived setting of a laboratory. Much of astrobiology is historical in character. Research into whether Mars once had standing lakes, Earth's early atmosphere was highly reducing, cyanobacteria existed on Earth 3.5 bya, and meteorite ALH84001 contains fossilized martian life provide salient examples. Moreover, most astrobiological research that is not explicitly historical nonetheless confronts similar problems. The hypothesis that Europa has a large subsurface water ocean is based on indirect evidence (e.g., fracture and tilt patterns observed on its icy crust). Thus, questions about the methodology of historical science and its differences from experimental science are of central importance to astrobiology.

Previously, I showed (Cleland, 2001, 2002) that historical and experimental scientists have access to different evidential relations. The source of this difference lies in an objective and pervasive feature of nature, a time asymmetry of

causation. More specifically, localized present events (the ultimate locus of all observation) *over*determine their localized past causes and *under*determine their localized future effects. This means that the present contains many disparate traces (e.g., pumice, ash, masses of basalt) of a past event (volcanic eruption). The work of historical scientists reflects this; they search the present for pivotal traces of past occurrences, and the overdetermination of the past by the present guarantees that such traces are likely to be numerous in both quantity and kind. In contrast, the conditions that experimentalists deliberately bring about in the lab are only partial causes of what subsequently happens; they underdetermine it. One can thus never fully exclude the possibility of a false positive or a false negative. This explains why experimental work is focused on "controlling" for interfering conditions. In short, the differing practices of historical scientists and experimentalists are grounded in an objective asymmetry in the evidential relations at their disposal.

I propose to develop the above framework into a full-blown philosophical theory of historical science. Understanding the nature of the evidential relation between a body of traces and a hypothesis is crucial to historical science, and particularly important to astrobiology given its extensive reliance upon "biosignatures". Most remote and *in situ* robotic investigations are designed to detect faint, indirect traces of life, e.g., isotopic records, biomolecules, geochemical anomalies. Without a clear understanding of what is required for a body of traces to qualify as a reliable evidential foundation for a hypothesis, it will be difficult to reach scientifically convincing conclusions about the presence or absence of life. Although the asymmetry of overdetermination guarantees that the present contains many traces of the past, traces become attenuated with the passage of time. Identifying traces of long past events typically requires sophisticated analytical techniques, and even then it is often difficult to interpret their significance. So much depends upon making correct assumptions about the environment that produced and sustained them until their discovery and subsequent analysis. Inadequate exploration of these assumptions can undermine what would otherwise be solid historical research. A pertinent example is the controversy over the use of carbon isotope ratios (in graphite inclusions from Akilia, Greenland) to infer the existence of 3.8 billion year old terrestrial organisms (e.g., Mojzsis and Harrison, 2002; Fedo and Whitehouse, 2002). In other words, successful historical research requires a stable evidential foundation, but by their very nature, highly indirect and attenuated traces seem unable to provide this.

I propose to investigate the logical, empirical, and social conditions under which indirect traces amplified by sophisticated analytical techniques become scientifically *trustworthy*. This will involve detailed case studies of scientific controversies hinging upon questions about the reliability of various putative "signatures", e.g., the controversy over the interpretation of carbon isotope ratios in quartz-pyroxene rock from Akilia (see above), the controversy over Schopf's interpretation of filamentous structures (found in Australian apex chert) as 3.5 billion year old terrestrial microfossils (e.g., Schopf and Packer, 1987; Schopf, 1999; Brasier et al., 2002; Schopf et al., 2002), and the ongoing debate over the significance of the prismatic magnetite in ALH84001 (e.g., Thomas-Keptra et al., 2000; Freidmann et al., 2001; Golden et al., 2001).

The use of computer models as surrogate experiments is another subject that warrants attention. Computer simulations are common in astrobiology. They are used to "test" conjectures about, e.g., the evolution of the solar system from the solar nebula, the chemical composition of the Earth's early atmosphere, and the structure of Europa beneath its icy crust. To the extent that they manipulate different assumptions

about the unobserved causes of observed traces, computer simulations resemble experiments; they yield "predictions" that can be compared with old observations and that suggest new observations. But they also differ from experiments because there is no guarantee that all the relevant conditions and processes operating in a targeted physical system are represented in the model; viewed from this perspective, they more closely resemble specialized theories than experiments. There is thus a pressing need to understand the nature of the support offered by computer models for hypotheses. I propose to address this problem by studying concrete examples (see above) of the use of computer models in astrobiology. My focus will be on the complex logical relations that obtain between a computer model, the evidence upon which it is based, the "predictions" that are derived from it, and the hypothesis that it reputedly supports.

Most remote and *in situ* searches for extraterrestrial life explicitly or implicitly presuppose a definition of "life". The Viking experiments, which were designed to detect carbon metabolism, provide a salient example. In Cleland and Chyba (2002, 2003), I challenged the utility of using definitions of "life" to guide research in astrobiology. Definitions associate concepts with words. They cannot reach beyond our current understanding and tell us about the way things really are. Accordingly, no definition of "life" can provide a scientifically compelling answer to the question "what is life?" What is required to answer this question is a *theoretical identity* (analogous to "Water is H_2O" or "Temperature is mean kinetic energy") grounded in a truly general theory of living systems. Unfortunately, because our theorizing has been limited to life on Earth and all life on Earth shares a common biochemistry, we do not yet have such a theory. This puts us in a position analogous to someone from the seventeenth century trying to define "water" before the advent of molecular theory. No analysis of the seventeenth century concept of water (as a transparent, odorless liquid that is a good solvent) could have revealed that water is H_2O. Yet this is what is required to answer the question "what is water?"

This presents us with a dilemma: Without a definition of life, how will we recognize alien life if we happen to encounter it? This dilemma is exacerbated when one considers the possibility of encountering very simple but really weird alien life. Considerations from the history and philosophy of science suggest possibilities for addressing and ultimately resolving it. Insofar as it emphasizes detecting biosignatures, the search for extraterrestrial life has an historical character. While Ken Nealson and his team recognize the importance of *diversity* of measurement (Nealson and Conrad, 1999, 2001), there has been little discussion of *trustworthiness* (discussed earlier). Moreover, in the absence of *any* examples of non-Earth-like life, abstracting from the biological details of terrestrial life is very problematic. Without a collection of trustworthy biosignatures for *terrestrial* life we are unlikely to reach a scientific consensus regarding the presence of Earth-like extraterrestrial life. Trustworthy terrestrial biosignatures can also play a crucial (albeit different) role in detecting really weird alien life so long as they are not taken as definitive of life. They can help us to discriminate insignificant false positives and false negatives from genuinely ambiguous cases. Genuinely ambiguous cases are the important ones. They provide us with Thomas Kuhn's famous "anomalies" (Kuhn, 1962, Ch. VI). Anomalies are the best grist for the theoretical mill. They alone stand out clearly against the backdrop of our current theories, forcing us to think beyond "the box" and come up with better generalizations about life. It is only in the context of such ideas that we will be able to formulate the necessary *theoretical identities* required for a truly non-Earth-centric approach to the detection of extraterrestrial life.

The above ideas are tentative. They need further exploration and fleshing out. I propose to begin this task by investigating biosignatures for detecting ancient terrestrial life: Which biosignatures are considered most and least trustworthy? On what considerations are these judgments founded; do they reflect appropriate and consistent logical and empirical considerations? I will then compare these biosignatures to those currently being proposed for searching for extraterrestrial life. Can the differences between the two classes be rationally justified? Are they driven by implicit definitions or otherwise unwarranted abstractions from terrestrial life?

Work Plan: My work on the historical science task will be spread out over the full five years of funding, with the first half of this period being devoted to the evidential role of "signatures" in astrobiological research and the second half being devoted to the use of computer simulations in astrobiological research. I anticipate some trips to other NAI institutions for extended discussions with appropriate scientific experts. My work on the "definition of life" task will be carried out in parallel with my work on historical science. Christopher Chyba (SETI Institute and Stanford University) will collaborate on the problem of searching for extraterrestrial life without a definition of "life". He will consult on the scientific aspects of searching for life elsewhere, in the context of the philosophical issues described above; his expertise spans both areas.

4.2. Societal Issues in Astrobiology (led by PI B. Jakosky)

The tremendous public interest in astrobiology is at a level far beyond its practical relevance, and suggests a broader public interest in exploration. We can use this interest as a way to educate the public about science in general and to inform them as to the nature of scientific inquiry (what science is and, of equal importance, what it is not). We will explore the underlying issues regarding the nature of science and its role in society and to encourage activities on the part of the astrobiology community that emphasize these connections (see Jakosky, 2000; Jakosky and Golombek, 2000; Bennett et al., 2002). As our objective is to engage the science community and to understand the nature of their science, this is a "science" rather than an "outreach" task. Specific activities will include:

(i) We will explore how astrobiology as a science operates and contrast the different approaches to doing science from the different disciplines (e.g., historical vs. experimental science). We will do this using specific case examples, with the goal of enhancing connections between disparate disciplines by helping researchers to understand approaches that differ from their own.

(ii) We will investigate the public's interest in astrobiology as a means of exploration, its interactions with the science community, and the role that exploration science plays in society. We will do this by identifying those parts of astrobiology that particularly attract the public's attention, relating them to the components that are most central to the science, and understanding the role of discovery and exploration science in astrobiology.

(iii) We will work with the astrobiology community to enhance the effectiveness of public outreach activities by addressing specifically these underlying issues that are of most interest to the public. We will do this by organizing science forums to discuss the societal connections at astrobiology workshops and conferences and by representing these goals in professional societies and advisory committees. We also will take the ideas directly to the public through various opportunities with the media and by working with students at all levels (e.g., the recently completed undergraduate textbook by Bennett, Shostak, and Jakosky).

Results will be presented at conferences and workshops, in popular science venues, and in a book on astrobiology, science, and society.

4.3. The Evolution of Astrobiological Thought (led by Co-I W. Friedman)

The launch of the NASA Astrobiology Institute in 1997 marked a major initiative to meld the fields of spaces sciences and biology in an effort to gain insights into the question of whether life exists elsewhere in the universe. This interface between biology (particularly the study of the history of life on Earth) and evolution of the universe actually has a remarkably rich intellectual history that goes back at least to the 1840s. Among the major 19th century individuals who can be viewed as the first astrobiologists are Baden Powell (1796 – 1860), Robert Chambers (1802 – 1871) and Alfred Russel Wallace (1823 – 1913). Each of these individuals wrote explicitly about the origin (or origins) of life on Earth and considered the question of the origin and evolution of life elsewhere in the universe (within, of course, the context of 19th century cosmology). What is most impressive is how many of the central questions associated with the NASA Astrobiology program in the 21st century were anticipated by these early thinkers. During the next five years, we propose to explore the intellectual history of early evolutionary and astrobiological thought, and to focus specifically on those who wrote and speculated about the likelihood and requisite conditions for the origin and evolution of life elsewhere in the universe. The goal is to understand the history of intellectual thought about life elsewhere and to relate it to current thinking. Results will be presented as a collection (and analysis) of the writings of these (and other) early evolutionists; negotiations for publication are in progress with the University of Chicago Press.

5. THEME 4: ASTROBIOLOGY TECHNOLOGY INITIATIVE

5.1. Introduction

One of the high-level components of the mission of the NAI is "providing scientific and technical leadership on astrobiology investigations for current and future space missions and for ongoing research programs". However, the NAI currently does not have a strong connection to the flight programs except through the separate activities of its individual members. We propose to form an NAI Focus Group on Astrobiology Technology (ATFG) that includes representation from the science, instrument technology, and mission technology components of astrobiology. The ATFG would (i) work with the community to define astrobiology science goals that can be addressed by flight missions still being defined, (ii) foster community development of appropriate technological capability to address the science goals, through both individual instrument concepts and mission concepts, and (iii) provide leadership in developing science community awareness and understanding of technology capabilities and advances and of the instrument and mission process within NASA.

Astrobiology instrumentation is in the germination state relative to NASA's space flight mission opportunities. This is in large part because of the relatively recent increase in the importance and role of astrobiology in space science. As a result, the infrastructure does not exist within the astrobiology community to define, develop, propose, build, and operate instruments and missions that emphasize astrobiology science goals. This problem is apparent, for example, in the current planning within the Mars program; it is not clear that the necessary instruments can be brought to appropriate technology readiness levels in time to propose for the 2009 Mars Science Laboratory mission.

This lack of a strong connection between the NAI and flight missions is of concern because it is through these flight programs that NASA

makes many of the astrobiology-related measurements. This is especially the case in that astrobiology science goals have moved to the intellectual center of the space science programs, as described, for example, by both the 2002 NRC Decadal Survey for Solar System Exploration and the recently completed Code S Space Science Roadmaps for all themes. The strongest formal connection at present between the NAI and the flight missions is through the NAI Focus Groups. The Mars and the Europa Focus Groups have emphasized providing short-term advice on missions and instruments. However, even these groups have not been involved in the long-range planning that is necessary for the community to develop specific instruments and missions that could address high-priority science goals. For example, the March 10-11, 2003, NAI-sponsored workshop on Mars life detection technology has been postponed twice in the last year, and now will occur relatively late in the planning for the 2009 MSL mission. And, the new opportunity to develop a Jupiter Icy Moons Orbiter (JIMO) will require strong integration between the astrobiology and flight mission communities in order to develop the scientific ratonale and intrument and mission concepts.

Our efforts will involve stimulation of the community through short courses, workshops, and the preparation of white papers on astrobiology technology that will help to meld the science and technology communities into a coherent group capable of proposing cutting-edge astrobiology investigations. Leadership will be provided by science researchers at CU who span the breadth of relevant disciplines, technologists at CU who have been involved in the development of low-cost space flight instruments and missions, technologists at Ball Aerospace Corporation (in Boulder, CO) who have been heavily involved in space flight instruments and missions that are pertinent to astrobiology, technologists at Lockheed Martin Astronautics Corp. (in Denver, CO) who have been intimately involved in development and operations of Mars orbiters, landers, and Scout mission concepts, and biotechnologists at Carnegie Institution of Washington (in Washington, D.C.) who are involved in state-of-the-art development of biotechnology hardware for space flight opportunities.

5.2. Building community through an astrobiology technology initiative

Building a rapport between scientists, instrument developers, spacecraft engineers, mission designers, and operations specialists has been key to some of the most outstanding success stories for planetary exploration. Such rapport invariably derives from common levels of understanding of the challenges facing each subdiscipline and a willingness to find appropriate solutions to enhance scientific success without compromising mission success. Growth in Principal Investigator savvy occurred long ago in the solar system and the astrophysics communities, enabling both fields to take great advantage of NASA missions. However, this growth has not yet occurred within the astrobiology community.

Despite the now-substantial investment in technology, the astrobiology community has not yet become fully engaged in the process. The NASA Astrobiology Science and Technology Instrument Development (ASTID) program and the Astrobiology Science and Technology in Exploration Program (ASTEP) were created to help address this technology gap. In addition, astrobiology-related instruments are being developed through the Mars Instrument Development Program (MIDP) and the Planetary Instrument Definition and Development Program (PIDDP). Each program is operating without substantial interactions with the community as a whole, and there is little overall community insight into what instruments and concepts are being developed, little community involvement in the directions necessary to achieve scientific

goals within astrobiology, little feedback to the community as a whole on how the development is going or what instruments and mission concepts exist, and no public forum for discussion of what directions or concepts are important in instrument development. It is apparent that many of those who are doing technology development do not fully understand the science and many of those who understand the science often do not know how to move forward with the technology. As a result, the efforts as a whole remain relatively immature, although there are a few groups that are able to work effectively. While NASA centers could be providing the oversight and leadership to ensure that necessary community and technology are being developed, this is not happening at present. As a result, there is substantial risk that the necessary technology and instruments will not be developed in a timely or effective manner.

We propose to act as a catalyst to foster and encourage discussion of the technology issues within the broader astrobiology science and technology community, to bring together members of the community for discussion of science and technology goals and issues, and to provide leadership to ensure that necessary and appropriate technology issues are addressed.

It is important to recognize that we are not proposing to, alone, provide direction for the program as a whole. No individual group will have sufficient background and expertise to do this, although we do bring together a combination of science and engineering expertise that may be unique in the astrobiology community. We also are not proposing to the NAI and NASA for funding to develop technology ourselves. Such an investment would not be appropriate for the NAI in general. Finally, we are not asking NASA for funding that we would distribute to other groups to develop technology. That is the purview of the technology programs at NASA and not of one group within the NAI.

By itself, formation of an NAI Focus Group on Astrobiology Technology would not be sufficient to address the technology and flight mission concerns raised here. While such a focus group would provide a forum in which technology issues could be discussed, it would attract interest mainly from those (primarily scientists) who are already members of the NAI, would not provide an opportunity to get participation from industry, and would not provide the leadership from the combination of science and industry perspectives that we envision. By proposing a technology initiative here, we will involve identified leaders from both science and industry right from the beginning who can provide the necessary leadership to promote development of the community as a whole.

5.3. Cross-disciplinary issues to be addressed

Our goal is to stimulate interactions between the communities representing astrobiology science, flight instrument design and operations, spacecraft and mission design and operations, and biotechnology. Below we address technology issues in instrument and spacecraft design and operation and in biotechnology that need to be understood within each group in order to develop appropriate instrumentation.

(i) Space instrument and mission technology

This topic includes a series of activities that are intended to develop the capability of astrobiologists to integrate technology into the discipline of space mission programs. Focus is oriented to maximizing scientific progress. Flight design parameters important to accommodation for spaceflight include not only weight, shape and volume constraints, but also field-of-view and stability requirements such as thermal, pointing, and dynamic mechanical environment. Many instruments are sensitive to condensable volatiles and/or particulate contaminants, and to electromagnetic interference and electrical grounding schemes. A variety of data processing methodologies are now possible, and inves-

tigators should become familiar with ways in which to accomplish these, as well as a familiarity with contemporary and advanced approaches to instrument programming, safing, diagnostics, and fault tolerance.

Workshop case studies will include practical examples of "best practices" methods as well as approaches to instrument design and implementation that can be counterproductive. Tutorials will include topics on instrument development scheduling, selection and back-up of advanced technologies, methods in risk mitigation, and use of form, fit, and function engineering models and experiment brassboards in early prototype testing.

Surveys of spacecraft characteristics will include those of flybys, orbiters, landers, rovers, and penetrators. Environments of particular interest to astrobiologists, such as the atmospheres of Venus and the gas giants, and the surfaces of planets and comets will be surveyed, with particular emphasis on Mars, Europa, comets and Titan. Understanding special challenges such as the unique radiation environments of deep space and planetary radiation belts will be essential for certain investigations.

For those instruments which sample the environment directly, whether gases or solids, the nature of sample needs in terms of quantity and quality can be paramount to the success of the investigation. Planetary atmospheres and surfaces are prime candidates for *in situ* investigation, but pose major challenges for reliable sampling. Sample acquisition and processing options are often as critical as the measurements to be made.

It would also be very useful to increase the astrobiology community's awareness of the power of remote sensing technology capabilities, from cameras or spectrometers near solar system targets, all the way to extrasolar observing platforms such as Astrobiology Explorer (characterizing potential biomolecular components in the interstellar environment), Kepler, and Terrestrial Planet Finder.

(ii) Biotechnology

One of the under-exploited areas of the interface between biology, engineering, and space sciences has been the application of biotechnology techniques to solar system exploration. The development of these techniques for space flight would fulfill a unique purpose in space exploration — the detection and characterization of organic and prebiotic chemistry distributed throughout the solar system, with implications for finding life elsewhere in our solar system. The current techniques used for solar system exploration have provided ambiguous results or failed completely to detect indications of life; this may be a combination of inadequate technology and the lack of understanding where and how to look.

Ideally, any organic detection instrument developed for space exploration must be able to detect and distinguish several classes of organic molecules of astrobiological interest. Biotechnology techniques have been used successfully to detect a large majority of the key biomolecules and, with the correct controls, could distinguish between spacecraft biomarker contamination and a true extraterrestrial signal. The obvious exceptions are molecules based on nonterrestrial biochemistries. Current immunoassays, such as Enzyme-Linked Immunosorbent Assay (ELISA), are known to be 150 times more sensitive and a great deal more specific than the best lab-based GCMS instruments (Li et al., 1999), and the Limulus Amebocyte Lysate (LAL) has proven to be a valuable resource for diagnosing extremely small quantities of microbial cell wall material (less than 0.1 picograms of initial biomarker signal) (Wainwright and Child, 1999).

Many of these techniques have been miniaturized for commercial lab applications and hence the marriage of nanotechnology and biotechnology has brought about the birth of "lab-on-a-chip" commercial instruments. BioMEMS and Microfluidics are fundamental to this new

paradigm of exploration science, but there is still development and research required to prepare this technology for flight.

These techniques have been developed primarily for clinical chemistry or medical diagnostics carried out in laboratory environments. Hence, although the fundamental technology (e.g., for Agilent/Caliper's RNA assay chip) is small in size, development of the support instrumentation to drive these systems has so far paid little or no attention to volume or mass requirements, or to concerns about the thermal, pressure, gravity or radiation environment to which these systems would be subjected during a space flight mission. Also, since these instruments would be primarily laboratory based, many require some amount of human interaction in the preparation of the sample prior to analysis. Many biotech companies have also developed their technology for a "disposable" society, and there are several systems that are made out of materials that would never survive a long-duration space-flight mission. On the other hand, Biotechnology, BioMEMS and microfluidics are uncharted territory for most aerospace engineers. The design and flight development rules that apply to traditional macro systems simply will not apply when you enter the micro/nano regime. Hence, a collaborative effort or an enhancement of communication between spacecraft engineers and biotechnologists will be crucial for astrobiologists to effectively integrate biotechnology into research and expedition science.

5.4. Specific tasks proposed

We are proposing the following activities in order to address our objectives:

(i) We propose to create an NAI-sponsored Focus Group on Astrobiology Technology. This group would be constituted under the standard NAI rules and procedures for creating focus groups, and would be open to both members of the NAI and non-members. This group would be the formal unit that would organize workshops and short courses (described below), write white papers, etc. The focus group will be integrated with the astrobiology biotechnology focus group that Dr. Andrew Steele (Carnegie Institution of Washington) already has begun to organize. Dr. Steele supports the combination of these into a single effort, and is a formal Collaborator on the present initiative.

(ii) We will sponsor a series of meetings that would provide a forum on astrobiology science and technology issues. As our goal is to stimulate community discussion, there are a number of different types of forums that might be productive, and we would organize the different types as an experiment. These would include:

-Workshop on status of instrument and mission development, in which those who are funded by PIDDP, MIDP, ASTEP, and ASTID, as well as those who are not, would have an opportunity to present their results to and interact with each other and with the broader community;

- Workshop on science and technology needs, in which the community could discuss what the current technology status and development needs are and thereby provide input to the members and to NASA HQ on productive directions. The output product would be a series of white papers aimed at NASA HQ, and the workshops would be repeated regularly with different emphases (e.g., Mars, Europa, Earth-orbiting telescopic, planet-orbiting spacecraft remote sensing, extrasolar missions, etc.);

- Short course on astrobiology for space science technologists, to bring them up to speed on the science issues and directions and to provide guidance as to the needs of this community; and

- Short course on development of flight hardware and missions and mission operations for astrobiologists, to educate the scientists who have good ideas but don't know how to proceed. These workshops will fulfill the needs of astrobiologists for information which enables them to

develop investigations which are realistic and compatible with the constraints of space flight.

(iii) We will work with the astrobiology discipline scientist and senior scientist at NASA HQ, the astrobiology integration office at NASA/ARC, and the NAI to identify areas that have specific needs in terms of technology development issues, and we will convene community workshops to address the issues and, where appropriate, write white papers to document the current state of the art and the community needs. White papers and workshop results will be posted on the web for broad dissemination.

5.5. Roles of key participants

Key participants are listed below. Together, they will comprise a working group to oversee the activities described above.

Bruce Jakosky (P.I. of this proposal) and John Bally (Co-I) will lead the science input into the technology development initiative, with involvement as appropriate from the other CU Co-Is.

Michael McGrath (Co-I, Engineering Director, CU Laboratory for Atmospheric and Space Physics) will lead the efforts to integrate flight technology issues from the perspective of small-scale instruments and spacecraft, with participation from other engineering and technical staff. The group he leads has developed and flown instruments, spacecraft components, and spacecraft on earth-orbiting and planetary missions. Through these efforts, LASP has been a pioneer in technology and operations for small space instruments.

Benton Clark (Co-I, Chief Scientist, Lockheed Martin Astronautics Space Exploration Systems) and James Crocker (Co-I, LMA Vice President for Civilian Space Program) will lead the efforts to include spacecraft and mission design and operations. Their group at LMA built and is operating the Mars Global Surveyor and Mars Odyssey spacecraft, has built Mars landers, and is the industry partner for all four Mars Scout proposal finalists currently under consideration.

Harold Reitsema (Co-I, Head, Ball Aerospace Civil Space Systems Space Science Directorate) and Steven Kilston (Co-I, Senior Staff, Ball Civil Space Programs) will lead the efforts to incorporate large and telescopic instrumentation and mission design concepts. Their group has built a large number of instruments relevant to astrobiology, including most of the Hubble Space Telescope cameras, the IRAS and SIRTF infrared telescopes, the upcoming Kepler mission to detect habitable extrasolar planets, and the HiRISE camera for the 2005 Mars Reconnaissance Orbiter.

Andrew Steele (Collaborator, Carnegie Institution of Washington) will lead the efforts to incorporate biotechnology with space science flight opportunities. He is a leader in the pioneering of biotechnology and microfluidics in astrobiology space flight instrumentation development.

Noel Hinners (Collaborator, currently CU/LASP, previously both Vice President for Civilian Space Program at LMA and Associate Administrator for Space Sciences at NASA) will consult on the industrial perspective on technology development.

6. MANAGEMENT PLAN

All research, outreach, education, and other programs supported through the NAI will be coordinated and run through the University of Colorado Center for Astrobiology, which is a Center (operating under the rules and regulations for Centers at CU) within the Laboratory for Atmospheric and Space Physics and reporting through the LASP Director to the Dean of the Graduate School and the Vice Chancellor for Research. The Principal Investigator of this Proposal also serves as the Center Director.

Individual research tasks will be planned and carried out under the supervision of each Co-Investigator, using research facilities provided by or in conjunction with their home de-

partments. While the individual task descriptions present a five-year research plan, it is anticipated that each Co-I will make revisions to their plans in response to discoveries and new results, both within their own group and in coordination with the larger NAI and astrobiology research program. Two new Co-Is are being added to our program; one (R. Pappalardo) will be added in year 1, and the second (T. McCollom) will be added in year 2.

The Principal Investigator and science Co-Investigators together will comprise a steering committee to oversee astrobiology research, education, and other activities at CU. As appropriate, they will make decisions to reallocate funds, if appropriate, to make recommendations to add or delete Co-Is, to invest resources in specific activities that are a part of strengthening the astrobiology community, etc. The steering committee will meet at least once per semester, as it has for the past four years. Each Co-I has reviewed this proposal and endorses their participation (see accompanying letters of endorsement in Volume 2).

The Principal Investigator and technology Co-Investigators together will comprise a steering committee to oversee the technology development activities. They will work in coordination with NASA HQ and the NAI to ensure appropriate participation and representation in these activities on the part of the astrobiology science and technology communities and will make a special effort to reach out beyond the NAI community.

Resources will be managed through LASP, and funds will be allocated for use by each Co-I. LASP will maintain financial oversight of each account and will prepare financial reports and provide them to NAI and NASA as necessary.

Subcontracts will be made to Ball Aerospace Corp. and to Lockheed Martin Astronautics for participation in our technology initiative. These activities will be managed and oversight provided by a steering group comprised of the Principal Investigator, the Co-Is from each organization, and Collaborator Andrew Steele (Carnegie Inst. of Washington).

Education and public outreach activities will be managed and oversight provided by Dr. Emily CoBabe-Amman (LASP Education and Outreach Coordinator), in cooperation with the Principal Investigator.

The Principal Investigator will have final authority on allocation of resources, implementation of the program, and oversight of research and other activities, and responsibility for financial management and reporting to the NAI and to NASA Headquarters on a timely basis, subject to the rules and regulations of the University of Colorado and of NASA. The P.I. or, in his absence, a representative appointed by him will serve as a member of and participate in the activities of the NAI Executive Council.

Science Collaborators are scientific colleagues who have agreed (see letters in Volume 2) to participate in the research to be carried out by the Co-Is, as described in the science task descriptions, at no cost to this proposal unless otherwise articulated in the individual science task descriptions.

Astrobiology Collaborators are scientists who are active in various component areas in astrobiology, who work in the vicinity of CU (at CU or at Southwest Research Institute in Boulder), and who have agreed to participate in the activities of the Center for Astrobiology. They will contribute to the intellectual environment surrounding astrobiology at CU, and they provide a way for us to leverage the activities supported by the NAI into a larger astrobiology research, teaching, and outreach program. Astrobiology Collaborators have provided letters (see Volume 2) stating that they agree to participate in the University of Colorado Center for Astrobiology as described here. The Astrobiology Collaborators and their fields of interest are listed in Table 6-1.

We will foster interdisciplinary interaction and integration across our diverse group by

holding regular meetings of various combinations of participants. We envision the following types of interactions: (i) We will form local working groups associated with each of our three research themes, and involving the appropriate Co-Investigators, Collaborators, and members of their research groups. These will meet informally on a regular basis to share results and discuss research directions. (ii) We will have regular meetings of all of the Co-Investigators and senior researchers, to discuss research programs and new results in a small and informal setting. (iii) We will have less-regular informal workshops (e.g., up to several times per year) involving all of the participants in the Center for Astrobiology. (iv) As discussed in the section on strengthening the astrobiology community, we will hold monthly astrobiology colloquia, to bring in keynote speakers from outside of the Colorado group and to hear about the latest scientific results.

We are aware of the separate proposal to the NAI from Southwest Research Institute (David Grinspoon, P.I.). As we understand it, our proposals are complementary to each other. If both are funded, we intend to collaborate and to integrate the research and the education and public outreach plans of the two groups in order to take full advantage of the proximity of the two groups and eliminate possible duplication of education and public outreach activities, while at the same time keeping the programs distinct so that they can each achieve their independent goals. The P.I. of the SWRI proposal has agreed to be a Collaborator on our proposal.

Table 6-1. Center for Astrobiology Collaborators

Star and planet formation and evolution

Thomas Ayres (CASA), evolution of stars and the solar wind
Veronica Bierbaum (CHEM), interstellar environment and planetary formation
Luke Dones (SWRI), planetary evolution
Hal Levison (SWRI), planetary formation, evolution and dynamics
Ted Snow (APS and CASA), interstellar environment and planetary formation
Glen Stewart (LASP), planetary formation and evolution
Brian Wood (JILA), time variability of the solar wind of sun-like stars

Planetary evolution

Dan Baker (LASP and APS), planetary/solar-wind interactions
Clark Chapman (SWRI), planetary impacts and evolution of the solar system
Mike Mellon (LASP), Mars geological evolution
Nick Schneider (LASP and APS), planetary evolution

Planetary habitability

Mark Bullock (SWRI), evolution of planetary atmospheres and exobiology
David Grinspoon (SWRI), planetary evolution and exobiology
Jose-Luis Jimenez (CHEM), experiments related to aerosols in the atmosphere of early Earth
Sara Martinez-Alonso (LASP), Mars mineralogy, remote sensing, and potential environments
Alex Pavlov (LASP), aerosol formation and methane planetary atmospheres
Margaret Tolbert (CHEM), aerosol composition and radiative transfer in planetary atmospheres

Microbial diversity and life in extreme environments

Ruth Ley (MCDB), molecular analysis of microbes in hypersaline ecosystems
Diane McKnight (ENGR), environments and ecology in Antarctica
Steven Schmidt (EPOB), microbiology in psychrophilic environments
John Spear (MCDB), molecular analysis of microbes in hypersaline and hydrothermal ecosystems

Origin and evolution of life

James T. Hynes (CHEM), theoretical studies of peptide bond formation under pre-biotic conditions
Veronica Vaida (CHEM), chemical evolution and origin of life

Societal and philosophical issues

Tom Yulsman (JOUR), origins, science, and society

[Unit abbreviations: APS = Dept. of Astrophysical and Planetary Sciences, CASA = Center for Astrophysics and Space Astronomy, CHEM = Dept. of Chemistry and Biochemistry, ENGR = School of Engineering, EPOB = Dept. of Environmental, Population, and Organismic Biology, JILA = Joint Institute for Laboratory Astrophysics, JOUR = Dept. of Journalism, LASP = Laboratory for Atmospheric and Space Physics, MCDB = Dept. of Molecular, Cellular, and Developmental Biology, SWRI = Southwest Research Institute in Boulder]

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8. STRENGTHENING THE ASTROBIOLOGY COMMUNITY

8.1 Introduction

The NASA Astrobiology Institute has several distinct objectives as part of its mission, as expressed in its mission statement. First and foremost is research — both of the highest quality within the component disciplines and as collaboration between groups and disciplines as befits a research Institute. The other objectives include training astrobiologists, providing astrobiology leadership and input into the NASA flight programs, developing and using innovative approaches to collaborative research, and education and public outreach. These non-research goals fall under the heading of the title of this section, strengthening the astrobiology community, and are just as important as research in the long run. To some extent, it is our collective willingness to participate in these "non-research" activities that provides the impetus to be a part of an astrobiology institute; without this participation, members of the NAI easily could go to the individual Research and Analysis Programs for funding on the individual research tasks that are being proposed. Thus, first-rate research programs are a necessary starting place for NAI participation, and the efforts on the part of each team to strengthen the astrobiology community, individually and collectively, provide the added value that molds the teams into a program and an Institute.

The CU group has been a leader in developing the astrobiology community from the beginning. We have taken and will continue to take a leadership role in defining astrobiology, developing its formal and informal connections to the themes and programs within Space Sciences at NASA, and working to forge a single community out of a disparate group of disciplines.

Here, we list a few of the ways in which we have been leaders in developing the astrobiology community in the last few years, and then develop these further, along with our specific proposed activities, in the remainder of this section.

- Our group (through P.I. Jakosky) led the effort on the part of the NAI Executive Council to develop a series of white papers on the relationship between astrobiology and solar-system exploration. This was done as input into the NRC report on a Decadal Strategy for Solar System Exploration in 2001-2002, and resulted directly in that group integrating the two fields very effectively. This is exactly the type of synthesis that must be done in order to gain support for astrobiology from non-astrobiologists (who often, incorrectly, see astrobiology as a very narrow and non-scientific discipline).
- Our group (through Co-I Pace) is playing an important role in the Ecogenomics Focus Group, which is investigating the interplay of organisms in a hypersaline ecosystem in Baja California. This effort among a number of the existing NAI teams represents the type of collaborative effort that only the NAI can do, by bringing multiple approaches and techniques to bear on a single problem that spans different fields. The CU contribution to this effort, of determining the nature and diversity of organisms that exist in the ecosystem, is a fundamental component that is central to the overall program.
- Our group (through Co-I Mojzsis) is co-leading the Mission to Early Earth Focus Group. This Focus Group was formed when a consensus emerged within the astrobiological community that progress in the area of "bioenvironmental reconstruction" is fundamentally sample limited. To make informed decisions about where and what to look for in the astrobiological exploration of the solar system and beyond requires us to draw on the geological record provided by

the only planet that unequivocally has life — Earth. The group has organized field workshops and is central to the currently planned program of drilling in ancient rocks.

Our group of Co-Investigators at CU is built largely of researchers who excel both at their science and in providing programmatic and intellectual leadership to the community. While we could *propose* to help to develop and strengthen the astrobiology community, instead we *have played* leadership roles in these activities over the past five years and will continue to do so.

In addition, the University of Colorado has a strong commitment to developing astrobiology as a research endeavor and as a program. This commitment has included new faculty hires, commitment of funds, and other commitments, and is detailed below.

In the following sections, we outline the roles that we have played and our proposed efforts in areas where this is appropriate.

8.2 Education and Public Outreach (E and PO)

Astrobiology provides a marvelous hook on which to hang public outreach and education about science. Everybody has an interest in it, whether the topic is about microbes on Mars, habitable planets around other stars, or even UFOs and alien abductions. Thus, it provides an excellent opportunity to interact with the public at all levels, to teach about the nature of science, and to discuss its role in our society. We heartily embrace these goals, and are pleased to be able to propose and participate in a vigorous E and PO program.

No single approach to E and PO is valid for all programs or all groups. This is analogous to informing people about the news — some are satisfied with the sound bites on CNN Headline News, some like the slightly more detailed discussions on the national network news programs, others prefer the details at a level heard on National Public Radio, and still others prefer the greater detail in the New York Times. So it is with E and PO. We take the attitude that we wish to provide some information to a large number of people, and then to reach smaller numbers of people who desire larger amounts of information or greater involvement.

The E and PO program that we are proposing is substantially augmented over our previous efforts. We propose the following tasks, each of which is described below: (i) Public symposia held at CU to delve in depth into a given topic for the local University, Boulder, and Denver communities. (ii) Extramural public/university symposia ("travelling show") to take this excitement to other institutions that do not normally have access to this information. (iii) Collaboration with the Denver Museum of Nature & Science to prepare a planetarium show and materials for dissemination to classrooms in Colorado that reach literally hundreds of thousands per year. [Note that our Co-Is participate in E and PO on an informal basis by giving public talks, presenting colloquia at other institutions, writing for the popular media, doing interviews with the media, etc. These are discussed elsewhere and are not a formal part of our E and PO effort. Also, we will discuss grades 13-16 (i.e., college level) along with graduate education in the section on training.]

To coordinate our efforts, LASP (the parent unit of the Center for Astrobiology) has hired an outreach coordinator, Dr. Emily CoBabe-Ammann. She will be a key person in implementing the proposed activities and in developing additional activities over the next five years.

8.2.1. Public symposia

The Center for Astrobiology has been running annual public symposia for more than four years that reach out to the local University, public community, and high-technology groups in the Boulder-Denver metropolitan area. For these, we have brought in nationally known ex-

perts and our own astrobiology faculty in exciting areas in astrobiology, and presented a coherent symposium on different aspects of a specific topic for an evening, and included visits to CU classrooms and interactions with students and faculty. Topics in the past have included the Mars exploration program, intelligent life in the universe, the nature of life, and the discipline of astrobiology. Each symposium has included a discussion of the science from multiple perspectives as well as discussion of the implications to the public or to society. Some of our speakers have been Don Brownlee, Ben Bova, Seth Shostak, Joel Achenbach, Norman Pace, William Calvin, Benton Clark, and Eugenie Scott. Public participation has been outstanding, with as many as 500 people attending each event.

We will continue these public symposia at an anticipated rate of 1-2 per year for the coming five years. These will allow us to continue to interact with the local community, something that fills the needs both of the University and the community. The emphasis of the continuing symposia will be on new and exciting topics. We will associate the symposia with spacecraft launches or arrivals (such as the Mars Exploration Rover missions to Mars or the Cassini mission to Saturn, both of which have strong astrobiology connections) or major discoveries that are announced. For example, CU presented a public symposium in 1996 on the potential for life in the martian meteorite ALH84001, held only three weeks following the public announcement, drawing on local scientists for analysis, and attended by over 600 people.

8.2.2. Extramural public/university symposia

The public symposia allow us to reach a large audience, but one that generally already is scientifically literate, technologically adept, and can (and will) take advantage of access to alternative sources of similar information. We also will develop a new program of extramural public symposia (informally called our "travelling show") to visit places that generally are not able on their own to support this type of activity. We are targeting 2- and 4-year colleges and universities that do not have sufficient resources to support visits by leading researchers, and schools that serve historically underrepresented populations. Our goals are to take cutting-edge science to places that often do not have access to it, and to expose students and faculty to the excitement of astrobiology. We will use the CU Co-Is to create public symposia on the exciting new results and their astrobiological significance, typically spanning the breadth of topics and disciplines in astrobiology. We will visit other institutions at no expense to them, put on an evening public symposium for their faculty, students, and the local community, visit in small groups with students and faculty, and, where appropriate, give more-technical seminars to the upper-level students and faculty.

We are planning to visit three institutions per year, both in Colorado and around the country. We have committed to a "tune up" symposium as a demonstration of concept and to work out the bugs of the logistics and the program on April 8, 2003, at Ft. Lewis College in Durango, CO. Ft. Lewis College is a 4-year undergraduate school located in southwestern Colorado and having a substantial population of Native American students. This first symposium will involve our Co-Is William Friedman (talking about the origin and evolution of life on Earth), Bruce Jakosky (on the possibility of life in the rest of our solar system), and John Bally (on habitable planets outside of our solar system). In addition, we have tentatively planned our first travelling show following selection to be at Hampton University in Virginia; they are a four-year historically black school with which we have informal ties through other venues.

8.2.3. Science journalist Workshops

We will educate and inform the public via a series of workshops intended to provide background material to science journalists on issues in astrobiology. The goal is to inform the writers of the deep background of the science issues, so that they can better report on the issues. Our first workshop will be held in December 2003, and will deal with the Mars landings to take place in December and January. Emphasis will be on the missions, the science to be done, and the astrobiological connections.

8.3. Professional Community

The CU Center for Astrobiology has been and will continue to be at the intellectual center of forging the discipline of astrobiology. This has been done by participating in activities designed to turn the disparate disciplines into a single coherent field, by representing astrobiology on high-level national advisory committees, and by other activities on the part of the individual team members. Our participation and leadership is shown by example, using a listing of the recent activities with which we have been and are currently involved, both locally at CU and nationally within the astrobiology community:

NAI Focus Group active participation

Mission to Early Earth Focus Group (Mojzsis, co-chair)

Mars Focus Group (Jakosky and Mojzsis, members)

EcoGenomics Focus Group (Pace, member)

Europa Focus Group (Pappalardo and McCollom, members)

Astrophysics Focus Group (Bally, member)

Advisory Committees (recent and current)

NAI Executive Council working group to provide astrobiology input to NRC Decadal Solar System Exploration Survey group (Jakosky, Chair)

NASA Solar System Exploration Subcommittee (Jakosky and Pappalardo, members)

NASA Mars Exploration Program Analysis Group (MEPAG) (Mojzsis, member; Jakosky, member and incoming chair)

NASA MEPAG Astrobiology Sub-Group (Jakosky and Mojzsis, members)

NASA MEPAG Pathways Sub-Group (Jakosky, member)

NASA Astrobiology Roadmapping team (Jakosky, member)

NASA Astrobiology Shared Foundations Working Group (Jakosky, co-chair)

NASA ASTEP (Astrobiology Technology in Exploration Program) program review panel (Jakosky)

NRC Committee on Planetary and Lunar Exploration (Pappalardo)

NRC Solar System Exploration Strategy Committee, Steering Committee Member and Vice-Chair of Large Satellites Panel (Pappalardo)

NRC Committee on the Origin and Evolution of Life (Pace)

NRC Mars Sample Return Task Group (Pace, Jakosky)

NSF Molecular Biochemistry Review Panel (Copley)

Board of Directors of the Monterey Bay Aquarium Research Institute (MBARI) (Pace, member)

USRA Lunar and Planetary Science Institute Science Council (Jakosky, member)

Key invited talks

AAAS Symposium on “The search for life beyond the solar system” (Pace)

NSCORT Annual Symposium, Rensselaer Polytechnic Institute, keynote talk (Pace)

"The solar system: A user's guide", NAI primer talk, 2003 NAI biannual meeting (Jakosky)

First Annual McDonnell Distinguished Lecture, Washington Univ. St. Louis (Jakosky)

Origin of Life Gordon Conference invited talk, 2002 (Mojzsis)

Origin of Life Gordon Conference session chair, 2003 (Jakosky)

Journal editorial boards and associate editorships

"Astrobiology" (Jakosky, Mojzsis)

"International Journal of Astrobiology" (Jakosky)

"Geobiology" (Jakosky, Pace, and Mojzsis)

"Environmental Microbiology" (Pace)

"Geophysical Research Letters" (Pappalardo)

"Bioorganic Chemistry" (Copley)

Other professional activities relevant to astrobiology

Organizing and leading NAI Insight course in planetary science for non-planetary scientists, summer 2003, Flagstaff (Jakosky; Pappalardo, participating lecturer)

Denver Museum of Nature and Science, curator for astrobiology planetarium show (Bally)

American Geophys. Union, annual fall meeting, organizer for session on Mars rivers and climate (Toon)

Division of Planetary Sciences, Amer. Astron. Soc., annual meeting science program cmte. (Pappalardo)

Author of "The Search for Life on Other Planets" (Cambridge U. Press, 1998, used as textbook in numerous courses and sold to the public) (Jakosky)

Co-author of "Life in the Universe" (Addison Wesley, 2002, non-majors undergraduate textbook on astrobiology) (Jakosky)

Seminar series

CU Center for Astrobiology monthly colloquium series

CU Center for Astrobiology monthly journal club series

NAI journal club series (initiated by CU in 2002 prior to becoming an NAI-wide activity)

Regular participation as both speakers and audience in the NAI monthly seminar series

8.4. Training

The University of Colorado has a vigorous program of training for undergraduate students, graduate students, and post-doctoral fellows. Each is described below:

Undergraduate program. CU offers numerous undergraduate courses in astrobiology and astrobiology-related areas and plans to explore the utility of an undergraduate minor. Our two key courses are:

(i) Undergraduate non-majors course in astrobiology, titled "Extraterrestrial Life". This course has been offered each semester for the past five years. It draws typically 75 students per semester from all across campus, including students who are majoring in the sciences, engineering, the humanities, the social sciences, business, and so on. Topics include the entire range of sciences that comprise astrobiology, along with the philosophical and societal issues, and, perhaps most importantly, the nature of science and the role of science in society. Course has used the book by Jakosky, and currently uses the book by Bennett, Shostak, and Jakosky.

(ii) Senior-level/graduate course on astrobiology, science, and society. Intended for science majors, we explore the nature of science, the history of astrobiology, the role of science in society, and the philosophical significance of searching for life elsewhere. Course is offered in alternate years (was taught in 2002, and will be taught again in 2004).

Other relevant undergraduate courses include planetary atmospheres; planets, moons, and rings; evolution; microbial ecology and life in extreme environments.

We will explore the desire on the part of undergraduates for an undergraduate minor, as an adjunct to majors in any of the biological or physical sciences. We have no plans to offer an undergraduate major, as it is in the best interests of the students to focus on a single discipline; however, a minor will expose students to the breadth of astrobiology and encourage them to pursue it at the graduate level.

Graduate program. CU offers a graduate certificate in astrobiology and several related courses.

Our graduate certificate program was approved in 2002 and continues on an ongoing basis. It is not a formal degree in astrobiology, but instead provides additional coursework and recognition in astrobiology, and can be thought of as a graduate-level equivalent of an undergraduate minor. We have no plans to offer a formal degree, as it is in the students' best interests to remain in their home department and receive in-depth training in a single discipline. The certificate offers the opportunity to become exposed to the entire breadth of astrobiology. Required courses include a graduate course in astrobiology, a course in history and philosophy of science, and two additional science courses from outside of the student's home discipline (e.g., a biology student will take courses in physical sciences, and vice versa).

Graduate-level courses specific to astrobiology include:

(i) A one-semester graduate course in astrobiology, intended for students in all of the relevant disciplines. That course is offered in alternate years, has been taught twice now, and typically has about 20 students enrolled. Topics include the entire breadth of disciplines that comprise astrobiology and appropriate integration to address the questions of life in the universe. Reading consists of approximately 100 papers (original research papers, review papers, book chapters, etc.).

(ii) A course in the nature of life from the philosophy of science perspective, taught by a formally trained philosopher of science (Co-I Cleland). Topics include the logic of definition, critical evaluation of popular scientific definitions of 'life', problems with defining 'life', and the nature of the justification of astrobiological hypotheses.

(iii) A reading group for graduate students, postdoctorals, and faculty to explore the writings of a select group of evolutionists who can be viewed as the first astrobiologists (e.g., Robert Chambers, Alfred Russel Wallace, Baden Powell). Each of these individuals wrote explicitly about the origin (or origins) of life on Earth and considered the evidence for the origin and evolution of life on Earth within the context of 19th century cosmology (e.g., the nebular hypothesis), and anticipated many of the central questions associated with astrobiology.

We also offer research training for graduate students. Students do dissertation research on areas related to astrobiology, working with any of our Co-Investigators or Collaborators. Funding comes from the NASA support through the NAI or through individual NASA, NSF, or other grants held by the Co-Is or Collaborators.

Post-doctoral program. Through our individual research programs, we train post-doctoral research associates in astrobiology. Most of the Co-Is have supported and continue to support post-docs, and we are regularly contacted by researchers wishing to do post-docs with us. We currently have three NAI post-docs in our program at CU, working in very different parts of our program (Ruth Ley, microbial ecology; Sara Martinez-Alonso, Mars remote sensing; Alex Pavlov, early Earth atmospheric physics and chemistry).

8.5. Teaming with Minority Institutions

CU is an active participant in the NAI Minority Research Program that brings in summer faculty fellows. As described in our Education and Public Outreach section, we will be actively working to expose underrepresented groups to the excitement and science in astrobiology through our travelling symposia. And, we have informal connections with faculty at Hampton University in Virginia (a historically black college) that we are working to formalize.

8.6. Staff

Co-Investigators in the CU Center for Astrobiology contribute substantial time in support of this proposal in addition to that which is funded explicitly through the NAI. This time is supported via state funds, through the academic-year salaries of each Co-I. The estimated fractions of time integrated through the work year that each science Co-I will spend on activities connected to this proposal are as follows: Bally 20 %, Cleland 30 %, Copley 8.5 %, Friedman 10 %, Jakosky 25 %, McCollom 20 %, Mojzsis 20%, Pace 20%, Pappalardo 15 %, Toon 10 %, Yarus 15 %.

CU and LASP are contributing one work month per year of senior IT/Tech person in support of our virtual institute and other hardware activities.

Estimated dollar value of staff, faculty, and IT/Tech commitments are included in the section on "Summary of institutional commitment of resources".

8.7. Flight missions

CU Center for Astrobiology Co-Is are actively involved in NASA flight missions that are pertinent to astrobiology, including participation in active missions, in missions currently under development (i.e., funded), and missions in advanced planning. Our participation includes (but not including participation on the part of our technology partners at Ball Aerospace and Lockheed Martin):

Active missions

Mars Global Surveyor (Jakosky, Interdisciplinary Scientist)

Mars Odyssey (Jakosky, Co-Investigator, Thermal Emission Spectrometer/THEMIS)

Galileo (Pappalardo, Affiliate member, Imaging science team)

Missions in development

Mars Airborne Regional Survey (ARES, one of four Scout proposals currently in Phase A development) (Jakosky, Co-Investigator)

MESSENGER (Mercury Discovery mission scheduled for 2004 launch) (McGrath)

SIRTF (Space Infrared Telescope Facility) Science Center Oversight Committee (Bally)

Ball Aerospace Terrestrial Planet Finder architecture study team (Bally)

Formal mission planning oversight

NASA/JPL Mars Science Laboratory Project Science Integration Group (Jakosky)

Europa Orbiter Science Definition Team (Pappalardo)

Europa Orbiter Analysis Team (Jakosky)

Mars Exploration Rover (MER) Landing Site Steering Group (Jakosky)

Mars Odyssey Project Science Group (Jakosky)

Mars Global Surveyor Project Science Group (Jakosky)

NASA Jupiter Icy Moons Orbiter Science Definition Team (Pappalardo)

8.8. Information Technology

The CU Center for Astrobiology will continue to participate in activities designed to take advantage of the "virtual institute" aspect of the NAI. We will participate in videoseminars, videoconferencing, development activities led by

the NAI of desktop video capability, and development activities led by the NAI of new approaches for file sharing and exchange. In particular, we have been utilizing the videoconferencing equipment for point-to-point discussions with individuals on other NAI teams, and will continue to do so. One example of this is the ongoing collaboration between the teams at CU, Penn. State Univ., NASA/Ames, and NASA/JPL on the physics, chemistry, and climate of Earth-like planets and implications for the early solar system and for extrasolar planets (CU participation led by Co-I Toon); this group is ramping up its use of videoconferencing for science group meetings and beginning to make active use of the equipment.

8.9. Linkage to Other Agencies

CU Center for Astrobiology Co-Investigators have substantial research programs that extend well beyond the NAI. As a result, the NAI gets the benefit of and participation from a much larger research program. Here, we list astrobiology-related programs that are not funded through the NAI and have not been mentioned elsewhere, with full details being provided in Volume 2 under current and pending research grants:

NSF Petrology and Geochemistry (pending), Clues to the early crust in the chemistry of ancient (3900-4400 Ma) terrestrial zircons: Geochemical and geochronological studies of the oldest known terrestrial solids, up to 4.38 Ga zircon grains from Western Australia (Mojzsis).

NSF LExEn, Constraining the timing and nature of life's emergence (Mojzsis).

NSF Instruments and Facilities (pending), Acquisition of the ion microprobe SHRIMP for geological research: Creation of a new laboratory for geochemical studies of samples of astrobiological interest, geochronology, micro-scale geochemistry and isotopic biomarker development (Mojzsis).

NSF, Molecular Diversity and Structure of Photoendolithic Ecosystems, examining molecular (rRNA) diversity among endolithic ecosystems, Antarctic and Rocky Mountain (Pace).

NASA Exobiology Program (pending), Applying Geochemical Constraints to the RNA World (Mojzsis).

NASA Exobiology Program (pending), A Mission to Very Early Earth: Exploring the chemical record of habitability in ancient terranes and applying this knowledge to the search for life in the universe (Mojzsis).

NASA Exobiology Program, Astrobiological and Geological Implications of Convective Transport in Icy Outer Planet Satellites (Pappalardo).

NASA Planetary Geology and Geophysics Program, Remote sensing and geochemistry of planetary surfaces (Jakosky).

NSF, Division of Molecular and Cellular Bioscience (Molecular Biochemistry), MAA Isomerase to TCHQ Dehalogenase: Evolution in Action (Copley)

NSF Division of Molecular and Cellular Bioscience (Metabolic Biochemistry), Studies of Degradation of a Xenobiotic Compound: A Window on the Evolution of a Novel Metabolic Pathway at an Early Stage of Development (Copley)

NIH Division of General Medicine, Recruitment of Enzymes to Serve New Functions (Copley)

NIH Division of General Medicine, RNA structure and function studies via gene construction (Yarus)

NIH Division of General Medicine, RNA catalysis of translational reactions (Yarus)

8.10. Commitment of University of Colorado to Astrobiology

The University of Colorado has a strong commitment to astrobiology, both as a research endeavor and as an integrated program. It has made a substantial commitment of personnel

and resources, both to the original proposal five years ago and to this proposal. The specific commitments are summarized here, with dollar values for the current proposal appearing in the next section:

Hiring of two new tenure-track faculty specifically in astrobiology (Pace, Mojzsis).

Hiring of two new faculty in areas relevant to astrobiology (Pappalardo [tenure track], McCollom [research faculty].

Providing start-up funds to support the astrobiology-relevant research program (Pappalardo).

Commitment of matching funds on equipment, in both the original proposal and in this proposal.

Providing a Graduate Teaching Assistant each semester to enhance interactions between the teaching and research programs in astrobiology.

Providing financial support out of its general funds (committed by each participating unit, through the Laboratory for Atmospheric and Space Physics).

Providing physical space to house an astrobiology program office and staff.

8.11. Summary of Institutional Commitments of Resources for this Proposal

The University of Colorado and its partner institutions are investing their own resources into our astrobiology program, allowing us to leverage the NASA funding to support a broader and larger program. Details of in-kind contributions are itemized below, although with the dollar value deleted.

Additional financial support for research, E and PO, colloquia, etc., from the University of Colorado through the Laboratory for Atmospheric and Space Physics.

Matching funds on purchase of equipment, from the University of Colorado.

Graduate Teaching Assistant support for astrobiology from CU Graduate School, total value over five years.

Contribution in kind from Ball Aerospace Corp. in support of our technology initiative over five years.

Contribution in kind from Lockheed Martin Aerospace in support of our technology initiative over five years.

Contribution of one month of senior IT/Tech support per year from CU LASP in support of the virtual institute and hardware aspects of our program.

Faculty contribution of time not supported directly through this grant.

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The Origin, History, and Distribution of Water and its Relation to Life in the Universe

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The Origin, History, and Distribution of Water and its Relation to Life in the Universe

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1 Executive Summary

Intellectual Focus & Proposed Research –

Water is the medium in which the chemistry of all life on Earth takes place. Water is the habitat in which life first emerged and in which all of it still thrives. Water has modified Earth's geology and climate to a degree that has allowed life to persist to the present epoch. We propose to create a research and education framework that links the biological, chemical, geological, and astronomical sciences to better understand the origin, history, distribution, and role of water as it relates to life in the universe. We focus on scenarios involving the sources and distribution of water in planetary systems and the delivery and incorporation of water into rocky planets that orbit within the "habitable zones" of their parent stars. Our framework will include and connect research on major aspects of planetary water – in effect we aim to understand the terms of a "watery Drake equation":

- Observations and modeling of the abundance and distribution of water in the interstellar medium, molecular clouds, and circumstellar disks;
- Surveys and studies of the numbers, water content, D/H ratio and dynamics of icy outer solar system bodies such as comets, Centaurs and Kuiper Belt objects;
- Laboratory experiments on complex molecule trapping and formation on interstellar water-rich grain mantles and planetary ices;
- Cosmochemical studies of meteorites that record the incorporation of water into silicate material in the primordial Solar System as an early step in its eventual inclusion in larger bodies, including planets;
- Models of the escape of water (as hydrogen) from the atmospheres of Earth-sized planets;
- Spacecraft- and meteorite-based research and theoretical studies on the role of water in forming the diversity of rocks and sediments on Earth, Mars, and Venus.
- Biological exploration of ice-covered habitats in Iceland, Antarctica, and North America with potential application to the search for life on Mars and Europa.
- Biological and chemical exploration of extreme aquatic habitats in and around the Hawaiian islands including the deep-sea, Kauhako Lake crater on Molokai and Lake Waiau near the summit of Mauna Kea.
- Development of concepts and prototype hardware for instruments that could be used to detect and characterize life on other planetary bodies.

Distinguishing Features –

By developing and testing models and exploring the outcomes of alternative scenarios we seek to determine what controls the abundance and distribution of water and hypothetical aqueous habitats in other planetary systems. This research will directly support the NASA search

for past or present life on Mars and efforts (such as the NASA Terrestrial Planet Finder) to directly detect and characterize Earth-sized exoplanets. The proposed framework of our research will support a group of postdoctoral scholars who will carry out independent, interdisciplinary research spanning two or more of the investigators' (or affiliated investigators') research specialties. The island setting and the unique facilities of the University of Hawaii offer an environment conducive to these investigations. The University of Hawaii houses world-class research groups active in astronomy, and in the chemical, geological and biological sciences. It is home to a substantial array of scientific facilities, including the telescopes of Mauna Kea Observatory, a fleet of research vessels for oceanographic and deep-sea investigations and facilities for exploration of the extreme environments found on the volcanoes and in the lakes and oceans around Hawaii. Through this Astrobiology proposal we aim to combine and capitalize on these many areas of research excellence to craft a new, interdisciplinary study of water and its relation to life.

Management Approach –

The UH-NAI PI will meet once a week with the Co-Investigators to discuss research progress, discuss any technical, financial or management issues, and to involve everyone in the E/PO activites. The timelines and status of the projects will be reviewed and updated at these meetings. In addition the UH-NAI will offer an astrobiology seminar series which will involve the entire local community. Additionally the development of our collaborative visualization tool will help foster interdisciplinary communications and highlight areas of new development which can be discussed at these regular meetings.

Education/Public Outreach Activities –

Our group has a strong record in education and public outreach, and we intend to build on that experience by providing innovative laboratory-based learning opportunities for teachers and engaging the public in our research. We intend to develop a program that trains teachers in the science of astrobiology, how to incorporate astrobiology into their classes, and in how to use the activities we will develop as part of the program. The focal point of our teacher-training program will be a summer professional development program. Using the summer workshop and research experience as a base, we will also develop a course for pre-service teachers. All projects will involve development of standards-based classroom activities. We also intend to actively engage the public in our discoveries.

Institutional Commitments –

The University of Hawaii and the Institute for Astronomy are strongly committed to the success of this project, and have commited a total of $4.5 million in faculty positions, in-kind salary support and cash contributions to the project.

Collaborative Networking Concepts –

We will design, implement and evaluate a software visualization tool to interface with the NAI ScienceOrganizer knowledge management tool.

2 Research and Management Plan

2.1 Water and Life in the Universe

"If there is magic on this planet, it is contained in water." – Loren Eiseley, *The Immense Journey*

We humans, and all life on Earth, are aqueous beings. Our cells are mostly water that has been exquisitely packaged in lipid membranes. They are cytoplasmic solutions of solvable molecules whose aqueous chemistry is responsible for cellular maintenance, growth, and reproduction. Water's chemical and thermodynamic properties make it an ideal medium for biological activity, so much so that it is considered essential for recognizable life. It has been called the "ultimate solvent" because of its ability to form multiple hydrogen bonds with solutes and its strongly polar character. It is also capable of serving as both an acid and a base. Strong hydrogen bonding between water molecules stabilizes the liquid phase over a wider range of temperatures compared to other cosmically abundant molecules, and it endows water with a high latent heat of vaporization. Unlike many compounds, water expands upon freezing and its ice floats, a property that is crucial for the persistence of aquatic habitats under freezing surface conditions. In addition to its direct role in biology, water is also a predominant determinant in the geology, geochemistry, and climate of our planet. It influenced the structure and early evolution of the Solar System itself, aspects of which dictate the habitability of Earth. Water and life are connected at many scales, from the interstellar medium to microbial habitats, and through many processes; astrophysical, geological, geochemical, and biological. We propose an Astrobiology research consortium that seeks to better understand these connections and their implications for the distribution, nature, and signature of life throughout the universe.

Water has been involved in life since its first appearance on the early Earth. The leading theories of the origin of life invoke prebiotic chemistry in low-temperature aqueous solutions, *e.g.*, a "warm little pond"[42] supplied with prebiotic molecules by atmospheric chemistry[143], or in the hydrothermal brines produced by high-temperature water-rock interactions[35]. The first three billion years of the drama of life on this planet was played out entirely in aquatic environments. The world's oceans and freshwater bodies remain the home for a large fraction of species at the present era and life has invaded and adapted to nearly every environment on the Earth's surface where there is some liquid water, almost regardless of temperature, pH, or chemistry.

Water is also involved in geochemical reactions that maintain surface conditions permissive of life. For example, aqueous weathering of silicate minerals in the presence of a CO_2-containing atmosphere produces alkalinity, which in turn allows CO_2 in the ocean-atmosphere system to precipitate as carbonate minerals and eventually become sequestered as carbonate rocks. Without these reactions, the buildup of volcanic CO_2 in the atmosphere would lead to intense greenhouse conditions similar to those on Venus. Instead, temperature-sensitive weathering and the concomitant deposition of carbonates constitute a negative feedback that may have regulated atmospheric CO_2 levels to compensate for the Sun's luminosity evolution over 4.5 billion years[214]. Water vapor itself is the most active greenhouse gas in the present-day atmosphere, amplifying the effects of other, incondensible gases by a factor of 2-3. Water also modifies the amount of solar radiation reflected by the Earth back to space, either as relatively absorbent oceans, as reflective ice, or as scattering clouds. These warm or cool the Earth and can create positive or negative albedo-temperature feedbacks. "Snowball Earth" epochs of runaway glaciation thought to have

been driven by such a feedback may have provoked major crises in the history of life[85, 111].

Finally, the presence of volatiles such as water significantly alters the rheology and thermodynamics of silicate minerals in Earth's crust and mantle. The presence of water in the mantle has a profound effect on its internal convective motions and the melting behavior. The formation of magmas involved in volcanism at hot spots and island arcs is thought to be driven by, or at least influenced by, the ability of fluxing water to depress the solidus of silicate rocks. Water may be responsible for a low viscosity zone beneath the brittle lithosphere[104] and may weaken faults, giving lithospheric plates increased flexure[168]. These last two may be critical to the operation of plate tectonics. Plate tectonics is responsible for much of the geologic activity on Earth's surface, including mid-ocean ridge and island arc volcanism at the margins of oceanic plates, uplift of mountain ranges by tectonic forces at converging continental plates, and deepening of basins where continental plates diverge. These geologic processes in turn drive many of the biogeochemical cycles important to Earth's habitability, particularly the weathering of uplifted silicate and carbonate rocks, the revolatilization and release of CO_2 from carbonates at subduction zones, and the burial of organic carbon in sediments and concomitant release of molecular oxygen.

Water is abundant on present-day Earth. Three-quarters of its surface is covered by oceans and models suggest that crustal and mantle rocks contain an even greater amount of water[200]. What was the origin of this water and how did it become incorporated in the Earth? The deuterium to hydrogen ratio (D/H) of seawater (1.56×10^{-4}) (and a similar value for mantle water) offers a constraint on the origin and subsequent history of Earth's water[119]. The total inventory and distribution of water on Earth was probably determined early in the planet's history and may have consisted primarily of water dissolved into a magma ocean in equilibrium with a dense steam atmosphere[174]. The D/H of seawater is similar to that found in certain primitive water-rich chondritic meteorites. This suggests that Earth's water was evolved from dehydration of hydrous minerals during the accretion of chondrite-like planetesimals (not necessarily represented by any of the extant classes of meteorites). Thus it is possible that Earth's water was evolved from dehydration of hydrous minerals during or after the accretion of chondrite-like planetesimals (not necessarily represented by any extant classes). The seawater D/H ratio is significantly lower than ratios measured in three long-period comets. While this appears to rule out long-period comets as primary contributors of Earth's water, the D/H ratios of other cometary types have not been measured. In particular, the short-period comets formed further from the sun and at lower temperatures than the long-period comets. Their nebular processing and resultant D/H ratios may have been significantly different from those of their long-period cometary counterparts. In addition, as we discuss later, the terrestrial planet abundances of the noble gases such as Ar, Kr and Xe are not easily explained by delivery from the asteroid belt, but more compatible with a cryogenic, presumably cometary source. For these reasons, it is too early to rule out a cometary source for Earth's water and other volatiles.

Water in precursor planetesimals could have derived either from hydration of silicate grains by water molecules in the primordial solar nebula, or at greater distances from the proto-Sun, by aqueous alteration of larger bodies composed of a rock/ice mixture[77] and their dynamical transport to accretion zone of the proto-Earth. Nebular water in turn traces its ancestry to the chemistry of the interstellar medium at temperatures of 10-20 K. The enrichment of Solar System water in deuterium with respect to the protosolar nebula (D/H = 2.5×10^{-6}), was the result of isotopic fractionation in the protosolar nebula, perhaps involving irradiation and ion-molecule reactions[186]. Irradiation of gas-phase or solid-phase water could also process organic material,

enriching it in deuterium, in the interstellar medium and proto-solar nebula. This processing may take the form of gas-phase reactions in the solar nebula, irradiation of icy grains, and aqueous alteration in the parent bodies of meteorites. Important prebiotic molecules such as amino acids and sugars could have been produced in these environments. This material would have been later brought to the Earth in comet-like bodies.

Earth has hosted surface waters throughout most of its history. The rock record preserves water-lain sediments at least as old as 3.5 billion years and the oxygen isotopes of zircon crystals support the existence of a hydrosphere as early as 4.4 billion years ago[223]. How has the total inventory of terrestrial water changed with time and how has it exchanged between reservoirs at the surface and in the interior? The current escape rate of water (as hydrogen) to space would have a negligible impact on the total inventory, even over 4.5 billion years, and is small compared to the fluxes between the surface and interior[119]. However, higher rates of escape may have accompanied an anoxic atmosphere and higher ultraviolet radiation from a younger Sun. The elemental and isotopic abundances of rare gases seem to require that a significant amount of hydrogen (perhaps some from water) was lost in a non-fractionating hydrodynamic wind[174]. Additional water (as hydrogen) could have been sequestered into Earth's core[169]. Because of volcanism, weathering, and the subduction of lithospheric plates, water is continually exchanging between the surface and mantle. A secular decrease of surface water (and uptake by the mantle) with time has been suggested, although this conclusion is highly model-dependent[18].

Like liquid water, life has a lengthy history on this planet. It is possible that for nearly as long as aquatic environments have been stable at the surface, life has been present to inhabitat them. Carbon isotopic fractionation in apatite grains from the 3.85 billion-year old Isua supracrustal belt (Greenland) has been interpreted as the product of biological carbon fixation, and microforms from Australian cherts have been described as the oldest microfossils[194]. Both of these interpretations have been challenged in the light of new geologic data[122, 19], and the exact temporal relationship between water and life on the early Earth remains unresolved. Though these early origins remain obscure, it is clear that life has subsequently expanded into, and adapted to, the entire range of aquatic habitats on the Earth, from anoxic seas to the extremely nutrient-poor open ocean, from seafloor hot springs to low-temperature brines in sea-ice, from highly alkaline lakes (pH $\sim$ 10) to the extremely acidic (pH $\sim$ 0) streams draining metal ore bodies. How has water physically and chemically interacted with the silicate component of the Earth over 4.5 billion years to create a physically and chemically diverse habitats for life? How has life evolved to adapt to those environments?

Humanity is extending its search for life beyond the Earth to other bodies in the Solar System, and that quest has been largely framed in terms of a search for water, especially water in its liquid state. Although the surface of present-day Mars is extremely cold and dry, a series of orbiting spacecraft have returned images containing geomorphic evidence for the presence of ground ice, and high concentrations of ice have now been detected in the uppermost meters of martian soil[57]. Spacecraft imagery has also revealed giant outflow channels and valley networks incised in ancient terrain, evidence that this water once flowed as a liquid[57]. Whether these landforms indicate that Mars once possessed a temperate climate, oceans and an Earth-like hydrological cycle is actively debated[33, 195, 71], yet it is clear that aqueous habitats existed, perhaps only transiently, near or at the surface of the planet. The more recent identification of small "gullies" on the walls of some craters and canyons[126], perhaps carved by expelled groundwater[69] or melting ice[36] has prompted speculation that suitable habitats for microbial life may persist to the present day. The search for

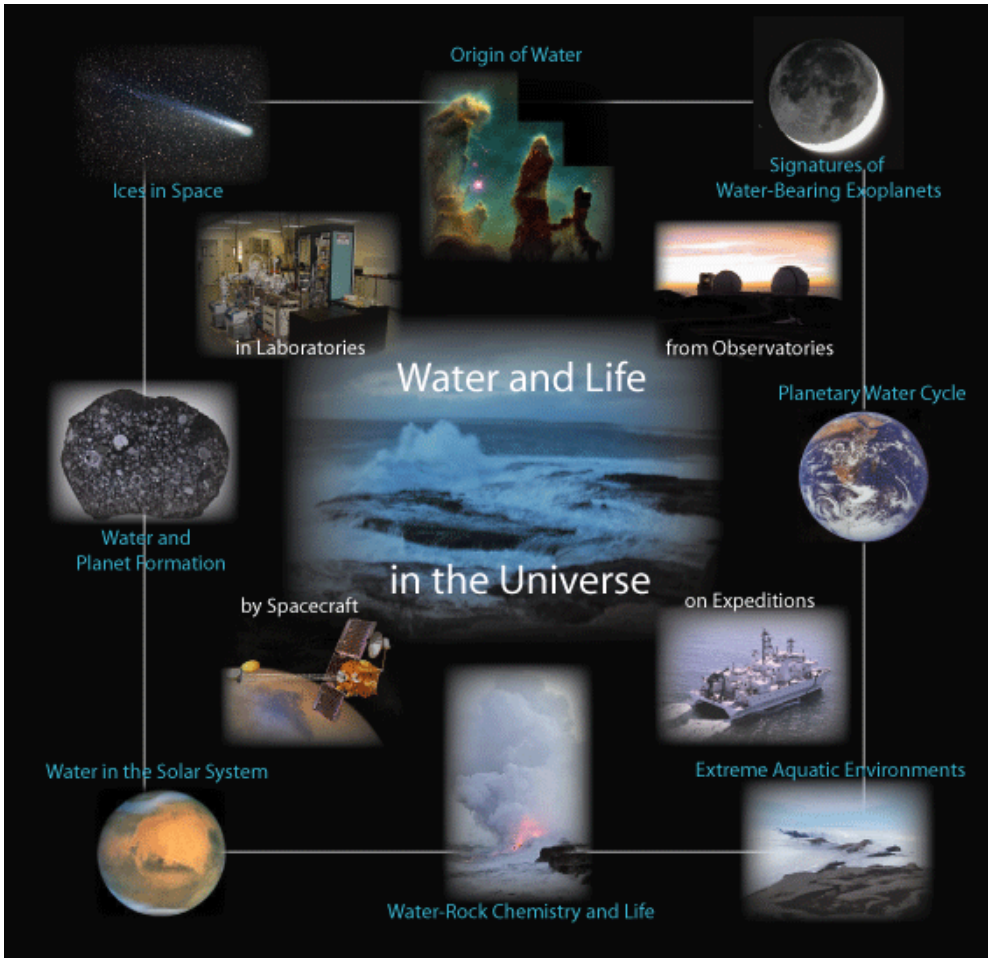
water is an integral piece of the NASA program of Mars exploration: current and future missions will use high-resolution and multi-spectral imaging to identify sites of past or present aqueous activity from orbit to be investigated by robotic landers and rovers.

The icy Galilean satellites of Jupiter, especially Europa, exhibit features indicative of active geologic resurfacing that may be related to the presence of interior liquid water maintained by tidal frictional heating in addition to heat released by the radioactive decay of long-lived isotopes[170]. The *Galileo* spacecraft's detection of an induced magnetic field around both Europa and Callisto can be explained by global interior oceans or layers of partially molten ice[226]. It is widely speculated that interior oceans could support life, assuming a suitable chemical source of energy replaces sunlight[184, 66, 130, 31]. The tidal-driven tectonics of the icy crust of Europa may allow liquid water to penetrate close to the surface[67]. Missions to orbit Europa, precisely measure the tidal flexure of its ice crust and probe its interior with radar have been considered. Longer-term concepts include plans to land on its surface and penetrate the crust to explore any ocean.

The scope of the search for life will expand dramatically as space observatories are developed to detect and characterize Earth-size planets around other stars[121, 11]. Current ground-based methods such as Doppler velocimetry are capable of detecting only giant gas planets[40]. Future, space-based searches will be based on the direct detection of a planet's reflected or emitted light. This will also allow their surfaces and/or atmospheres to be characterized both spectroscopically and photometrically (changes in full-disk light as the planet revolves around the parent star and rotates on its axis). Planets within the "habitable zone" of their parent star (the range of orbital semimajor axes where surface temperatures permit liquid water) will be of particular interest. Searches for potential atmospheric biosignatures (*e.g.*, methane or molecular oxygen)[47] will be accompanied by observations to determine the physiochemical conditions on the planet's surface and especially the thermodynamic state of water. The latter can be elucidated by searching for the spectral signature of atmospheric water vapor and the photometric variability produced by the planet's rotation and albedo differences between water, land masses, water vapor clouds, and ice. Further into the future, giant space observatories may perform a census of thousands of nearby stars and image the closest Earth-like planets to determine directly if they have oceans and continents, clouds and polar caps, a hydrological cycle and aquatic habitats like those on Earth.

Cosmically, water is not uniformally abundant, its incorporation into Earth-sized planets not necessarily constant, nor is its planetary manifestation as aquatic habitats suitable for life a given. The search for life in the universe must account for these variations and the uncertainties that accompany them. Water is formed in the interstellar medium and in the denser molecular clouds that give rise to star-forming regions. Differences in elemental abundance, gas-phase chemistry, and grain chemistry will result in measurable variation in the abundance of water in those regions. Water is a significant source of infrared opacity in collapsing cloud cores and protostellar disks; the efficiency at which the collapsing gas cools may control the stability against gravitational fragmentation and the formation of binary or multiple systems (including brown dwarf companions). The abundance of water in protoplanetary disks is a sensitive function of the oxidation state of the nebula and, to first order, the elemental ratio of carbon to oxygen: the C/O ratio has been observed to vary significantly from star to star, implying that there may be very "wet" as well as "dry" planetary systems[67]. Furthermore, the cosmochemical record in meteorites shows that a large range in oxidation states and water abundance existed in the early Solar System, perhaps as a result of removal of water from the warm interior of the primordial nebula and its condensation at greater distances[41]. Presumably, planets formed from different mixing ratios of this primitive

material would be endowed with different initial inventories of water. The total planetary inventory of water may also depend on the presence of giant planets and the efficiency of its dynamical transport as icy bodies from the outer regions of a planetary system[150].



The efficiency of incorporation of water into planets will also depend on the accretion history and subsequent escape of water back into space via hydrodynamic flow powered by the extreme ultraviolet radiation of the young parent star. Stars of different mass, metallicity (heavy-element abundance) and initial rotation rates will emit different levels of EUV radiation. The convection of planetary silicate mantles as they reject the heat from long-lived radioisotopes and the gravitational energy of formation will manifest itself as geologic activity and will drive the exchange of water between the surface and the mantle of these planets. Depending on the size and composition of a planet, that exchange will result in different partitioning of water between the surface and interior with obvious consequences for habitability. Further, the thermodynamic state of water on the surface (*i.e.*, liquid, vapor or ice) will depend on surface temperatures and atmospheric pressures which are determined in large part by the geochemical cycling of volatiles such as carbon driven by geologic activity and, ultimately, mantle convection. The tempo and mode of mantle convection appears to be sensitive to the amount of volatiles, particularly water, stored in the mantle itself. Finally, the process of biological adaptation may allow life to colonize and flourish not only in a variety of temperate aquatic environments, but also extreme environments such as low-nutrient oceans, high UV exposure lakes, high temperature deep sea vents, low temperature

subglacial lakes without sunlight, and even ice or settings where the only stable phase of water is steam. These adaptations will ultimately constrain the limits, distribution, and history of life relative to that of water in the universe.

We seek to investigate the astrophysical, cosmochemical, geological, and biological processes that link the history and distribution of life in the universe to that of water. This research will have eight interdisplinary foci:

- *The origin of water and the formation of stars and planetary systems*: This research will focus on mapping the distribution of water in the molecular clouds and cloud cores that are the precursors of stars and planetary systems; understanding the role of water as active molecule and potential tracer in the evolution of collapsing cloud cores, protostars, and protoplanetary disks, and measuring and modeling the participation of water in the protoplanetary disk chemistry that is recorded in pristine meteoric and cometary material.

- *Observational and experimental investigation of water ices in space*: Comet ices preserve a chemical record of the interstellar medium and early Solar System that can be explored by spectroscopy and imaging as the ices volatilize near orbital perihelion. Interpretations of these observations, however, requires laboratory measurements that include solid-phase reactions. Water ice serves as the energy-transfer medium and active participant in a variety of radiation-driven organic chemistry reactions thought to be important in either the interstellar medium or the primordial solar nebula.

- *The origin and distribution of planetary water*: The distribution of water in planet-forming disks, its incorporation of water into planet-forming material, and its eventual inclusion in planets such as the Earth is a fundamental cosmochemical problem. The D/H ratio of planetary atmospheres and potential sources such as meteorites and comets is a powerful means of constraining models of early Solar System water and continued measurement of D/H in additional comets and primitive meteorites will aid that effort. The record of aqueous activity in the parent bodies of chondritic meteorites provides information about the abundance and distribution of water in the primordial solar nebula and its subsequent conversion into the hydrous minerals that are a likely source of most of Earth's water.

- *Water and aqueous alteration on Mars*: Mars is the planet most resembling Earth, it contains unambiguous evidence for the activity of past and present water, and is probably the most likely to host or have hosted extant or extinct life. Studies of the history and action of water on this body compared to Earth are thus of great importance in this regard. These include understanding the potential for the hydrothermal and low-temperature alteration of crustal minerals and rocks by water, and the mechanisms whereby liquid water might be generated on, or brought to the surface of the planet at different epochs.

- *Water-rock chemistry and habitats for life*: The reaction between silicate rocks and water, particularly at high temperature, produces aqueous fluids and altered mineral surfaces whose thermodynamic disequilibria are potential energy sources for life. The role of both subaerial and submarine hotsprings influenced by such water-rock reactions has been well established, but the diversity of potential chemistries – and concomitant biological communities – has only begun to be explored. These include subseafloor hydrothermal systems in the oceanic crust near mid-ocean ridges, low-temperature systems further from the ridge

axis, cool springs generated by interaction of water with ultramafic rocks at subduction zones, and the interaction of lava and seawater at the surface of the earth.

- *Extreme aquatic habitats on Earth and their analogy to potential habitats elsewhere in the Solar System*: Evidence for water near the surfaces of Mars and Europa suggests there are potential aquatic habitats for life waiting to be explored. Yet these habitats may be very different from those typically found on Earth in terms of lack of sunlight, nutrient levels, high UV levels, or even the state of water itself. Approximate terrestrial analogs to such habitats do exist and will be explored to determine if and how communities of organisms have adapted to those conditions, and to evaluate strategies for the detection of life in those environments.

- *An integrated model of planetary water and its early history on Earth-like planets*: The processes of impacts, atmospheric escape, volcanic outgassing, weathering, and burial or subduction of hydrous minerals result in changes in the total inventory of planetary water as well as the exchange of water between surface and interior reservoirs. The importance of each process, and its effect on the time-dependent abundance of surface water (and hence the planet's ability to support life) will depend on the initial inventory of water, the astrophysical environment of the planet (*i.e.*, late rate of accretion, ultraviolet radiation from the parent star), its size, and its composition. A model of planetary water can be constrained using known or estimated fluxes and reservoirs of water on the present-day Earth, and can be used to explore the time-evolution of water on the early Earth, as well as Earth-size planets whose space environment or composition differ.

- *Signatures of water-bearing exoplanets*: The search for life – and water – will expand outside the Solar System with the eventual deployment of space observatories capable of detecting and characterizing Earth-size exoplanets and their environments. The escape of water from such bodies in young planetary systems may be directly detectable. In addition to searching for signatures of life on a planet, it will be desirable to measure the abundance, distribution, and state of water on its surface using photometric and spectroscopic data. Earthshine, the light from Earth reflected from the dark side of the Moon, provides a practical means of evaluating our capability to identify and describe similarly water-covered worlds around other stars.

With regards to the theme of Water and Life in the Universe, the University of Hawaii is unique in (1) having personnel whose research encompasses the role of water over the entire range of astrophysics, planetary science, chemistry, geology, and biology; (2) having unequaled access to the telescopes of the world's premier astronomical observing site to carry out water-related observations; and (3) being located near a variety of aquatic habitats, many found nowhere else near a major research university, (*e.g.*, the open ocean, a high-altitude lake, fumaroles, and a shoreline where lava enters the sea). To carry out our research on Water and Life in the Universe, we propose to create an interdisciplinary network of investigators and a core community of postdoctoral fellows. This organization will be based at the University of Hawaii but will have collaborators in several other countries. Each postdoctoral fellow will carry out independent, interdisciplinary research that connects two or more of the areas of expertise of the individual investigators involved in this proposal. The fellow will not be based with any one investigator but will instead be physically located in their own group, which we call the "Water Hole".

2.1.1 Origin of Water and the Formation of Stars and Planetary Systems

Objectives – (1) quantify the presence of water ice in the interstellar clouds, out of which stars form. (2) understand the structure, kinematics, composition, and physical properties of the circumstellar disks out of which planets form, thus defining the general environment in which water will exist. (3) determine the specific role of water ice in these chemical and physical processes.

Connections – §2.1.3
Astrobiology Roadmap Links – Goal 3; Objective 3.1
Researchers – Reipurth, Williams, Ceccarelli

Background – The interstellar medium contains the basic material out of which stars and new planetary systems form. Molecular clouds contain cores of gas and dust, which are cold and dense, and which can collapse, leading to the formation of a protostar and a surrounding rotating disk. By studying star and planet formation as it occurs at present in nearby dark clouds, we open a window on the processes that shaped our own solar system 4.6 billion years ago. The Institute for Astronomy at the University of Hawaii has just inaugurated the Center for Star and Planet Formation in order to develop and strengthen interdisciplinary connections between early solar system studies focusing on comets, Kuiper belt objects, and meteorites, and the analysis of present-day star formation events and newborn stellar objects. One of the prime goals of this effort is to trace the evolution of water from its origins in molecular clouds, its role as a major coolant in the collapse process, its inclusion into and processing within circumstellar disks, to its eventual incorporation into icy solar system bodies such as comets. Ultimately this will lead to planetary water reservoirs such as the oceans on Earth, thus forming the basis for the development of life as we know it.

Interstellar Chemistry and the Origin of Water

In the cold environment of dense interstellar clouds, volatile molecules freeze out onto dust grains and form icy mantles surrounding the silicate and carbonaceous cores. Grain surface chemistry allows for the formation of more complex molecules than is possible in the gas phase. These molecules, including water, are then liberated from the grain surfaces through heating by a young star. Determining the solid state processes in dense clouds is thus essential for an understanding of the chemistry and evolution of water ice. Observationally, there are three closely related approaches to explore the role of water ice in the star formation process.

Mapping the Distribution of Water Ice in Dense Cloud Cores

Water ice exists in the interior of molecular clouds; it is estimated that about 10% of oxygen atoms in molecular clouds are bound up as water ice[4]. For visual extinctions of less than $A_V \sim$ 2 - 5 mag, UV radiation from the interstellar radiation field destroys water molecules. For higher extinctions, the UV field gradually becomes sufficiently attenuated for ice to survive, and a linear relation is found between the optical depth of water ice τ_{ice} and A_V[221, 157]. Such observations can be carried out by observing background stars in the near-IR wavelength range thanks to a strong water ice absorption band at 3.1 μm (Fig. 2.1.1a).

Few studies have been made, since terrestrial water absorption interferes with observations. We will launch a major infrared study extending the early water ice studies between $5 < A_V < 20$ mag (Fig. 2.1.1b) to the much higher extinctions (up to $A_V \sim 100$) in the dense cores out of which solarlike stars are forming. This requires the superior high-altitude dry conditions at Mauna Kea and the availability of 8 - 10 m class telescopes, and the Institute for Astronomy is thus ideally positioned to successfully carry out such a project. Nothing is currently known about water ice absorption at such high column densities, and we will for the first time determine if the linear relation found at low extinctions continues.

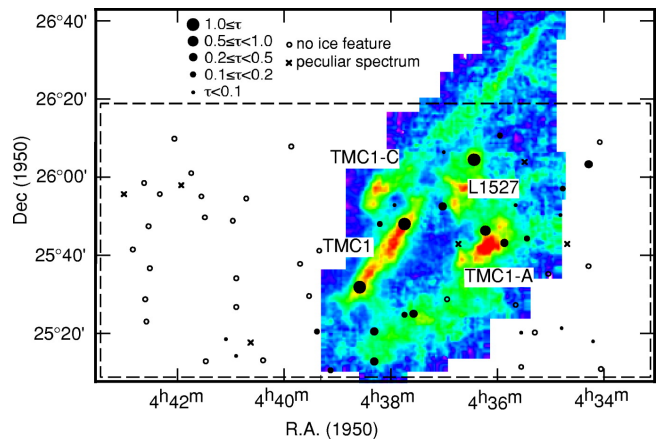


Fig 2.1.1a–Water ice map overlaid on a $C^{18}O$ map of parts of the Taurus molecular clouds[157]. Both filled and open circles show the position of observed field stars and the diameter of filled circles is proportional to the value of τ_{ice}.

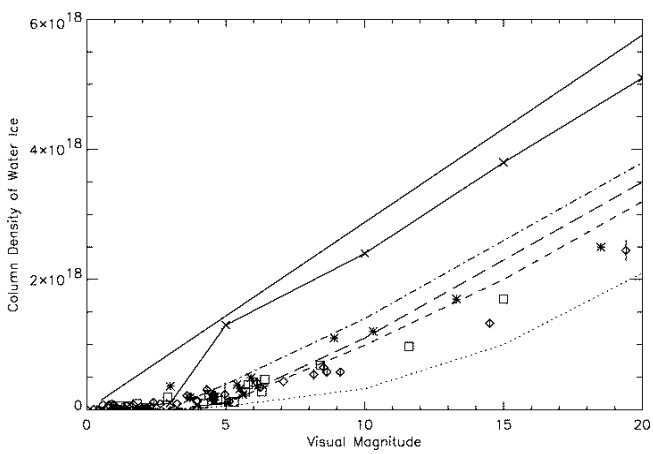


Fig 2.1.1b–Calculated and observed column densitiesof water ice as a function of visual extinction over the range $0 \leq A_V \leq 20$ for Taurus. The models are discussed in[167].

One may expect that the slope changes as the UV field decreases and molecules increasingly condense onto grain surfaces. Physical conditions in the cloud cores can be determined by mapping them with heterodyne detectors and sub-mm continuum arrays at Mauna Kea facilities like the JCMT and the SMA, for direct comparison with the water ice maps. Since the C/O ratio differs between various molecular clouds, we will also explore if the abundance of water ice changes in different star forming regions. If so, the eventual incorporation into planets may not necessarily be constant.

The Physical and Chemical Properties of Circumstellar Disks

It is possible to learn much about water chemistry via observations of related species such as CH_3OH, HCO^+, and their deuterated counterparts, which have observable line emission outside of the broad terrestrial water bands. A rich chemistry has been observed in star forming cores at (sub-)millimeter wavelengths[116]. The next step is to extend the study of chemistry in protostellar envelopes to protoplanetary disks. Dutrey[50] have surveyed a variety of molecules in a nearby disk in Taurus but there are few other observations to date due to the weakness of the line emission. Observations of molecules in disks are technically more challenging than in cores be-

cause of the low resolution of single-dish telescopes operating at millimeter wavelengths and the consequent beam dilution. To achieve higher resolution requires operating multiple antennas as an interferometer. Interferometers also have the advantage of automatically filtering out uniform emission from unrelated molecular gas in the cloud. The first such array of telescopes operating at sub-millimeter wavelengths has recently started operations on Mauna Kea. Once it reaches its targeted specifications in late 2003, the Sub-Millimeter Array (SMA) will provide the ability to map molecular line emission in disks and study their chemical state.

Through the UH access to telescopes on Mauna Kea, it is possible to dedicate large quantities of time to survey for sub-millimeter line emission from water related species in nearby protostellar cores and protoplanetary disks and follow their chemical evolution. The single dish Caltech Submillimeter Observatory (CSO) and James Clerk Maxwell Telescope (JCMT) telescopes have a resolution in the range 10-20″ and are well suited to line surveys in protostellar cores. Even in the closest stellar nurseries in Taurus, protostellar disks have radii of only a few arcseconds so line detection is very challenging with these single dish telescopes. The SMA will be a unique resource for imaging at arcsecond resolution in the sub-millimeter regime. By matching the beam and source size more closely, many lines are expected to be detectable and it will become possible to explore the structure, dynamics, and chemistry of protostellar disks. A pioneering study of chemistry in the LkCa 15 disk was made at millimeter wavelengths using the OVRO interferometer[180]. Our proposed observations are at shorter wavelengths than this work and target warmer gas where both line and continuum emission are stronger. In addition to the line observations, the thermal dust emission provides information on the grain mass opacity and thereby the composition of the (icy) mantles.

The combination of the single dish observations of cores and interferometer measurements of disks will show the chemical evolution from the early stages of star formation in cores to the beginning of planet formation in disks. This is a challenging project that will require large amounts of telescope time due to the multitude of species and relative weakness of emission. UH is in a unique position of being able to bring three sub-millimeter telescopes to bear on this problem, including the only one able to image at arcsecond resolution. Therefore, this survey can be expected to make a significant contribution to our knowledge of the initial chemical conditions of star-forming clouds. The SMA will be dedicated in November 2003. It will be linked to the JCMT and CSO in 2005, thereby doubling the sensitivity and resolution. These telescopes are a unique resource both for research and for training. The skills that students and postdocs learn in carrying out this work will prepare them to take full advantage of the Atacama Large Millimeter Array (ALMA). Scheduled for completion in 2011, this 64-element sub-millimeter interferometer will revolutionize the field of astrobiology by imaging the formation of stars in unprecedented detail. It will have, for example, the capability to image the gaps in disks formed by protoplanets and to study chemical gradients at AU scales.

Hot Water Emission and the Chemistry of Circumstellar Disks

Water is found in the form of water ice at temperatures below about 150 K, but it exists as water vapor at temperatures up to the thermal dissociation limit around 2500 K. Whereas the interiors of dark clouds are always below the ice condensation limit, circumstellar disks around newborn sun-like stars are within the temperature range of water vapor between roughly 0.1 and 5 AU. In this region of a disk, water is a strong and efficient molecular coolant (Fig. 2.1.1c). Furthermore,

not only is water abundant in circumstellar disks, but it has a large number of radiative transitions sampling a wide range of temperatures. Water is therefore an excellent diagnostic of the properties of circumstellar disks at radii relevant for planet formation[159].

Groundbased observations of water are complicated by the presence of water in the Earth's atmosphere, causing telluric absorption. Observations from dry high-altitude observatories like Mauna Kea greatly reduce this problem. Additionally, because higher excitation states of water have transitions far from the vibrational band centers, it is possible to make groundbased low-resolution spectroscopic observations of water, if it is much hotter than the Earth's atmosphere, when observing in the wings of the telluric water bands at near-infrared wavelengths. Najita *et al.*[159] have demonstrated that it is possible to use high spectral resolution to study individual, resolved near-infrared lines far from the vibrational band centers.

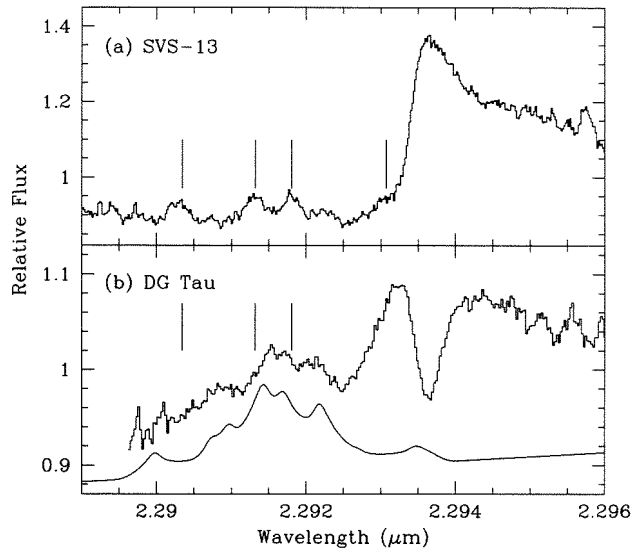


Fig 2.1.1c–Hot water emission (vertical lines) in two young solar-type stars near a CO bandhead[159]. A synthetic spectrum is included for DG Tau.

They find that the water lines are narrower than the CO lines (as expected for a rotating Keplerian disk since water has a lower dissociation temperature than CO) and therefore extend further out into more slowly rotating parts of a disk. Such spectroscopic studies require very high signal-to-noise in order to define the broad weak molecular features, and we envisage to employ the 8-10m telescopes at Mauna Kea for the first detailed and comprehensive study of water vapor emission in the circumstellar disks of newborn sun-like stars.

Water Masers in the Protostellar Environment

Many ortho and para rotational levels of water lie close to each other and thus tend to be easily inverted. Accordingly, if these levels correspond to allowed radiative transitions they can give rise to maser amplification. The strongest water maser emission is that of the $6_{1,6} - 5_{2,3}$ transition which is easily detected from the ground at 22 GHz[25]. The strong 22 GHz maser emission can be explained by the collisional pumping of dense neutral gas which has been heated by shocks.

In recent years a number of surveys have demonstrated that water masers are commonly observed around newborn stars, with detection rates of 40% for the youngest Class 0 sources, 4% for slightly more evolved (50,000 to 100,000 yr older) Class I sources, and 0% for still older, optically visible Class II sources[64, 65]. This dramatic decrease in water maser detection over a relatively short evolutionary time span is likely to be caused by the rapid dissipation of dense gas around the central objects. In most cases the masers appear to align along the axis of outflow from the newborn stars[32], supporting other evidence suggesting that water is efficiently and abundantly produced within warm gas, heated by shocks that are converting gas-phase oxygen into water[164]. The high spatial resolution of the Very Large Array (VLA) interferometer (0.08 arcsec at 1.3 cm in the A configuration, corresponding to 10 AU in the nearest star forming regions) is thus pro-

viding insights into the launch of protostellar jets which may be the source of chondrules and CAI's in the early solar nebula[199]. This offers a unique connection between the formation of other young stars and the study of our own origins through meteoritic analysis. In rarer cases, the masers appear to trace the distribution of gas in circumstellar disks, see Fig. 2.1.1d[211, 98, 171].

Recently, proper motions of water masers have been determined by studies with Very Long Baseline Interferometry. Such observations represent an improvement of two orders of magnitude with respect to the best angular resolution achieved with the VLA in its A-configuration[210]. Interferometric water maser observations are the *only* observations with sufficiently high spatial resolution to allow a detailed determination, on solar system scales, of physical conditions and kinematics in the circumstellar disks out of which planets will form. For the last decade, we have used the VLA extensively to study deeply embedded, newborn, sun-like stars[181]. We will employ this facility to study the distribution and temporal evolution of water masers, using the water maser emission as a tool to explore physical conditions in protoplanetary disks versus of age.

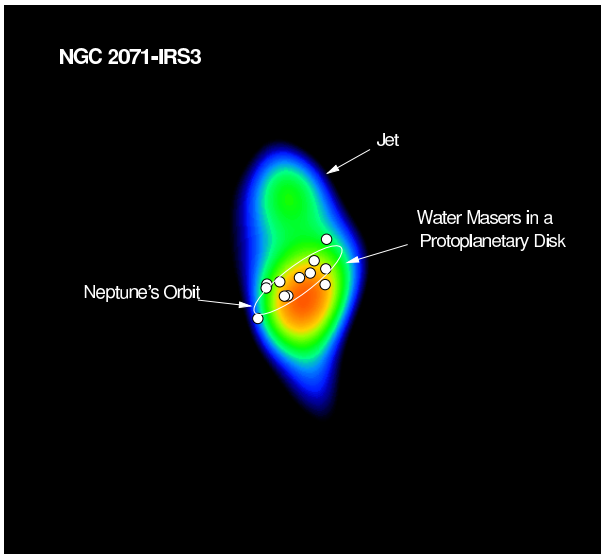


Fig 2.1.1d–A 1.3 cm radio continuum image from the Very Large Array[210]. Dots indicate the position of the water masers. The ellipse shows the size of Neptune's orbit for comparison purposes.

Analysis of Water in Star Forming Regions with Space Missions

Water vapor is so abundant and so rapidly variable in the Earth's atmosphere that detailed observations are rather difficult. The Infrared Space Observatory (ISO) and the Submillimeter Wave Astronomy Satellite (SWAS) opened mid- and far-infrared windows with numerous strong water lines[24, 128, 140], and indicated the advances we can expect in this area of research from future missions. Two major projects, the Stratospheric Observatory for Infrared Astronomy (SOFIA) and the Space Infrared Telescope Facility (SIRTF), are in the final stages of preparation, and will soon be available to the astronomical community. Later this decade we will get the Herschel mission, a 3.5 m mid- and far-infrared telescope that will be placed in a solar orbit at the L2 point. It will be extremely sensitive, and will permit detailed studies of the relation of water ice to circumstellar chemistry in newborn stars[25]. Meanwhile SIRTF, due to be launched this year, will have far higher sensitivity and resolution than ISO and SWAS, although it, too, will be unable to resolve circumstellar disks. We will use SIRTF to measure the global water content in young star forming cores and disks as a constraint on modeling our resolved ground based observations of water related molecules.

2.1.2 Interstellar Ices $\longrightarrow$ Comets

Objective – We would like to understand the volatile (especially water ice) abundances of comets and related bodies and to know to what extent cometary water has been processed since its ac-

cretion in the precursor molecular cloud. As ice-rich bodies, the nuclei are thermodynamically stable against sublimation only at heliocentric distances > 5 AU. Therefore, we must address the compositions of objects in the middle and outer solar system in order to understand the sources and delivery mechanisms. This requires the application of astronomical techniques using the largest telescopes. As the cometary ices are likely to have been altered by prolonged irradiation by energetic particles, we must also use laboratory experiments to understand the nature and rates of chemical reactions occuring in exposed ices. We will mount a systematic program to study irradiated ices both astronomically and in the laboratory.

Connections – §2.1.4 §2.1.3
Astrobiology Roadmap Links – Goal 3; Objective 3.1
Researchers – Bar-Nun, Jewitt, Kaiser, Meech, Owen, Prialnik

Background– Until the late 1980's, the standard paradigm held that comets were pristine relics, "planetesimals", from the era of planet formation. Indeed, the inventory of species detected to date in the ISM is very similar to that found in the comets[156], broadly consistent with this paradigm. However, we now understand that comets can have different sources. Dynamical evidence suggests that the short-period comets (SP) must have had a low-inclination source in the trans-Neptunian region (beyond 30 AU), while long-period comets (LP) formed at smaller distances (*e.g.* the Jupiter-Neptune zone), were perturbed outwards and are now stored in the Oort Cloud[49, 58]. The different comet dynamical types have experienced different thermal, collisional and irradiation histories; it is natural to expect that these differences will be reflected in their compositions.

The Comet – ISM Connection

The inventory of species detected to date in the ISM is very similar to those found in comets[53], leading to the suggestion that much of the interstellar material is incorporated unaltered into comets. However, during the earliest stages of the evolution of the planetary disk, infalling interstellar material may have been heavily processed due to shock-induced sublimation from icy grain mantles and subsequent volatile re-condensation[124]. At the low ambient temperatures expected in the nebula, the water ice would recondense in an amorphous form, trapping other more volatile species. Nevertheless, observations of recent bright comets have shown that there is evidence for preservation of an interstellar ice component within nuclei, yet at the same time the cometary material has undergone processing during its formation. Measurements of the D/H ratio in P/Halley[10, 52], in C/1996 B2 Hyakutake[17] and in C/1995 O1 Hale-Bopp[139] show an enrichment by a factor of ten in water compared to the protosolar ratio. The enrichment is a result of ion-molecule and grain-surface reactions in molecular clouds. Laboratory experiments have shown that this ratio cannot have been re-equilibrated in the solar nebula[118].

The Oort Cloud, the Kuiper Belt and Related Bodies

The Oort Cloud contains about 10^{12} comets, with a combined mass probably comparable to that of Neptune. The planetesimals grew collisionally until they were big enough to decouple from the nebular gas (at sizes of 10's to 100's of meters)[218]. Planetesimals ejected to the Oort Cloud

may have undergone collisional evolution en-route, but have since been stored in a collision-free environment at only 10K temperature[201]. They have been subject to the full intensity of ionizing galactic cosmic rays for the past 4.6 Gyr. The Kuiper Belt, contains perhaps 10^5 bodies larger than 100 km in diameter and $\sim 10^{10}$ larger than the typical cometary nucleus ($\sim$ 1 km: Jewitt and Luu 2000). The current total mass is only $\sim 0.2\ M_\oplus$ but this is thought to be only about 1% of the initial Kuiper Belt mass. Material has been lost from the Kuiper Belt through dynamical erosion and through collisional shatteriing and ejection. It has been conjectured that the late heavy bombardment of the solar system, most clearly recorded in the cratering history of the Moon, might have been caused by the clearing out of the massive initial Kuiper belt.

Presently, objects escape from the Kuiper belt through dynamical chaos, and are scattered amongst the planets. Those that are not ejected from the solar system to the interstellar medium are scattered inwards to fall under the gravitational control of Jupiter. Once inside Jupiter's 5 AU radius orbit, water ice in the surface layers begins to sublimate, giving rise to an atmosphere or "coma", and these objects are observationally relabelled as "comets". It is thus appropriate to think of a stream of objects emanating from the Kuiper belt and being scattered between the planets, leading sometimes to the appearance of active comets near the earth and, on rare occasions, to collisions with the Earth and terrestrial planets. Bodies that have left the Kuiper belt but which are not yet warm enough to strongly sublimate are known as "Centaurs". About 50 such objects are currently known. In contrast to the Oort Cloud objects, comets from the Kuiper belt are thought to be collisionally produced chips from larger bodies. They have been in a warmer thermal environment (50K), have been at least partly protected from cosmic rays by heliospheric shielding, and may have been heated and shocked during ejection from their parent bodies.

Spectral Properties

Observational evidence for the effects of irradiation of the comets is found in their remarkably low albedos. Even though they are known to be ice-rich, the cometary nuclei are amongst the darkest objects in the solar system, with albedos typically of only $\sim$4%. Albedos of Kuiper Belt Objects and Centaurs, although few are known with confidence, also fall in the few to 10% range. Such low albedoes, together with the reddish colors measured in the optical spectral region, are consistent with hydrogen-depeleted, carbon-rich organics.

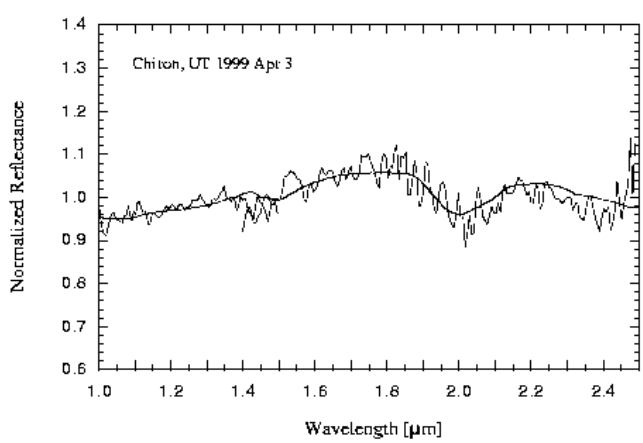


Fig 2.1.2a–Water ice bands at 1.5 and 2.0μm in the reflection spectrum of Centaur 2060 Chiron[?].

Spectral features on these bodies have proved difficult to find, indicating the low abundance of bonds capable of generating measurable vibrational features in the near infrared. In one spectacular counter-example to this claim, telescopic observations have revealed the presence of several near-infrared absorptions in the spectrum of Centaur 5145 Pholus. One model can fit these features using a surface composition including complex organics ("tholin"), water ice and a light hydrocarbon (possible methanol[39]). Water ice is seen also in the reflection spectra of other Cen-

taurs and KBOs (see Fig. 2.1.2a). The existence of organics in comets is known from optical spectroscopy of radicals (C_2, C_3) and from near infrared and radio spectroscopy of molecules (HCN, H_2CO). In-situ measurements of dust grains in P/Halley detected grains composed largely of the elements CHON.

Whether the processed organics are confined to a meter-thick surface layer or "mantle", or are to be found throughout the bulk of the cometary nucleus remains unknown (see Fig. 2.1.2b). Cosmic rays have a stopping length of only meters but irradiation of solid matter prior to incorporation in the cometary nucleus could lead to this matter being found at all depths[138]. Observations of amorphous ice in comets[44] show that the comets cannot have been heated much above 137K. This places limits on the allowable abundance of ^{26}Al and on timescales from chondrule formation to comet formation to allow the ^{26}Al to decay by 2 orders of magnitude from the abundance inferred in the Allende meteorite. The heating from ^{26}Al would occur in the first few $\times 10^6$ to 10^7 years, or possibly longer for larger bodies[176]. Delayed formation is a feature of accretion in the low density Kuiper Belt, so that it seems natural that ^{26}Al would be largely absent in these bodies.

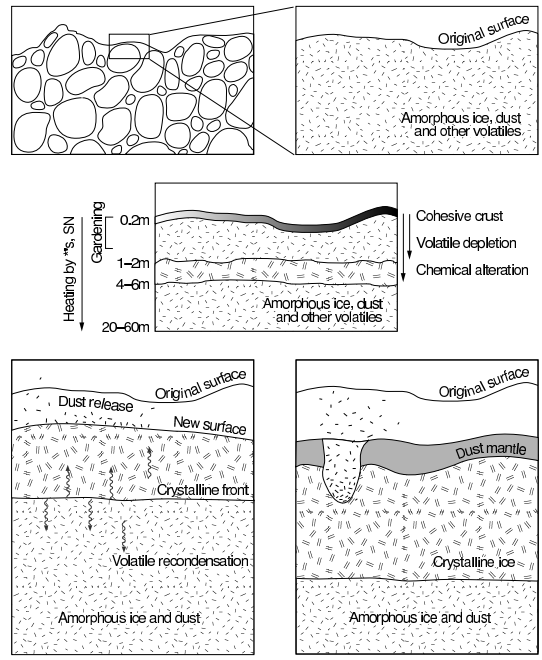


Fig 2.1.2b–Sequences of aging in the upper layers of a comet nucleus from the pristine state, through alterations undergone from heating in the Oort cloud from bright stars, supernovae and decay of ^{26}Al to the active phase of volatile loss.

Comets formed closer to the sun at higher densities could have grown more rapidly and might have incorporated a larger fraction of ^{26}Al and other short-lived isotopes, possibly with important consequences for thermal and chemical processing histories. Organic molecules when exposed to charged particle and UV radiation produce radiolysis and photolysis. Laboratory evidence shows that carbon-containing frozen mixtures will form complex organics when exposed to radiation and that complex organics will break down under irradiation (see §2.1.3). The effects of progressive irradiation include weakening of the IR bands owing to CH bonds, a change in the slope of the visible reflectance spectra (0.4-0.8μm) and a progressive lowering of the albedo. Hence there is much speculation that red outer solar system materials result from these processes[204], and that bright, neutral-blue surfaces correspond to newly exposed icy surfaces. The technology has just become advanced enough to begin to study a statistically meaningful sample of comets and Centaurs to look for the water-ice signature, and the chemical composition of the surface, including organic materials.

Proposed Observations

We aim to capitalize on University of Hawaii access to Mauna Kea to undertake a systematic investigation of the abundances and abundance differences in the ice-bearing bodies of the solar

system. Such a study is timely because (*i*) new surveys have revealed a large number of observable but currently unobserved target Centaurs and Kuiper belt Objects and (*ii*) new telescopes and new detector technologies allow high quality observations that were previously impossible to secure. As a counterpart to the acquisition of astronomical spectra, we will obtain laboratory reflection spectra of relevant ice-dirt mixtures, all subject to appropriate degrees of irradiation damage by high energy particle sources. See §2.3.4 for details.

2.1.3 Role of Water in the Formation of Biologically Important Molecules in Space

Objective – To use ultra-high vacuum scattering lab experiments to investigate the charged particle versus photon induced formation of interstellar $C_2H_4O_2$ isomers in astrophysically important, water-rich ice mixtures.

Connections – §2.1.1
Astrobiology Roadmap Links – Goals 3, 4; Objective 3.1, 4.3
Researchers – Kaiser, Ehrenfreund, Bar-Nun, Owen

Background – Cutting-edge laboratory experiments present a unique opportunity to address for the very first time the important questions of how the basic life ingredients can be formed abiotically in extraterrestrial environments such as molecular clouds and the crucial role that water has played in their formation. Water ice presents the main constituent not only of interstellar grain material, but also of cometary matter[146]. Due to its high abundance, it plays a vital role in the underlying physicochemical processes which lead to the formation of astrobiologically significant molecules like carbon hydrates (sugars) in the interstellar medium and in our solar system.

Basic processes of ice formation and destruction in interstellar clouds

There are four basic processes which influence the ice chemistry in the ISM. Figure 2.1.3a shows a schematic overview of an icy grain and its catalytic surface. Accretion is a very efficient process in cold environments because most of the gaseous species (with the exception of H_2 and He) stick onto the grains with almost 100% efficiency. This accretion process occurs on a timescale of $\tau \sim 2 \times 10^9/n(\mathrm{H_2})$ yr, assuming a sticking efficiency of unity. The accretion process occurs to specific grain sites, known as binding sites. Water ice acts as a matrix which embeds complex organic molecules such as freshly synthesized sugars and amino acids. Due to this matrix isolation, ionizing radiation (nuclei, electrons, photons) from the galactic cosmic radiation field and the solar wind interact predominantly with the main constituent of these ices: water molecules. Therefore, water molecules protect astrobiologically significant molecules inside the icy grain material from being destroyed by ionizing radiation. Without this water ice, no organic molecule can survive neither on interstellar grains nor on comets.

Secondly, the actual formation of astrobiologically important molecules generates chemical energy, which is stored as vibrational energy in the newly formed molecules. This excess energy must be diverted from the molecules; otherwise organic molecules fragment and cannot be stabilized. The water matrix can divert this excess energy via phonon coupling. Without this energy transfer of water ice, the astrobiological evolution of extraterrestrial environments would stop right at the beginning. However, the actual effect of both processes and the role of water

on the formation of astrobiologically important molecules has never been investigated in laboratory experiments. Here, we unravel for the very first time how these astrobiologically relevant building blocks are actually formed in water rich ices (via ionizing radiation, charged particles, and photons). The formation of the sugar glycolaldehyde and its isomers acetic acid and methyl formate in water ice acts as a prototype example with fundamental astrobiological implications. The production routes are explored quantitatively as a function of temperature (10 K – 300 K) to simulate the chemical processing in cold clouds and hot molecular cores. We also account for the different chemical reactivities in various water ice phases (crystalline versus amorphous). By coupling these laboratory investigations and the production routes of glycolaldehyde, acetic acid, and methyl formate with sub-mm observations, we will then infer conditions in the cores and cold clouds where the chemistry in water-rich ices is occurring.

Untangling the synthetic routes to form basic sugars, which are the building blocks of RNA, in water rich extraterrestrial ices is particularly significant because they serve as an energy source to living organisms (glucose) and as a structural skeleton (cellulose)[51]. Carbohydrates also play a role in vital amino acid producing chemical reactions. These species may have formed on early Earth via the polymerization of two formaldehyde (H_2CO) molecules. However, the validity of whether or not these conditions were likely on a primitive Earth has been seriously questioned, because under any conditions where monosaccharides will form, they would subsequently degrade or react on short time-scales.

Early in Earth's history, it experienced a period of heavy bombardment during which fragile carbon-based life could not have survived[30]. Geological surveys have found 3.5Gy old cyanobacteria fossils and evidence of chemical processing dating to 3.8Gy. Thus, the evolution of pre-biotic molecules on Earth took less than 300 million years, suggesting that these biologically important molecules might have been already available. Astrobiologically important molecules such as glycolaldehyde, a monosaccharide sugar, could have been produced in extraterrestrial environments and then subsequently survived solar nebular processing or were introduced to the Earth during the period of heavy bombardment.

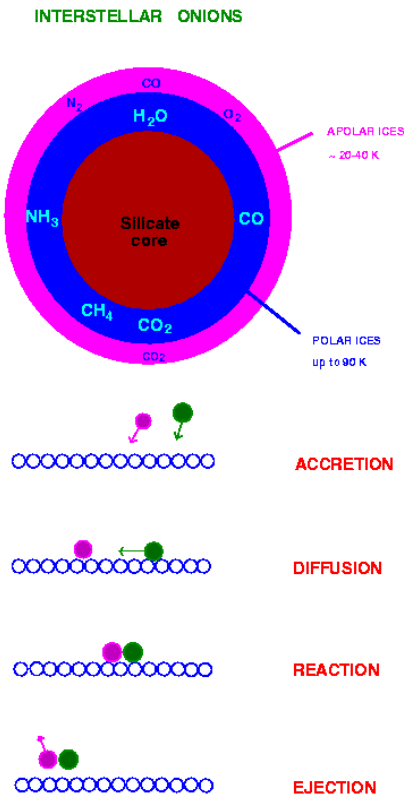


Fig 2.1.3a– A schematic view of an interstellar grain covered with polar (water-dominated) and/or apolar (highly volatile, H-deficient) ice layers. Four major processes act to form and equilibrate interstellar icy grain mantles. A more detailed explanation is given in the text.

An analysis of meteorites such as Murchison and Murray also indicates a large proportion of complex organic matter including amino acids, lipids and sugars to support this claim. In addition, several interesting organic molecules (such as methyl formate and acetic acid) have been tentatively identified in comets.

Hot molecular cores and star forming regions provide a rich laboratory for understanding complex molecular evolution. The transition from the cold molecular cloud to the hot core phase

depends strongly on the molecular composition. A detailed understanding of the synthesis of three $C_2H_4O_2$ isomers [acetic acid (CH_3COOH), methyl formate ($HCOOCH_3$), and glycolaldehyde ($HCOCH_2H$)] is of particular pertinence since they play a key role in astrobiology. Acetic acid, a precursor to the simplest amino acid glycine, was first detected in the hot core Sagittarius B2(N) (Sgr B2) , and later also toward W51e2[89]. The second isomer, methyl formate, is ubiquitous in the interstellar medium and has been observed not only in hot cores like Sgr B2 but also in molecular clouds such as OMC-1. The recent detection of glycolaldehyde in Sgr B2 is a significant astrobiological milestone since it represents the first member of monosaccharide sugars and denotes an important biomarker.

Despite the key role of these $C_2H_4O_2$ isomers in astrobiology, their formation is not understood. Chemical reaction network models of pure gas phase chemistry which focus on ion-molecule reactions with methanol and formaldehyde yield abundances 50-100 less than observed. Grain-surface reactions on sub-micrometer sized silicate- and carbonaceous-based nanoparticles at T $\sim$ 10K in cold molecular clouds have been proposed as an alternative formation mechanism. The molecules can be liberated into the gas phase via sublimation in hot cores when the surrounded matter is heated above 100 K by the embedded protostar. Millar & Hatchell[142] extended previous reaction networks to simulate these grain sublimation processes, however, the models could not adequately fit the observed abundances. This suggests that key production routes to form $C_2H_4O_2$ isomers involving interstellar grains are still missing.

Understanding Grain Chemistry

The crucial role of the grain mantles to produce molecular hydrogen (H_2) and simple hydrides such as water and methane has been recognized explicitly, but no consensus has been reached whether complex astrobiologically important molecules are actually formed on grain surfaces or inside icy grains[120, 142]. In the cold molecular clouds, interstellar grains have temperatures of 10K. Thus, grain particles trap all molecules and atoms except H, H_2, and He with unit efficiency upon collision. This results in the formation of nm-thick icy layers which consist predominantly of water (H_2O), methanol (CH_3OH), carbon monoxide (CO), carbon dioxide (CO_2), and of minor components like ammonia (NH_3), formaldehyde (H_2CO), hydrogen cyanide (HCN), carbonyl sulfide (OCS), and methane (CH_4). These ice mantles are processed chemically by MeV cosmic-ray (CR) induced internal UV radiation which is present even in the deep interior of the dense clouds. Because current reaction models assume that this frozen grain mantle material is chemically inert, they have limited validity.

Despite the importance of high energy particle and photon induced chemical formation of molecules in extraterrestrial ices, these processes have never been comprehensively included into astrophysical reaction networks modeling the formation of $C_2H_4O_2$ isomers in cold clouds and hot cores. Studies (including the NASA decadal survey) investigating the effects of charged particle processing of interstellar ices to form heavy hydrocarbons in molecular clouds call for extensive laboratory investigations of the underlying elementary processes.The interstellar medium modeling communities are also calling for laboratory experiments. Novel laboratory experiments on the CR and UV triggered formation of $C_2H_4O_2$ isomers in extraterrestrial ices are clearly desired. Once the prebiotic synthetic routes to form these molecules have been exposed quantitatively in laboratory experiments, we can then predict where sugars and their isomers (or their precursors) can be formed, searched for, and ultimately be observed spectroscopically (via telescopes) or in

situ (via space missions).

Experimental Objectives

We will use ultra-high vacuum scattering lab experiments to investigate the charged particle versus photon induced formation of interstellar $C_2H_4O_2$ isomers in astrophysically important, water-rich ice mixtures. These experiments will generate quantitative data under controlled conditions and provide temperature, kinetic energy, and wavelength-dependent synthetic routes to form acetic acid, methyl formate, and glycolaldehyde in water-rich ices as present in cold molecular clouds, hot cores, and in cometary ices. This unique approach addresses for the first time specific mechanisms and generalized concepts on photon versus particle induced formation of complex molecules in astrophysical ices rather than attempting solely to reproduce infrared spectra of astronomical observations.

Production rates of $C_2H_4O_2$ isomers will be explored systematically as a function of ice temperature in molecular clouds and hot cores (10 to 100-300 K), ice composition, photon wavelength, nature of the charged particles (electrons, hydrogen, helium, oxygen, and carbon ions), and flux and kinetic energy of the irradiating particles. Since photons penetrate only the outer layers of the grain, whereas cosmic ray particles can penetrate deep inside, a depth-dependent molecular differentiation is expected. The laboratory experiments combined with kinetic models predict the existence of these isomers quantitatively in various extraterrestrial environments. Using JCMT, CSO, and SMA at Mauna Kea (§2.1.1), we can then attempt to observe these isomers in molecular clouds and star forming regions in the microwave region; the microwave transitions of all three isomers are well known. It will be possible to compare the T – dependent production rates and outputs of the kinetic models with results from astronomical observations.

These experiments may also be important for understanding the data from future space missions (*e.g.* Deep Impact Mission) and spectroscopic observations with telescopes (Stratospheric Observatory for Far Infrared Astronomy; Space Infrared Telescope Facility). This unique synergistic approach combines for the very first time sophisticated laboratory experiments, electronic structure theory, kinetic models, and actual astronomical observations to address generalized concepts in understanding the formation of astrobiologically important molecules in water rich ices in our solar system and in the interstellar medium.

Chemical Processing in the Clouds – Physical processes

An understanding of the chemical processing of ices by photons and charged particles, will enable us to predict the nature of the ice mixtures where $C_2H_4O_2$ isomers might be synthesized. Dictated by optical selection rules, a photon can be absorbed by a single molecule in the ice. This process can be followed by a selective bond rupture. If a hydrogen atom is released in the photodissociation process, it may have kinetic energies up to a few eV. The corresponding radical formed is internally excited and might react with a neighboring molecule. Since the internal energy can be coupled into the reaction coordinate, entrance barriers can be passed and endothermic reactions are feasible.

UV photons are absorbed within about 100Å of the surface, but CR particles (such as 10 MeV H^+) can penetrate deeper and deposit up to 1 MeV inside the icy mantle. This exceeds the chemical bond strength (1 eV) and the stability of the molecule. Upon absorption, a CR can interact

inelastically or elastically with either the electronic or nuclear part of molecules, respectively. Consequences of this interaction include the electronic excitation, ionization, and/or bond ruptures. The energetic species thus formed are not in thermal equilibrium with the surrounding 10K ice. Once particles are slowed down in successive collisions to kinetic energies of a few eV – energies in the order of chemical bond strengths – they can react with a molecule in the ice via one of three mechanisms to form new molecules: (*i*) hydrogen abstraction, (*ii*) insertion into a single bond, or (*iii*) addition to an unsaturated bond or to a non-bonding orbital.

The power of suprathermal reactants is based on the ability to impart their kinetic energy into the reaction coordinate. Reaction barriers can be overcome, and endothermic reactions are open resulting in rate constants up to 16 orders of magnitude larger than thermal reactions. Most importantly, calculations show that although the CR flux in dense molecular clouds is two orders of magnitude below the internal UV flux, each MeV particle generates about 100 suprathermal species in a 0.1 μm thick icy layer. Hence the flux advantage of the UV field is eliminated by the ability of one CR particle to generate multiple suprathermal species.

2.1.4 Origin and Distribution of Planetary Water

Objective – Mineralogical, geochemical and isotopic studies of aqueously-altered meteorites will help to understand, firstly, the time and physico-chemical conditions of aqueous alteration in the asteroids, and secondly, how and when the water accreted into asteroids and planets from these diverse sources.

Connections – §2.1.1 2.1.2
Astrobiology Roadmap Links – Goal 3, 5; Objective 3.1
Researchers – Owen, Jewitt, Keil, Krot, Scott

Comets to Earth: Isotopes

The origin of the Earth's water is one of the key issues that we will address under this investigation. It is likely that the Earth formed too hot for much water to have been incorporated directly, or as chemically bound water in minerals, into the body of the planet. The presence of $\sim 10^{-6}$ $M_\oplus$ of water on our planet instead indicates delivery from an external source or sources. The other terrestrial planets are likewise thought to have harbored substantial water inventories.

Mars might still do so in the form of permafrost ice. For a long time, the water-rich nuclei of comets have been suspected as major carriers of planetary water. Measurements of the D/H ratio in comets P/Halley, C/Hyakutake and C/Hale-Bopp have indicated a problem with the simplest version of this idea, however. The D/H ratio in these comets averages $D/H = 3\times10^{-4}$, which is twice the value for Standard Mean Ocean Water, $D/H_{SMOW} = 1.6 \times 10^{-4}$. The three measured comets are from dynamical classes thought to derive from the Oort Cloud, and to have originated in the middle solar system. The comets in the Kuiper Belt, (so-called short-period, Jupiter-family comets), remain unmeasured. The figure at the right shows the D/H ratios of Hale-Bopp, P/Halley and Hyakutake as a function of the (current) inverse semimajor axis, a.

Accordingly, the strongest conclusion that can be drawn from the available data is that the Earth's water does not consist only of melted comets from the middle solar system. There could be other contributions from cometary sources with D/H ratios quite different from the 3 measured

so far. It has been specifically suggested that the measured comets are not representative of the isotopic compositions to be found in the outer solar system. It has also been suggested that Earth's water could have come from the outer asteroid-belt. Measurements of planetary noble gas abundances cannot be easily reconciled with "hot" sources from the outer belt, and a cometary carrier still seems likely. However, we lack the data to definitively understand the origin of the Earth's water.

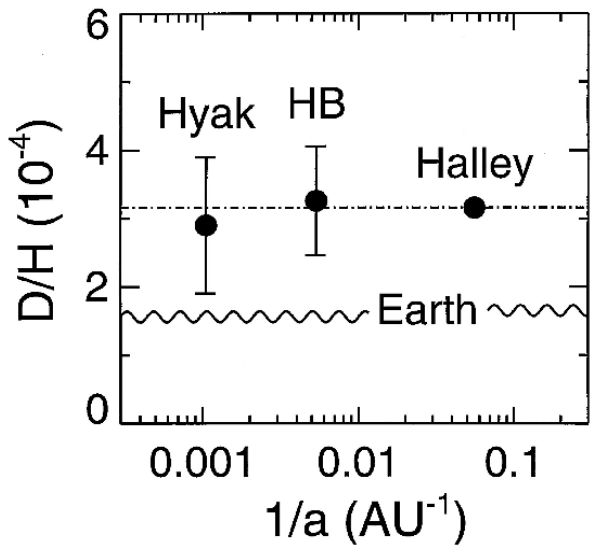


Fig 2.1.4a– D/H ratio of Hale-Bopp (HB), Halley, and Hyakutake (Hyak) versus semi-major axis. The wavy line shows the SMOW value, and the dashed-dotted line, the in situ value for Halley. Depicted are 1σ standard deviations. Figure from [139].

Comets to Earth: Isotopes

There is a long tradition of assuming the Earth is made of matter that is identical to or at least closely resembles the chondritic meteorites. Starting from the observation that the value of Kr/Xe in the Earth's atmosphere is 20 times greater than the value in the meteorites, and that

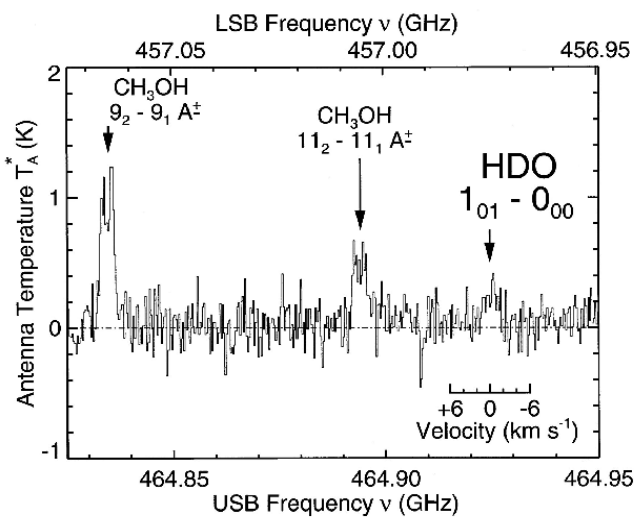


Fig 2.1.4b–Mauna Kea spectrum of Hale-Bopp showing the HDO 101-000 line together with two methanol transitions. The CH_3OH 112-111 A transition originates in the lower sideband (LSB) (top frequency scale); all other lines belong to the upper sideband (USB). The double-peak structure of the lines is caused by the velocity distribution in the comet[139].

the Xe isotope abundances in our atmosphere are also fundamentally different from those in meteoritic xenon, the term "planetary component" for noble gases in meteorites is a misnomer. If meteorites – and by extension asteroids – did not deliver the noble gases, they cannot have brought in the water, nitrogen and carbon either. If they had, we would have a very different situation for xenon in our atmosphere. Fractionation does not help.

This leads to the suggestion that comets may have played some role. Amorphous ice could trap noble gases in the proportion that we find them on Earth, but the isotopic pattern in atmospheric xenon cannot be duplicated. Furthermore, we now know that the water in Oort cloud comets has twice the value of D/H that we find in seawater on Earth. Hence we cannot make the

oceans out of melted Oort cloud comets alone.

Suppose comets indeed are a minor carrier of water to Earth, even though they may have brought the noble gases. Then what brought in the water? Presumably it was the rocks that formed the planet, which may have been significantly different in composition from the chondritic meteorites. Mars is an important test bed for this idea, because there is very little mixing between the surface and the interior, and the atmosphere is very thin. Hence external contributions of volatiles will be likely to stay on or near the surface, while internal volatiles should retain their original properties.

Records of Aqueous Activity on Meteorite Parent Bodies

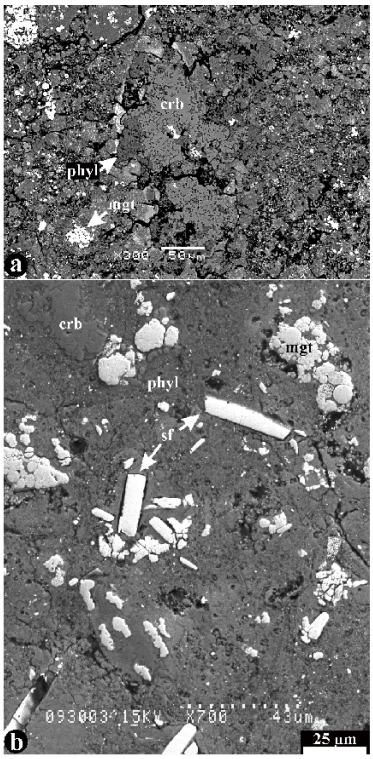


Fig 2.1.4c–Backscattered electron images of the Tagish Lake carbonaceous chondrite (a) and QUE94411 carbonaceous chndrite. Both contained secondary minerals produced during aqueous alteration, including carbonates (crb), magnetite (mgt), FeNi-sulfides (sf), and phyllosilicates (phyl).

Chondritic meteorites (chondrites) are fragments of mineralogically and chemically diverse asteroids that formed at 2-3 AU from the Sun prior to accretion of the planets in the inner solar system (Mercury, Venus, Earth, Mars). They are considered to be analogs for the building blocks for these planets. Asteroids, like planets, formed by aggregation of solids and gas from the protosolar disk. Remote sensing of asteroids and laboratory studies of chondrites show that asteroids at 2 AU largely consist of anhydrous silicates, metal and sulfides, whereas at distances $\sim$3 AU most asteroids are largely composed of hydrated silicates like clay minerals, organic materials, carbonates, sulfates, magnetite, and other iron oxides (Figs. 2.1.4c, 2.1.4d). Experimental studies and studies of meteorites suggest that the hydrated minerals, carbonates, sulfates, magnetite, etc. were not produced in the protosolar disk, but resulted from aqueous activity on asteroidal bodies. The water on asteroids is thought to have multiple sources, including interstellar ice, hydrous silicates from asteroidal and cometary fragments that formed further away from the Sun, and minor amounts of ice condensing in the solar nebula (Fig. 2.1.4e). Mineralogical, geochemical and isotopic studies of aqueously-altered meteorites will help to understand, firstly, the time and physico-chemical conditions of aqueous alteration in the asteroids, and secondly, how and when the water accreted into asteroids and planets from these diverse sources. In order to address these questions, we propose to study secondary mineralization (*e.g.*, phyllosilicates, magnetite, halite, carbonates, nepheline, sodalite, fayalite, andradite) that resulted from asteroidal aqueous alteration of various chondritic meteorites (H, L, LL, CI, CM, CO, CR, CH, CV).

We will conduct this work through detailed mineralogic, petrologic and isotopic studies using optical, scanning and transmission electron microscopy (SEM, TEM), electron probe microanalysis (EPMA), ion probe microanalysis (SIMS), Raman and IR-spectroscopy, thermal ionization and

inductively coupled plasma-mass spectrometry (TIMS, ICP-MS).

We will carefully characterize secondary mineralization in chondritic meteorites using optical microscopy, SEM, EPMA, and TEM. Using X-ray elemental mapping and backscattered electron imaging (Figs. 2.1.4c, 2.1.4d), we will identify phyllosilicates, magnetite, halite, carbonates, nepheline, sodalite, fayalite, andradite, and search for fluid inclusions in carbonates and salts. Chemical composition of the identified phases will be measured using EPMA. Based on the occurrences of the secondary phases, their chemical compositions and textural relationships with primary minerals of high-temperature chondritic components (*e.g.*, chondrules and Ca, Al-rich inclusions), we will infer setting (nebular or asteroidal) and chemical changes resulting from aqueous alteration.

Physico-chemical conditions of aqueous alteration will be estimated using thermodynamic modeling[113]. The identified fluid inclusions will be studied using Raman and IR spectroscopy, and SIMS to define composition (pH, D/H, oxygen isotopes) and redox conditions (Eh) of a fluid phase phase[227]. In order to understand sources of water in aqueously-altered asteroids, we will study hydrogen isotopic compositions of their phyllosilicates[?]. As a result of this complex mineralogical, petrological and isotopic studies of aqueously altered chondritic meteorites, we will be able to infer environment, temperature, pressure, water/rock ratio, and other physicochemical conditions (Eh, pH, fluid composition) of aqueous alteration. Our recent results show that aqueous activities on the chondrite asteroids started within 1-2 Myr after formation of Ca, Al-rich inclusions ($\sim$4567$\pm$.6 Myr[5]) and lasted for at least $\sim$15 Myr[207, 55, 93, 94, 95, 87, 113, 115, 90, 20, 21, 175].

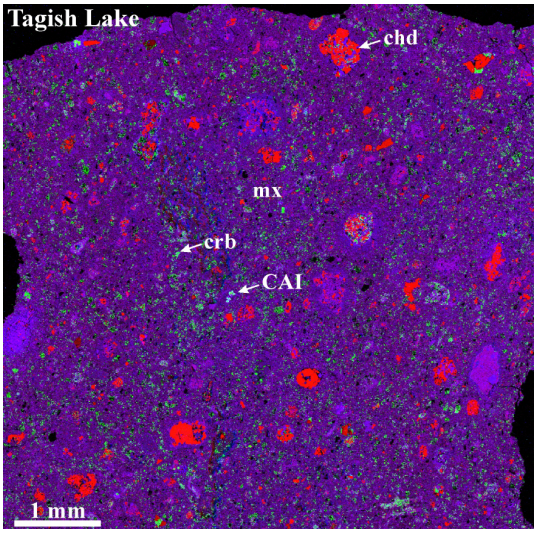


Fig 2.1.4d–Combined X-ray elemental map in Mg (red), Ca (green) and Al Kα (blue) of the Tagish Lake carbonaccous chondrite, which experienced extensive aqueous alteration that resulted in formation of abundant carbonates (crb), magnetite and phyllosilicates. These secondary phases replace chondrules (chd), Ca, Al-rich inclusions (CAls) and matrix (mx) materials.

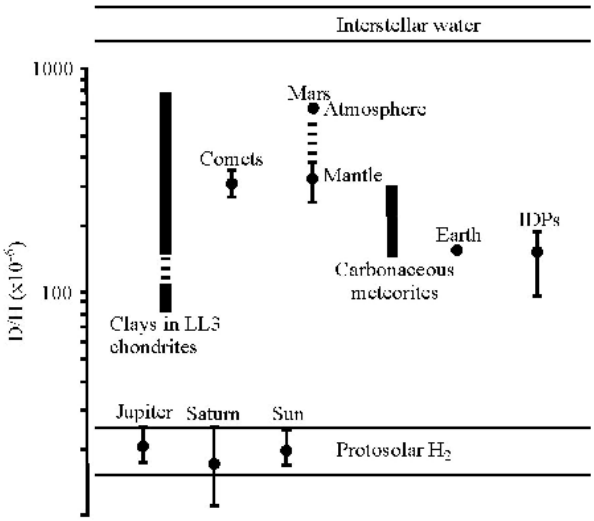


Fig 2.1.4e–Distribution of the hydrogen isotopic composition in solar system bodies.

Physico-chemical conditions of aqueous alteration will be estimated using thermodynamic

modeling[113]. In order to constrain timing of aqueous alteration of H, L, LL, CI, CM, CO, CP, CH, and CV chondrites, we will date some of the secondary minerals (carbonates, fayalite, magnetite, phyllosilicates) using SIMS and TIMS based on short-lived radionuclide systems such as ^{26}Al-^{26}Mg ($t_{1/2} \sim 0.7$ Myr), ^{53}Mn-^{53}Cr ($t_{1/2} \sim 3.7$ Myr), and ^{129}I-^{129}Xe ($t_{1/2} \sim 16$ Myr).

Dating of carbonate and fayalite formation will be done *in situ* using SIMS[95]. Dating of magnetite and phyllosilicates formation will be done using TIMS on mineral separates[175]. If we find that aqueous alteration of chondritic meteorites occurred within the first few Myr of the solar system, it would indicate that accretion of the planets in the inner solar system (Mercury, Venus, Earth, Mars) must have involved aqueously altered asteroidal bodies. This would provide important information on the sources of water in the inner solar system planets.

2.1.5 Water and Aqueous Alteration on Mars

Objective – Develop predictive models for the transport and state of water in the Martian crust, its alteration of crustal rocks, and the production and deposition of sediments.

Connections – §2.1.6, 2.1.7
Astrobiology Roadmap Links – Goal 2; Objective 2.1
Researchers – Taylor, Gaidos

Aqueous Processes on Mars

The Martian crust was constructed by igneous processes and subsequently modified by aqueous and aeolian processes. The products of this geologic activity contains the record of the early differentiation of Mars, the evolution of magma composition and production rates, the nature of interactions among the atmosphere, hydrosphere, and lithosphere, and, perhaps, a history of (microbial) life. The Martian crust preserves extremely ancient material but it also records very recent events. Combining data returned from spacecraft instruments with geochemical models it is possible, in principle, to reconstruct the history and importance of these processes, particularly those involving water, over 4.5 billion years of Mars history.

We propose to continue an extensive project (funded largely by other sources) that combines theoretical calculations and published data on terrestrial analogs to develop predictive models for the transport and state of water in the Martian crust, its alteration of crustal rocks, and the production and deposition of sediments. We will make predictions that can be tested by data from past, present, and future orbital and landed missions. Specifically, we intend to make detailed predictions about what assemblages of minerals will be diagnostic of a given aqueous process. This will be coupled with predictions of the effect of these processes on the chemical composition of the surface (including trace element concentrations). A good example of this approach is predictions based on terrestrial analogs of how the large hematite deposits on Mars form[28, 97]. The hematite problem can be tackled by modeling as well, including modeling reaction rates to determine the likelihood of hematite formation by aqueous reactions. For geochemical modeling we will use Geochemist's Workbench[14], which allows thermodynamic based geochemical reaction modeling. Finally, we will use these models to study possible aqueous activity on present-day Mars, its chemical signature, and its potential for hosting extant life. This research will have three foci, as described briefly below.

Products of Past Hydrothermal Alteration

Hydrothermal processes might have pervasively altered the ancient highlands of Mars[165, 166]. The Noachian was marked by active hydrologic systems, vigorous magmatism that would have provided heat to drive hydrothermal systems, and a high impact rate that would also have provided heat for hydrothermal processes. Later magmatism and impacts would also have produced localized hydrothermal systems. These products might have ended up deep in the crust, as sedimentary rocks, or in a global soil/dust layer.

- Products produced by hydrothermal activity – Hydrothermal systems produce some specific types of minerals, depending on physical-chemical conditions and the composition of the starting rock. This is testable by geochemical modeling. The suspected igneous rocks will serve as input into assessing the likely products that would be produced by hydrothermal processes.

- Changes in the nature of hydrothermal systems with time – Because of changing atmospheric composition and pressure and possibly less water being available through time, the nature of hydrothermal alteration might change dramatically. This can be assessed by modeling rock alteration under varying CO_2 pressure and water/rock ratio.

- Mars volatile inventory trapped in hydrothermal deposits. Griffith & Shock[75, 76] have drawn attention to the possibility that much of the H_2O and CO_2 on Mars might be sequestered in the crust. The mineral assemblages produced depend in part on the activities of these volatiles during alteration. More extensive modeling is called for to map out the compositional space of starting rocks and the conditions under which the hydrothermal alteration took place.

- Hydrothermal deposits – effects on compositions / oxidation states of magmas – Hydrothermal mineral assemblages are likely to be assimilated readily by ascending magmas. This process has been proposed for chemical and isotopic properties of Martian meteorites[213, 137, 81]. This would alter magma compositions, water contents, and possibly oxidation state. This process can be modeled by assimilating the alteration products suspected to be made in hydrothermal systems on Mars. This also illustrates that igneous and alteration processes are intimately related.

Ancient Weathering and Sedimentation

There is clearly a gradation from low-temperature alteration at the surface to hydrothermal alteration. We separate them here largely for convenience and include all low-temperature, water-driven processes operating within a few tens of meters of the surface. Weathering is the process in which rocks react with water and atmosphere to form new minerals. The new minerals may be eroded, transported, and deposited as part of sedimentary deposits.

- Assessing the weathering products of the major igneous rocks on Mars – The starting rock composition greatly affects what weathering products are made, so we also will work extensively on determining the full range of possible compositions of igneous rocks on Mars

to guide the choice of starting rock compositions[74]. This is an important question because many remote-sensing instruments will analyze weathered rock. We might be able to determine the nature of the unweathered rock from characteristic weathering products. Terrestrial analog studies will be very useful in this aspect of our study[163], as will be considerations of production of alteration products in the Martian regolith[74, 136, 216]

- The weathering products of hydrothermal deposits – Hydrothermal deposits can be weathered when exposed to the Martian atmosphere and surface or near-surface water. If hydrothermal deposits are as widespread as suspected, then their weathering products will be, too. Thus, it will be useful to determine the nature of the mineral assemblages for a suite of hydrothermal deposits produced under different conditions.

- Martian sedimentary mineral production – When sediments were deposited on Mars, they would have reacted with water for however long it was still present. Diagenetic processes would have operated to produce lithified sedimentary rocks. These processes will likely be the most difficult to model, but it is important to do so because sedimentary rocks will be prime targets for exploration. Water-rock reaction rates are slow and the loss of water was probably relatively rapid, so evaporation might be the major process that produces cementing minerals in the sedimentary environment[134].

- Fractionation during sedimentary transport – An intriguing and important possibility is that heavy minerals might be deposited before less dense minerals, forming distinct patterns in sedimentary basins. This can be examined by knowing the mineral assemblages present after weathering or partial weathering and applying depositional models to them to see the extent to which fractionation is possible. Fractionation by sedimentary transport has been proposed to explain aspects of the Martian soil[133].

- The effect microorganisms might have had on sedimentary mineral assemblages – In principle, a vigorous microbial population could influence what minerals are produced during alteration. In many ways organisms act as catalysts for reactions, but unique, nonequilibrium phases are also produced. This work is speculative but still worth doing, especially if it helps set limits on the role of organisms in producing the observed characteristics of the Martian soil. Geochemists Workbench allows a certain limited amount of modeling, but most of this effort will concentrate on the extensive literature of terrestrial soils.

Water, Aqueous Chemistry, and Habitats on Present-Day Mars

The detection of copious ground ice[57] and recently carved gully-like landforms[126] raises the possibility that liquid water is appearing close to or at the surface of present-day Mars. Continued aqueous activity on Mars has implications both for the geochemical evolution of the surface as well as the potential for discovering life[71]. Detailed and integrated modeling of liquid water formation, transport, and aqueous geochemistry is needed to understand these phenomena:

- Expulsion of groundwater by permafrost processes – Groundwater may be expelled from deep, freezing aquifers and rapidly erupted to the surface of Mars[69]. A mechanical and geochemical model of groundwater expulsion[69, 71] will be improved and applied to realistic Martian geologies.

- Melting of ground ice and ice fields on present-day Mars – Melting ground ice or ice fields during periods of high obliquity is currently a favored mechanism for the formation of the gullies seen on the walls of many canyons and craters[36]. However, the frost point of the Martian atmosphere (the temperature above which ice is unstable) has probably never reached the melting point of water, and melting must compete with surface sublimation and refreezing at depth to produce significant liquid water. We will further develop the model of Clow[34] to examine these issues.

- Martian hydrothermal systems at low ambient pressure The presence of ground ice and evidence for recent geologic activity (*i.e.*, young crystallization ages for some Martian meteorites) suggests that hydrothermal systems may still be active on present Mars. However, the atmospheric pressure on Mars is near the triple point of water and liquid water boils at a very low temperature (or is unstable entirely): Water driven to the surface at sites of hydrothermal circulation is most likely to boil below ground and reach the surface as steam. Nevertheless, such fumarolic systems will produce geochemical signatures and be important targets of exobiological exploration: We will model a hydrothermal/fumarole system in a Martian regolith to determine its likely physical and chemical characteristics.

2.1.6 Water-Rock Chemistry and Habitats for Life

Objectives – (*i*) to understand the role of microbially mediated reactions in diverse seafloor environments that may exist on other planets, including hot springs along the mid-ocean ridge axis, warm springs on ridge flanks, and ultramafic-hosted springs in subduction zones, and (*ii*) to assess the interplay between these reactions and the transfer of water between mantle, crust, oceans.

Connections – §2.1.5, 2.1.7, 2.1.8
Astrobiology Roadmap Links – Goals 4, 5; Objectives 4.2, 5.1
Researchers – Cowen, Mottl, Gaidos

Background – Chemical reactions between water and silicate minerals, particularly at high temperature, produces fluids that are in thermodynamic disequilibrium which, if quenched at low temperature, provide a source of free energy for life. Microbial life also accelerates mineral dissolution reactions whose effect is to release important nutrients such as phosphorus. Also, rock-water interfaces provide surfaces that can be colonized by structured communities of microorganisms. Water-rock chemistry, some of it catalyzed by microorganisms, is involved in much of the geochemistry of the Earth's crust and oceans. It also plays an important role in the formation of hydrous minerals and the planetary water cycle. On the early Earth before the appearance of substantial continental crust, the chemical reaction of water with basaltic rocks was the dominant geochemical process, and it is still important in much of the seafloor of the present-day Earth. Such submarine settings, shielded from high levels of solar radiation and the thermal insults of impacts, were likely to have been important for early life. They may also be critical to the sustenance of subsurface ecosystems, if they exist, on Mars or Europa. The role of microorganisms in subaerial weathering reactions and the production of hydrous minerals [162] and their ability to exploit the chemistry of high-temperature water-rock reactions have been clearly established. However, the role of biology in the geochemistry of seafloor alteration is only beginning to be explored. Most alteration of seafloor crust takes place within the first few$\times 10^6$yr [185].

The microbiota place a significant role in geochemical transformations within the oceanic crust both at mid-ocean ridges[206] and in cooler, off-axis crust[37]. Known biology (T $< 115°$) could persist as deep as 4 km in off-axis oceanic crust. We propose to investigate several environments dominated by water-basaltic rock chemistry, including some that are particularly accessible from Hawaii.

Hot springs along the mid-ocean ridge axis

Basaltic magma crystallizes along the mid-ocean ridge axis to form the oceanic crust. This magma also supplies heat to drive hydrothermal circulation of seawater through the crust, producing spectacular "black-smoker" springs at temperatures up to about 400°C. UH scientists who are Co-Is for this proposal have active field programs at several such sites on the seafloor, including the Endeavour main vent field on the Juan de Fuca Ridge and the Lau Basin behind the Tonga volcanic arc. The former is a typical basalt-hosted system with some evidence for sediment input, whereas the latter has systems in rocks ranging from basalt to rhyolite. The range in rock type has a large effect on the chemistry of the springs, particularly their metal and volatile content, which are doubtless important for the microbial communities in the different settings. These field programs will provide a range of opportunities for post-doctoral researchers within the proposed NAI.

One particularly unique opportunity to study the adaptation of microbial life to extreme hydrothermal environments and to freshly emplaced but rapidly evolving basalts is offered by a program headed by UH to detect and respond to mid-ocean ridge eruptive events. Ridge axis diking and eruptive events are episodic perturbations that trigger a sequence of interrelated and rapidly evolving physical, chemical, and biological processes associated with the formation of ocean crust. Dikes and lava flows develop rapidly and instantly alter the local hydrothermal flow regime. Volcanic activity produces hydrothermal discharge with a distinct geochemical signature and triggers specific geochemical and microbial responses in the adjacent crust and overlying water-column. Significant change in a variety of processes can be expected to take place over limited time spans. In effect, diking-eruptive events offer short-term natural "experiments" unachievable in the laboratory. Observing and quantifying co-variation among related processes provides opportunistic time-zero points for observations of water-basalt reactions and the development and role of microbial communities in low water/rock environments.

Diking and eruptive events dramatically alter the intensity of hydrothermal discharge from the seafloor, epitomized by the generation of so-called event plumes (Fig. 2.1.6a)[9], which involve the near instantaneous release of enormous volumes of hydrothermal fluids. Event plumes appear to form only during the short-lived period of intense seismic activity and the event-associated chronic discharge can attenuate quickly. Furthermore, the chemical and biological characteristics within hydrothermal plumes change rapidly, with the steepest rate of change occurring over seconds to days following their discharge and subsequent mixing in the deep sea. Because of these rapid changes, early on-site investigation of event plumes and associated chronic venting is essential to derive the wealth of information that they can provide, including:

- Chemical, biological, and physical constraints on subseafloor chemistry, biology and hydrology; in effect opening a window to the subsurface. It is important to take advantage of this information before aging processes degrade the initial signals.

- A unique opportunity to study and isolate subseafloor extremophiles, perhaps discharged from otherwise inaccessible regions of the crust.

- Knowledge of the heat and chemical fluxes also provide insight into the potential production and physiological diversity of subseafloor microbial communities.

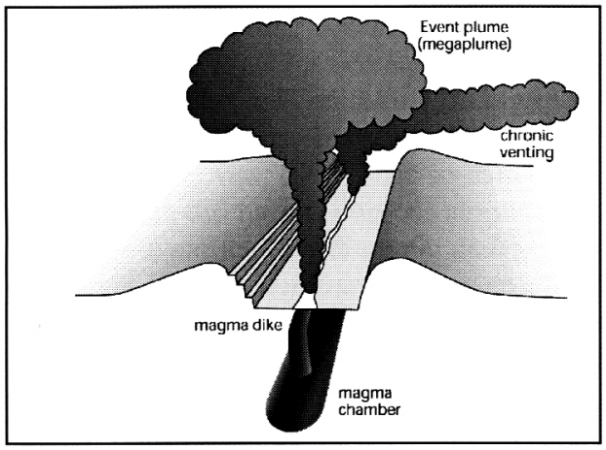


Fig 2.1.6a–Active mid-ocean ridge showing formation of event plume at location of magma dike extrusion. Continuous "chronic" style venting also indicated [37].

Diking-eruptive events provide unique opportunities to detect and characterize subseafloor microbial communities that are ejected from the ocean crust[88]. Models of the response of hydrothermal systems to a magmatic event indicate that the flux of heat and volatiles increases enormously at the time of an intrusion and then decays slowly with time. In addition, the output of subsurface bacterial biomass undoubtedly also changes. Presumably, the hydrological (*e.g.*, hydrothermal circulation) changes induced in the oceanic crust by diking-eruptive events tap pre-existing microbial crustal habitats as well as create new ones. Massive bacterial output from the associated fissures and vents created during diking/eruptive activity has accompanied the early stages of all three eruptive events studied to date[80, 88, 205]. The so-called snow blower vents associated with some of the events which discharged copious amounts of microbially-derived sulfur-based particulate matter were particularly dramatic demonstrations of potential subsurface microbial activity and biomass. Both familiar and new species and genera of hyperthermophilic anaerobic Archaea were also detected and isolated at each event studied.

Volatile output has been very high in the early phases of most diking events observed. Ratios of He, H_2S, H_2 and CO_2 to heat were all relatively high in the venting fluids during the early period of an event, recovering to lower, more steady-state levels over weeks to several years. The subsurface microorganisms are subjected to these changing, intense chemical and thermal conditions. The most intensely affected parts of the hydrothermal system are probably sterilized and would subsequently have to be recolonized. These processes are likely analogous on a small scale to sterilizing events during early Solar System bombardment of the terrestrial planets.

The unpredictability of these research opportunities accentuates the value of a sensitive and reliable event detection tool. Directed rapid response to volcanic events depends on remote real-time detection of seismic events. In 1993, the T-phase Monitoring System was developed by NOAA-PMEL to access the U.S. Navy's Sound Surveillance System (SOSUS) and allows real-time monitoring of acoustic T-waves generated by seafloor seismic activity[61].

The T-phase Monitoring System depends on U.S. Navy hydrophone arrays located off the coast of Oregon, Washington, and British Columbia and covers the northeast Pacific ridge system from the southern limit of the Gorda Ridge (GR) to the northern limit of Explorer Ridge (Fig. 2.1.6b).

Since the real-time T-Phase Monitoring System was brought on-line in June, 1993, three major eruptive episodes have been documented on the northeast Pacific spreading centers (1993 CoAxial, 1996 North GR, and 1998 Axial Volcano). Another likely magmatic event, the 2001 Middle Valley event, apparently did not penetrate the thick sediment cap overlying that ridge valley. In total, at least 6 eruptive events have occurred along the Juan de Fuca/Gorda Ridges over 15 years, or 4 remotely detected events over 6 years. Given this recent record, there is a very high probability that at least one major eruptive event will be detected along the Juan de Fuca/Gorda Ridges during the lifetime of this proposed NASA program; UH is the lead institution on the response team which is funded by NSF to maintain readiness for rapid response to SOSUS detected events through 2007.

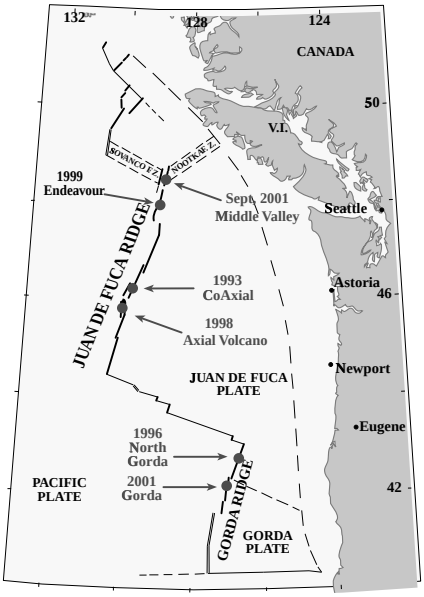


Fig 2.1.6b–Map of Juan de Fuca and Gorda Ridges (and Explorer Ridge to north). SOSUS system covers the entire MOR shown.

The close involvement and leadership of the University of Hawaii in this Event Response program will insure excellent opportunities for participation by Institute postdoctoral associates who are interested in pursing research into the identification/isolation/functionality of mesophiles, thermophiles, hyperthermophiles, halophiles and other "extremophilic" microorganisms, as well as into water(fresh) basalt interactions. Rapid and follow-up field response efforts (including submersible) will provide abundant water, fluid, and rock sampling opportunities over and at the new eruption sites.

Warm springs along mid-ocean ridge flanks

The possibility of a significant biosphere extending throughout the immense volume of aging crusts under the global system of mid- ocean ridge flanks and ocean basins is controversial. Since most ridge flank and ocean basin crust is buried under a thick, impermeable layer of sediment, the fluids circulating within the underlying oceanic crust are usually inaccessible for direct studies.

However, CORK (Circulation Obviation Retrofit Kit) observatories[45] (Fig. 2.1.6c) affixed to over-pressured Ocean Drilling Program (ODP) boreholes[46] on the flanks of the Juan de Fuca Ridge, offer unprecedented new opportunities to study biogeochemical properties and microbial diversity in circulating crustal fluids with very low water/rock ratios over a range of temperatures ($\sim$1.5 to $>$ 100°C). Basement waters collected from one such hole (ODP Hole 1026B) are chemically similar to fluid venting from nearby rocky outcrops[153, 219, 220]; the chemical characteristics and temperature (65°C) of the fluids escaping from Hole 1026B are conducive to microbial growth, supporting a diverse community of bacteria and Archaea[37].

Small subunit rRNA genes cloned from microorganisms collected on BioColumn filters (Fig. 2.1.6c) revealed a diverse assemblage of phylogenetic groups (including both Archaea and Bacteria) that are often most closely affiliated with cultured thermophiles, many that are known for their metabolic capacity to reduce sulfate or nitrate[37]. Though speculative, the data suggest that microbial processes with these electron acceptors (nitrate and sulfate) may play an important role in this region of the oceanic crust. However, stoichiometric inconsistencies in the crustal fluid chemistry remain unexplained. Critical questions remain regarding the age and flow rate of the crustal fluid[59], and the potential presence of novel metabolic pathways that may sustain microbial life in low water/rock, slow recharge environments.

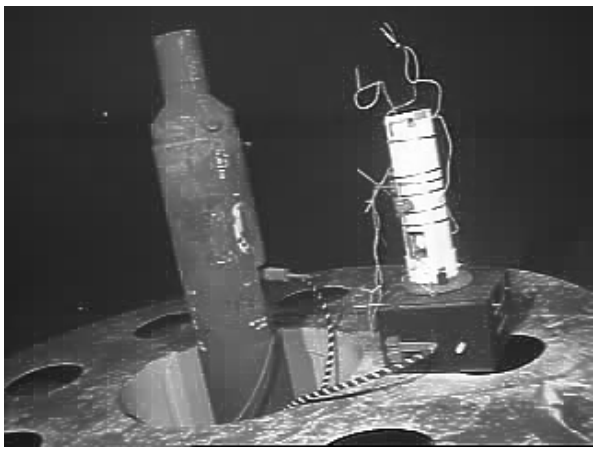


Fig 2.1.6c– Circulation Obviation Retrofit Kit (CORK) structure on top of ODP borehole 1026B. Cylinder and box to right are sampling device "plumbed" into the fluid access port of the CORK.

To what extent does H_2 production as well as organic compound synthesis via abiotic reactions between seawater and basalt[198] fuel this deep subsurface biosphere? The opportunities are great for Institute postdoctoral researchers to uncover new insights into "low water/rock" microbial communities and to discover new organisms and novel metabolic pathways.

Ultramafic-hosted springs in subduction zones

Ultramafic rocks make up the mantle of most rocky bodies in the Solar System. When ultramafic rocks come in contact with liquid water they are altered to serpentinite over a wide range of temperatures, from freezing to about 500°C. On Earth, new crust is primarily mafic basalts, however plate tectonics still provides opportunities for the reaction of water with ultramafic rocks: 1) along the mid-ocean ridge axis, especially where the basaltic crust is thin such as at fracture zones in slow-spread crust; 2) in subduction zones, where dehydration reactions in the subducting slab release water that ascends into and serpentinizes the mantle wedge of the overriding plate; 3) most controversially, in the outer rise just seaward of the trench, where the plate flexes upward as it begins to bend before plunging into the mantle[173]. On planets around other stars with somewhat different compositions or where mantle melting is less efficient, considerable more ultramafic rock may come in contact with water.

Recent work in the Mariana forearc by a Co-I[154], including manned submersible dives and deep drilling by the Ocean Drilling Program, indicates that slab dehydration and the resulting serpentinization produce unusual habitats for extremophilic microbes, including both Bacteria and Archaea, that may be a model for some extraterrestrial environments. The Mariana subduction complex is formed between the northwestward subducting Pacific plate and the overriding Philippine plate. Volatiles released from the downgoing Pacific plate hydrate the overlying mantle wedge of the Philippine plate and convert depleted harzburgite to low-density serpentinite. The resulting serpentinite mud, containing variably serpentinized harzburgite clasts, ascends buoyantly

along fractures and extrudes at the seafloor, where it forms large (30 km diameter, 2 km high) mud volcanoes along the outer Mariana forearc, in a band that extends from 50 to 120 km behind the trench axis[63]. These mud volcanoes are built from flows of poorly consolidated sedimentary serpentinite fed through a central conduit. Cold (∼2°C) spring waters fresher than seawater have been sampled on several of these mud volcanoes.

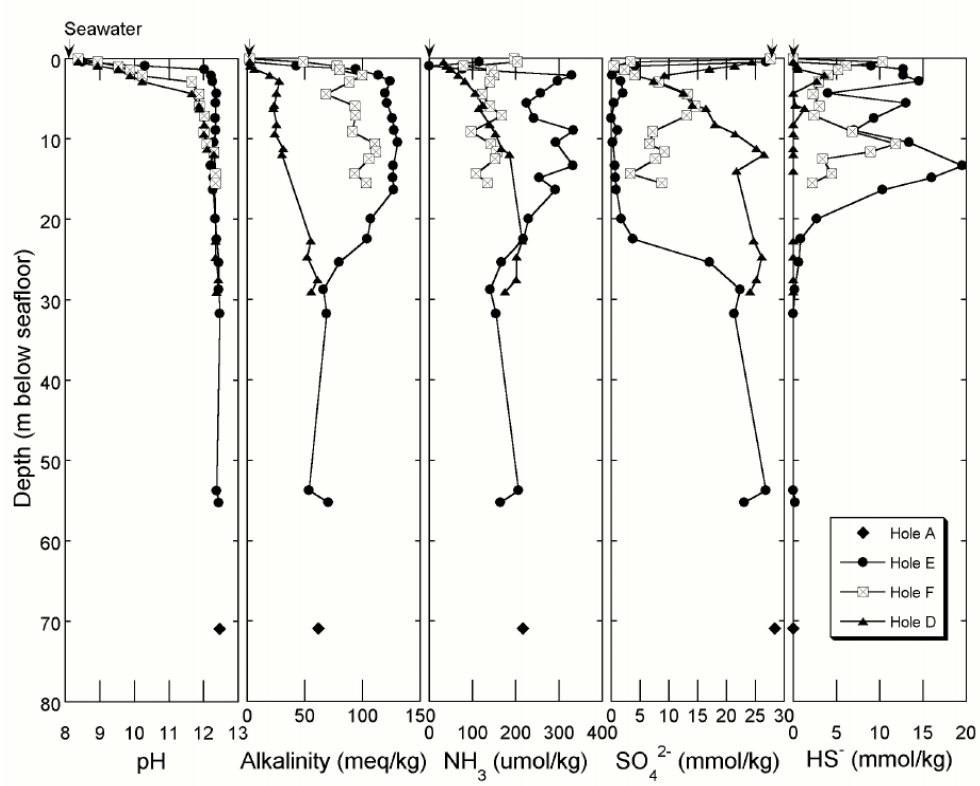


Fig 2.1.6d–Composition of pore water squeezed from serpentinite mud at ODP Site 1200, summit of South Chamorro Seamount, an active serpentinite mud volcano in the Mariana forearc at 13°47.0N, 146°0.2E[154]. Holes A and E were drilled at a cold (2°C) spring and Holes F and D are 20 m and 80 m to the north, respectively. Sulfate reduction at 1 to 3 and 13 mbsf, mainly by Archaea as indicated by the concentration of phospholipid-derived diphytanyl diethers (DE), produces alkalinity, bisulfide, and ammonia, all at pH 12.5. The carbon source is methane, which is present at 2 mmol/kg in the fluid ascending from the top of the downgoing plate, which is about 27 km below the seafloor at this site.

The chemistry of these springs is highly unusual: they are among the least saline waters ever recovered from the deep sea and have the highest pH, 12.5, which results from ongoing serpentinization. Relative to seawater, these springs have higher to much higher sulfate, alkalinity, pH, Na/Cl, K, Rb, B, light hydrocarbons, ammonia, δ^{18}O, and δD; and lower to much lower chloride, Mg, Ca, Sr, Li, Si, phosphate, and Sr isotopic ratio. Ba, Mn, Fe, and bisulfide are low[152]. Within the upper 20 m below seafloor in the vicinity of these springs, a microbial community operating at pH 12.5, are made up overwhelmingly of Archaea, is oxidizing methane from the ascending fluid to carbonate ion and organic carbon, while reducing sulfate to bisulfide and probably dissolved nitrogen to ammonia (Fig. 2.1.6d). There is essentially no sedimentary organic carbon in this setting. The microbial community that feeds the macrobiota at the summit of S. Chamorro Seamount

is therefore not only extremophile, but subsists on a source of chemical energy delivered from as deep as 27 km below the seafloor. Additional cruises to the Mariana forearc during the period of this grant will permit Institute scientists, including post-doctoral researchers, to characterize these unusual microbial communities and to assess the likelihood that similar communities might have evolved in extraterrestrial habitats. We will culte these organisms in the laboratory at high pH in a serpentinite matrix.

Microbial "colonization" of lava-seawater-generated plumes

Direct entry of lava into the ocean on the shoreline of Kilauea Volcano, Hawaii, creates a surface hydrothermal plume enriched in dissolved F, Cl, Fe, Al, Ti, Mn, Si, V, certain rare earth elements, dissolved gases such as H_2 and even sulfate[190, 191, 182, 183]. The plumes are acidic and contain high levels of suspended silicate glass particles produced by the explosive disintegration of lava as it is quenched from temperatures near 1400 K to that of boiling water. Surface waters are also influenced by input of aerosols generated in the resulting steam plume[192]. These plumes are a unique biological habitat because of the high concentrations of biologically important elements, particularly P and Fe, which were measured at 2 and 5 orders of magnitude, respectively, above average seawater concentrations[183], extremely high concentrations of potentially toxic metals (Al, Co, Cu, Pb, Cd, V), the high density of suspended glass particles available for colonization (up to 65 mg L^{-1} [191]), *and* they are within the photic zone (unlike deep-sea hydrothermal vents). Strong thermal and chemical gradients are produced by entrainment of ambient water into the plume[191]. These could represent analogs to aquatic microbial "oases" on the early Proterozoic Earth when rising oxygen levels and the insolubility of iron in surface waters deprived photosynthetic organisms of critical nutrients[111]. They also may be representative of processes occuring on a wider scale during the emplacement of large igneous provinces[222] during the Phanerozoic.

Although large zooplankton blooms have been observed, the biology of these plumes has remained unexplored. We propose to survey the microbiology associated with the plume water column and that attached to the glass particulates, using the ambient seawater as the basis of comparison. We are especially interested in tracing the dynamics of both populations as the plume waters are a dynamic entity, being mixed out over a distance of about 1 km. We will carry out spectrophotometric analyses of the plume waters to identify potential photosynthetic pigments. Filtered particulates and water samples will be analyzed separately with microscopy, including low-vacuum scanning electron microscopy for the former. DNA will be extracted and amplified using polymerase chain reaction (PCR) and analyzed both at the community level , *e.g.*, denaturing gradient gel electrophoresis (DGGE), and at the individual sequence level. We predict that because of the high levels of H_2 and ferrous iron in the plume, gnallgas and iron-oxidizing bacteria will be well-represented in these communities.

2.1.7 Extreme Aquatic Environments on Earth and their Analogy to Potential Habitats in the Solar System

Objective –In this project we will focus on a comparative study of microbial biodiversity, biomass and metabolic activity in a variety of "extreme" aquatic habitats.

Connections – §2.1.5, 2.1.6

***A**strobiology Roadmap Links* – Goal 5; Objective 5.3
Researchers – Gaidos, Karl, Thorsteinsson

Background – Life, especially microbial life, has successfully radiated into nearly all the aquatic habitat space on Earth. It is usually assumed that life as we know it absolutely requires liquid water and that the fundamental constraint for growth is the activity of water or the availability of free water. Besides water, life also requires a source of carbon and nutrients, and an environment that is conducive to the propagation of genetic information, *i.e.*, the error-free replication of DNA. Many aquatic environments on Earth are challenging or "extreme" from the point of view of these other requirements. By studying these we can better understand what may limit the origin and persistence of life in aquatic habitats elsewhere in the universe.

The scope of potential habitats is expanded further when one considers indications of possible aquatic environments elsewhere in the Solar System. There is significant geomorphological evidence for past (or even present) flowing water on Mars, and geophysical evidence for an interior ocean beneath the crust of Europa (and possibly Callisto). Ground ice is abundant on present-day Mars[57] and the outflow channels and valley networks carved in ancient terrains show some of that water was liquid, at least transiently, in the past[23]. Apparently much more recent gullies carved in the walls of canyons and craters raise the exciting possibility that surface or shallow groundwaters occur at the present epoch[126],[69],[36]. The geomorphic evidence for a subsurface ocean on Europa[170] has been strengthened by the detection of an eddy current-induced magnetic field around the satellite[226].

However, these extraterrestrial aquatic environments may be far more extreme than most encountered on Earth: Liquid water is not, and may never have been stable on Mars, and any long-lived bodies of water were probably covered with thick ice crusts that isolated the surface from sunlight and the atmosphere [71]. During episodes of high martian obliquity, summer surface temperatures on poleward facing slopes on Mars can exceed the melting point of water ice, but mean surface pressures and temperatures will limit the amount of liquid that can be generated. Martian meteorites with young crystallization ages[135] and low crater counts on some martian terrains[79] suggest that magma is still reaching relatively shallow depths on Mars. The near-ubiquitous presence of ground ice[57] supports speculation that hydrothermal systems may still be active on present Mars and are potential targets for exobiological exploration. However, the atmospheric pressure on Mars is near the triple point of water and liquid water boils at a very low temperature (or is unstable entirely): Water driven to the surface at sites of high planetary heat flow is most likely to boil and reach the surface as a vapor. Europa's ocean is isolated from sunlight by an ice crust between 10 and 100 km thick and the amount of free energy for chemotrophic organisms will be meager [66, 31]. Tide-driven shear and viscous dissipation can warm the shallow crust of Europa and create liquid water within reach of sunlight[67] , but the melt fraction will be low[168] and any aquatic habitats will probably consist of brine channels within an ice matrix.

The limits to life with respect to temperature have been intensely explored since the pioneering work of Brock[22]. Hyperthermophilic prokaryotic archaea with measurable growth rates at 115°C have been cultured from deep-sea hydrothermal systems and there has been tantalizing evidence of biological activity at still higher temperatures. Accelerated hydrolysis of tri-phosphates above 160°C may constitute an ultimate temperature limit to life based on known biochemistry. What are the limits to life with respect to the activity of water, *e.g.*, can life exist under conditions where the stable phase of water in the environment is vapor? Hyperthermophiles growing above

100° are found in environments where the pressure is well above ambient and the boiling point well above the maximum growth temperature. Certain microorganisms florish in superheated hot springs 1-2°C above the boiling point but their ability to survive in superheated steam conditions is unknown. Any life in these truly extreme conditions must adapt to the limited activity of water. These truly extreme conditions create life that must adapt. By studying analogs of such environments on Earth and their potential biota we obtain basic information on the strategies of, and limitations to, biochemical and physiological adaptation.

In this project we will focus on a comparative study of microbial biodiversity, biomass and metabolic activity in a variety of "extreme" aquatic habitats including: (1) the open sea near Hawaii, (2) Lake Kauhako, Molokai and (3) Lake Waiau, Hawaii, (4) subglacial habitats in Iceland and Antarctica, and (5) fumaroles in Hawaii and South America. Each study area has unique physical and chemical characteristics and each is expected to select for a different assemblage of microorganisms. By studying the similarities as well as the differences we hope to provide basic information on the strategies of biochemical and physiological adaptation and on gene evolution.

1. Scientists in the UH-NAI program will have access to numerous open ocean habitats ranging from the nutrient stressed surface waters of Station ALOHA (22°45′N, 158°W) to the deep abyss. These study areas are visited approximately monthly as part of the ongoing Hawaii Ocean Time-series (HOT) program that uses the university's research vessel as a floating laboratory. Samples collected at sea can either be processed on site or returned to the university for more extensive or sophisticated analyses.
2. Lake Kauhako (Fig. 2.1.7a), Molokai is a deep (248 m) meromictic lake that occupies the crater of an extinct volcano (21°11.5'N, 158°58'W). This lake has the highest relative depth (ratio of depth to surface area) of any lake in the world. The upper 4.5m of the lake is well stratified but below 4.5m the lake is nearly uniform in temperature and salinity (26.25°C, 32 per mil). The lake is anoxic below about 2 m with very high concentrations of hydrogen sulfide appearing below about 6m[48].

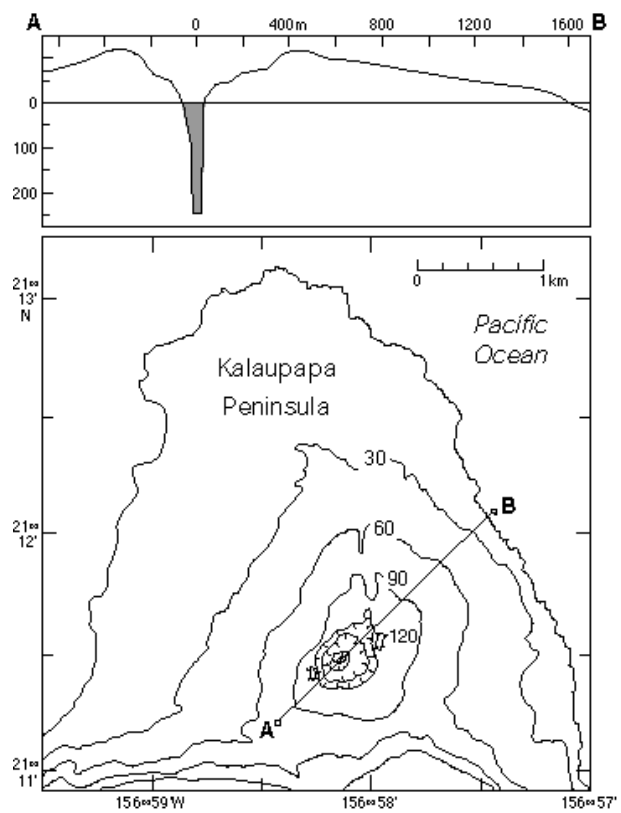


Fig 2.1.7a–Lake Kauhako on Molokai. Upper panel shows a vertical profile along A to B on the bottom map. The shaded portion in the top figure is the lake itself with depth interval in meters. Bottom panel: contour elevation map in meters.

In many respects this lake has conditions that are analogous to the permanently anoxic basin, the Black Sea[158] and thus serves as a convenient and relatively accessible habitat for comparative studies of aquatic microbiology. Kauhako Crater was designated a "special Ecological Area" in 1994.

3. Lake Waiau, Hawaii, is located at an elevation of 3980 m near the summit of Mauna Kea (19°48′N, 155°29′W). The lake lies in a shallow crater and is approximately 90m in diameter and 3 m deep, although its dimensions vary with precipitation patterns. Weather at this site is extreme with especially high winds; the typically isothermal lake temperature ranges annually from 0-13°C, with wintertime ice cover. Lake Waiau ultraviolet radiation levels are about 30% higher than at sea level for comparable latitude and is thus of interest to studies of organismal adaptation to the high radiation environments of the early Earth and on other planets. Like Lake Kauhako, Lake Waiau is located in a restricted conservation area which minimizes human impact and interference. For the open Pacific Ocean, Lake Kauhaku, and Lake Waiau we will make comparative observations and measurements of key indices of microbial biomass (by total ATP, lipopolysaccharide, chlorophyll and epifluorescence microscopy[106] and habitat chemical characteristics) temperature/salinity, dissolved oxygen and CO_2 gas concentrations, inorganic and organic nutrient concentrations, particulate matter carbon/nitrogen and phosphorus content[105] at each site.

   Metabolic activity will be assessed for the total microbial assemblage by measurement of respiration (in vitro oxygen consumption) as well as for specialized physiological functions such as nitrogen fixation (by the acetylene reduction method) and other selected enzyme activities (phosphatase, peptidase). We will also endeavor to isolate representative microorganisms from each site, with a focus on prokaryotes.

4. The existence of a large, deep freshwater lake beneath the ice of central East Antarctica was reported in 1996[103]. The lake was named Lake Vostok because of its proximity to Vostok Station (near 77°S, 105°E). During the same year an inventory of 77 such Antarctic subglacial lakes was published[196] proving them to be a relatively common occurrence. Lake Vostok is located beneath approximately 3800m of ice; below approximately 3500 m the ice appears to be refrozen from Lake Vostok, accreted to the bottom of the glacial ice column (*i.e.*, so-called accretion ice). Although microorganisms have been detected in the accreted ice portion of the 3623 m long Vostok ice core (drilling was terminated at a depth of approximately 120 m from the ice-lake water interface[107, 179]) no samples have yet been recovered from any Antarctic subglacial lake.

   Besides the liquid water in the lake itself, both the glacial ice column and the accreted ice may also provide unique habitats for the growth of microorganisms. For example, bacteria, yeasts, fungi and microalgae at depths ranging from 1500 to 2750 m have been found[2] in Vostok glacial ice. Some of the microbes were claimed to be viable. Price (2000) has also reported viable cells to depths of 3600 m in the Vostok core. He hypothesized that the microorganisms inhabit a microenvironment consisting of interconnected liquid veins along three-grain boundaries in ice wherein psychrophilic, halophilic bacteria can move and obtain energy and carbon from ions and compounds in solution. We plan to test this hypothesis using ice column mesocosms in the laboratory. We will also test the hypothesis concerning the role of brine channels in sea ice as a unique habitat for microbial growth using a laboratory based mesocosm approach similar in design to the one recently described by Mock[147].

5. Iceland is located on the Mid-Atlantic Ridge and at the Arctic Circle, and consequently hosts numerous active volcanoes and glaciers. We are investigating Icelandic subglacial

volcanism as unique terrestrial environments and analogs to potential habitats on Mars and the galilean satellites of Jupiter. Some martian landforms have already been identified as possibly of glaciovolcanic origin[26]. Subglacial systems where volcanic heat drives hydrothermal circulation are more like to support a flourishing subsurface community because (1) liquid water is produced from the melting of the overlying ice by high geothermal heat; (2) free energy is provided by the reducing hydrothermal fluids produced by high-temperature water-rock chemistry mixing with oxidizing gases such as O_2 or CO_2 released by melting of ice; and (3) the extreme thermal gradients between the ice and hot rock or water will create a wide range of habitats. We expect that, because of differences in temperature, geology, and ice flow, subglacial hydrothermal environments will manifest significant physiochemical and biological diversity similar to that seen in the hot springs within the Yellowstone caldera.

Grimsvötn is one of two active volcanoes under the Vatnajökull ice sheet. Intense hydrothermal circulation within the 100 km^2 caldera drives an average heat flow of 50 W m^{-2}, far exceeding the 2 W m^{-2} typical of the Yellowstone caldera and in vent fields on mid-ocean ridges. Glacial melt in a 300 km^2 drainage basin feeds a geothermally sustained lake covering 20 km^2. Continuous geothermal activity and eruptions cause the lake to episodically – and catastrophically – drain, triggering jökulhlaups, the largest floods on present-day Earth. The lake was previously sampled for geochemical analyses in 1991[3]. We carried out a preliminary biological investigation of the Grimsvötn subglacial caldera lake in June 2002 expedition[70]. Self-sterilizing hot-water drilling was used to penetrate the 300 m ice shelf and sample the lake (Fig. 3.1.7b). We sampled the lake water, the tephra at the bottom of the lake, and the ice and snow over the lake. Chemical analyses included pH, alkalanity, and total dissolved solids. Cell counts were made on replicate samples. Lake water samples were incubated with ^{14}C-labeled bicarbonate and acetylene to assay for carbon fixation and nitrogen fixation, respectively. DNA was extracted and amplified using universal bacterial primers and the polymerase chain reaction (PCR). An analysis of the whole community was done using the denaturing-gradient gel electrophoresis (DGGE) technique.

There were significant cell counts in both lake waters and especially high counts in the tephra sediments. We measured significant carbon uptake and nitrogen fixation by lake samples. The DGGE analysis showed that (at the molecular level) the lake community was distinct from the organisms trapped in the overlying snow and ice. We have preliminarily concluded that an endemic, autotrophic, and nitrogen-fixing community of microorganisms exists in the lake, the product of the unique environment selecting on an assemblage of "innoculating" microbes that are released into the lake from melting ice.

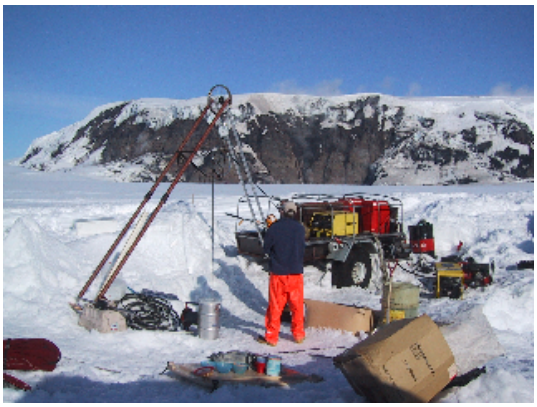


Fig 3.1.7b– Hot-water drilling apparatus in operation on the lake ice shelf within the Grimsvötn volcanic caldera, Iceland.

The discovery of high cell counts in the lake tephra is consistent with findings in many settings that microorganisms preferentially colonize particle surfaces relative to the water column. At Grimsvötn and similar environments in Iceland, volcanic tephra and hyaloclastic breccia is emplaced as extremely hot (900-1400 K) and thus sterile debris during subglacial eruptions. This material is, of course, immediately quenched by melting and vaporization of the surrounding ice, but temperatures are likely to remain at the boiling point (230°C under 300 m of ice) and well above plausible limits for life for a brief period of time that depends on the water to rock ratio and the vigor of subsequent hydrothermal circulation. Eventual cooling will allow organisms released from the surrounding ice to colonize the mineral surfaces on the tephra. Our objective is to understand the mechanisms and sequence of events associated with the establishment and maintenance of a microbial community in an initially sterile, abiotic subglacial environment. The processes of colonization and succession on subaerial[209] and submarine[155] basaltic lavas, particularly involving plants and macrofauna has been extensively studied including on Hawaii. The subglacial process is arguably simpler and better defined because potential colonists must come from the overlying ice and can be surveyed, and plants and macrofauna are absent. The process may be crucial to the continuity of life on planets (such as Mars) with inhospitable surfaces. We are interested in addressing the following question: Is there a single colonization event or continued succession in the microbial community over time? In the absence of sunlight and phototrophy, is the colonization process related to the suite of chemotrophic metabolisms available to, and employed by, microorganisms? Is the process identical at every subglacial site or is it a function of local conditions? Is it deterministic or depends on some stochastic element? What are the important potential biomarkers of colonization that could be used to identify past biological activity at similar sites on other Solar System bodies?

Our plans for future field work include a return to Grimsvötn in the summer of 2004 for additional drilling and sampling close to the caldera rim where normal faulting associated with the collapse of the caldera is expected to permit more intense hydrothermal circulation. We will also investigate the Skáfta "cauldrons", two sites of subglacial geothermal heating whose surface expression are 2 km-wide depression in the Vatnajökull ice sheet. These occupy separate drainage basins adjacent to that of Grimsvötn. Additional cauldrons have been identified on the Mýrdalsjökull ice cap, which occupies the extremely active Katla caldera[16]. Samples will be examined using both epifluorescence microscopy and environmental scanning electron microcopy. DNA will be extracted and amplified for both community-level analysis and the construction of clone libraries for sequencing. Fluorescent probes will be designed to target organisms with DNA sequences of interest and image them in the tephra particle environment. We will remove pristine samples of ice and fresh tephra for use in laboratory microcosm experiments: The tephra is reheated to temperatures sufficient to sterilize and volatilize any organic carbon and then added to the ice. Sampling of these incubations will allow time-dependent phenomenae to be observed.

6. On Earth, high-altitude fumaroles provide natural environments in which temperatures within the known range of biological tolerance exceed the boiling point. For example, water boils at 92°C in Yellowstone (2225 m elevation) and at the Geysers del Tatio in Chile (4321 m elevatio) the boiling point is 85°C. At Sol de Manana, Bolivia (up to 5000 m eleva-

tion) water boils at a "mere" 83°C, well within the range of non-photosynthetic life. Organisms surviving in such environments would have to evolve two adaptations; (1) preventing its cytoplasmic fluid from boiling, and (2) preventing dehydration due to the difference in the vapor pressure between liquid water inside and outside the organism. Microorganisms with cell walls maintain their volume by creating an internal (turgor) pressure. This is done by maintaining a difference in osmolality (equivalent solute concentration) between the internal cell and its environment. Gram-negative bacteria can maintain turgor pressures up to several bars. The corresponding boiling point elevation is approximately 60°C. Cellular cytoplasm is also a briny fluid and the presence of anti-boil compounds could elevate the boiling point. Finally, boiling requires the nucleation of bubbles. The scale of most bacteria is 1 μm, raising the potential of suppression of boiling by suppression of nucleation. Dehydration could be prevented by suppressing the vapor pressure of the cytoplasm (by antiboil compounds), or by expending energy to actively pump water into the organism against the osmotic gradient. In fumarolic environments where steam temperatures are outside the tolerable range of life, microorganisms could adapt endolithic lifestyles and use the insulating properties of the fumarole rock walls to protect them from heat and retain water.

We have developed a program to search for biological activity, and organisms, in low-temperature fumaroles associated with the Kilauea Volcano in Hawaii (1200 m elevation). We will expand our program to identify high-altitude fumaroles in South America, including the Geysers del Tatio (4321 m) and Sol de Manana sites. Past and present fumarolic activity has been identified as high as 6000 m in the Andes, where the boiling point is about 80°C. Fumarole wall samples will be retrieved for optical and electron microscopy, isotopic analysis, DNA extraction for molecular identification of organisms. We will also carry out colonization experiments, leaving sterilized silicate surfaces in place for a period of 1-2 years (Fig. 3.1.7c).

Fig 3.1.7c– Colonization experiments tested in fumaroles at Kilauea Volcano, Hawaii. Basalt sample were thin-sliced, attached to glass slides using high-temperature epoxy, and roughly polished. This provides a natural substrate for potential organisms.

2.1.8 An Integrated Model of the Planetary Water Cycle

Objective – Develop and refine an integrated model of planetary water, including mantle degassing, crustal hydration, dewatering at subduction zones, and escape to space. Use this model to explore the sensitivity of planetary water evolution to planetary mass, composition, and initial inventory of water.

Connections: §2.1.4, §2.1.9

***A**strobiology Roadmap Links* – Goals 3, 5; Objective 3.1
Researchers – Gaidos

Background – Earth's most visible inventories of water are its oceans, fresh-water bodies, and continental ice sheets, however an additional 40% is present as hydrous minerals in sediments and crustal rocks, and several oceans is contained in the hydrated mantle[200]. Mantle convection and plate tectonics mediates the exchange of water between surface and interior reservoirs. Mantle water partitions into the silicate melts created by the divergence of lithospheric plates and is volcanically outgassed at mid-ocean ridges. Water enters crustal rocks and sediments as hydrated minerals during continental and seafloor weathering and hydrothermal alteration of the oceanic crust. Much of this water is re-released during high pressure metamorphism and arc volcanism at subduction zones, but some fraction is returned to the mantle. The difference in these fluxes controls the evolution of the surface and mantle inventories of water over Earth history.

Although the current escape rate of water to space (as H) by thermal and non-thermal mechanisms is $\sim 10^{-6}$ oceans per billion years[225] and is negligible compared to the geological fluxes, more significant amounts of hydrogen (and equivalent water) may have been lost during the Archean (prior to 2.5 billion years ago) when molecular oxygen did not limit the mixing ratio of molecular hydrogen[108] or biogenic methane[84] in the trophosphere. Greater amounts may also have escaped to space via hydrodynamic space powered by giant impacts or higher solar ultraviolet radition early in Earth's history[92]. Conversely it has been proposed that additional water was brought to Earth subsequent to accretion by carbonaceous chondritic or cometary bodies[29]. These processes would obviously have effected the size of Earth's surface and interior inventories of water.

A minimum reservoir of surface water is probably required for planetary habitability, *e.g.*, to promote the precipitation of atmospheric CO_2 as carbonate minerals and its sequestration as crustal carbonate rocks so as to avoid a runaway greenhouse. In addition, surface water entering the crust can lubricate faults, weakening the brittle lithosphere and possibly enabling plate tectonics itself to function[168, 151]. The abundance of water in the mantle influences its rheology and solidus and thus exerts a powerful control on mantle dynamics and degree of mantle melting[82]. In turn, this affects the exchange of water between the surface and interior. Thus the partitioning of planetary water between mantle and surface reservoirs may be crucial to long-term habitability, especially if the initial surface inventory is removed early on and replenished by degassing of the mantle. In addition, hydrous melting is involved in the production of buoyant felsic crust that serves as the source material for continents. Continental growth is important factor in rates of weathering and atmospheric CO_2 levels, sequestration of organic carbon and stabilization of atmospheric O_2[13]. High oxygen levels and stable continental platforms may be prerequisites for the appearance of complex terrestrial life, and, perhaps, intelligence.

The inventories and cycling of water in Earth-size planets around other stars may be quite different than our own. The initial abundance of water may depend sensitively on the redox chemistry of the planet-forming nebula and water content of the planetary source material[68], the presence of giant planets which facilitate (or impede) transfer of water from the outer to the inner regions of a planetary system[125], and variation in the efficiency of loss processes such as impacts[141] or ultraviolet radiation-driven hydrodynamic escape[217]. The fluxes of water between the surface and interior can differ because of differences in the composition and melting properties of the planet's mantle and the abundance of heat-generating long-lived radioactive isotopes.

We are developing a model of the dynamics of water in and on a solid planet. Our objective is to understand the implications of differences in planetary mass, composition, environment and initial inventory on the long-term evolution of planetary water. Such models will be useful in predicting the potential diversity of Earth-mass companions to stars that might be directly detected by future space-based observatories such as those envisioned by the Terrestrial Planet Finder project[11]. We have developed an initial parametric model of an ideal, perfectly uniform mantle. Similar parameterized convection models include those of[131, 208, 18]. The thermal evolution of the mantle is set by the balance between the heat generated by the decay of long-lived radioisotopes, heat rejected by mantle convection, and mantle cooling. The convection involves a temperature- and water content-dependent viscosity[131] and the usual parametric relationship between heat flow and the mantle Raleigh number is assumed. The total spreading rate of the lithosphere is then calculated as a function of heat flow[131].

The evolution of the surface and mantle water reservoirs (W_s and W_m) is specified by the difference between the outgassing flux and the regassing and escape fluxes;

$$\frac{dW_s}{dt} = F_{out} - F_{recycl} - F_{esc} \tag{1}$$

$$\frac{dW_m}{dt} = -F_{out} + F_{reg} \tag{2}$$

The rate of outgassing is

$$F_{out} = C_{mw}\rho_m SR \int_0^{d_m} X\,dz, \tag{3}$$

where the concentration of water in the mantle C_{mw} is W_m/V_m, ρ_m is the average mantle density, SR is the spreading rate, d_m is the depth of melting, and X is the melt fraction. We assume that water partitions into the melt fraction X up to a specified solubility limit. We use an empirical melting parameterization to calculate the melt fraction and depth of melting[132].

The rate of recycling of water back into the mantle is the rate of subduction of water in lithospheric slabs corrected by a factor R_{reg} that accounts for the devolatilization and escape of water from unstable minerals;

$$F_{recycl} = (1 - R_{reg})C_{cw}\rho_c d_c SR, \tag{4}$$

where C_{cw} is the concentration of water in crustal minerals, ρ_c is the density of the crust, and d_c is the thickness of the hydrous crust. The amount of water in the crust will depend on the depth at which hydrothermal circulation penetrates, and on the mineralogy of the crust[99].

Fig. 2.1.8a shows the results of initial simulations with three different initial total inventories of water (measured in present-day Earth oceans). In each simulation, the initial surface reservoir is zero and the initial mantle temperature is set so that the mantle is in thermal equilibrium with the initial rate of heat production by long-lived radioactive isotopes. Using these assumptions, we find that the surface reservoir asymptotically increases with time. This result is consistent with the absence of any indication of a dramatic change in continental freeboard in the geologic record. The model (Fig. 2.1.8a) also correctly produces the current mantle potential temperature of the Earth but the estimated global spreading rate must be corrected by a factor of 1.7. Currently the model simulates only planets with contiguous oceanic crust; we will add the formation of continental crust to a later version. We will continue to improve the model, constrain the model

parameters using the terrestrial case, and then use the model to explore the effect of varying the abundance of planetary mass, abundance of heat-producing long-lived radioisotopes, mantle composition, initial water inventory, and rate of hydrogen escape on the evolution of the planetary water cycle.

Fig 2.1.8a– Planetary surface water reservoir versus time for an Earth-like planet simulated using an empirical model of mantle convection coupled with a geologic water cycle model. Only the initial total water inventory is varied. See text for details.

2.1.9 Signatures of Water-Bearing Exoplanets

Objective – Model the water-rich comae of super-comets and planets experiencing atmospheric escape and determine their detectability. Evaluate the feasibility of the time-series spectroscopic determination of the abundance, distribution, and state of water on Earth-like planets around other stars using observations of earthshine reflected by the Moon.

Connections – §2.1.1, 2.1.8
Astrobiology Roadmap Links – Goal 1; Objective 1.1, 1.2
Researchers – Gaidos, Meech

Background – Nebular water will be removed with the rest of the primordial disk gas from a young solar system either by a T-Tauri wind or by incorporation into giant planets. However, volatiles, including water, will be retained for much longer periods of time in icy bodies or water-rich planets, depending on their masses and orbits. This water may be subsequently lost to space by direct devolatilization (in the case of an icy body) or by the ultraviolet radiation-driven escape of water-rich atmospheres. This process is still ongoing in our solar system, although it is now important only for comets. In the early Solar System, (dissociated) water would be escaping from the runaway greenhouse atmosphere of Venus[27] as well as from satellite-sized icy planetesimals scattered closer to the Sun by the giant planets. Are the extended, hydrogen/water-rich atmospheres/coma of equivalent objects around other stars detectable? Transient, doppler-shifted absorption lines in the spectrum of β Pictoris, surrounded by a circumstellar disk, have been proposed to be the result of comet-like "falling, evaporating bodies" crossing the stellar disk[15]. We propose to model the comae of "super-comets" and planets with hydrodynamically escaping atmospheres and determine if it is possible to detect them either in absorption or emission.

Space-based observatories have been proposed to directly detect and characterize Earth-sized planets around nearby stars[121, 11]. The ultimate goal of these projects is to ascertain whether a extrasolar planet has life by identifying "biosignatures" in a planet's spectrum, *e.g.*, atmospheric O_2, O_3, CH_4, or biogenic pigments such as chlorophyll. Time-series photometry and spectroscopy of

an Earth-like planet may also reveal important physiochemical details about the surface and its habitability. These include the presence of oceans, clouds, ice caps, and deserts[60]. The thermodynamic state of water on a planet and its variability is fundamental to its climate and seasonality. Earth-like planets need not resemble the Earth as it is today: Large continental glaciations, episodes of intense warming (and cloudiness) and so-called "snowball Earth" events[85] would have dramatically altered the albedo and spectrum of the past Earth.

In the absence of known Earth-like extrasolar planets (and a space observatory to observe them), a suitable test of an observational strategy is to observe the Earth itself from a distance. Sagan[189] observed the Earth with the *Galileo* spacecraft and detected the chlorophyll "red edge" of terrestrial vegetation at $\sim$750 Å. A more cost-effective technique is to measure the intensity and spectrum of earthshine reflected by the dark side of the Moon. Two groups have derived the terrestrial albedo as a function of wavelength and report the detection of Rayleigh scattering at blue wavelengths and absorption features of ozone, molecular oxygen, and water[224, 6]. They also report the tentative detection of the chlorophyll red edge. The Earth exhibits significant albedo variations as a consequence of exposure of the Antarctic ice sheet to austral summer sunlight and changes in cloud and vegetation patterns[73]. But with limited temporal and spectral resolution, limited signal-to-noise, and no spatial resolution, how can clouds and ice sheets be distinguished? How can water-ice and CO_2-ice clouds be distinguished, particularly in the absence of independent temperature data? Can seasons be inferred solely by detecting changes in cloud cover patterns or the waxing and waning of ice sheets? These signatures may be subtle and difficult to deconvolve. While the absorption bands of water vapor may be obvious (at least from space), the spectrum of ocean water is blue and resembles that produced by Rayleigh scattering and an atmospheric aerosol component.

To answer these questions we are carrying out a long-term monitoring of the spectrum of earthshine with the UH 2.2 meter telescope and the HARIS spectrograph. The first observations are to be carried out during late March of 2003. The earthshine spectrum, modulo a constant, is obtained by dividing the spectrum of the dark side of the Moon by that of the illuminated portion. Care will be taken to remove from each spectrum the temporally interleaved spectra of the sky (which changes with time) and correcting for detector effects, including CCD fringing.

2.2 Extending the State of the Field – Relevance to Past, Current, Future NASA OSS Programs

We have chosen water, and its relationship to life, as both a unifying paradigm and interconnecting theme. Our consortium will advance the state of the field by placing the research of individual investigators into a framework in which the results in any given field will advance, or serve as the foundation of, research in other fields: Observations of the abundance and chemistry of water in star- and planet-forming regions provides the boundary counditions for experiments on space ices, which in turn are critically important to correct interpretation of observations of comets and the interstellar medium. Modeling of Martian hydrothermal systems is necessary for selection of terrestrial environments as appropriate analogs, exploration of which will help the search for potential habitats for and signatures of life on Mars. Modeling of the planetary water cycle requires information about water in planet-forming material provided by the study of meteorites and comets, and in turn informs searches for the signatures from water-bearing exoplanets.

In addition, the bulk of the funding requested in this proposal will support postdoctoral fellows

who will be given access to facilities, the academic freedom, and the independence necessary to pioneer innovative and interdisplinary rsearch that includes, and builds upon, the research described in this proposal. In funding a corps of respected junior colleagues, allowing them to create a unique and independent environment (the "Water Hole") and connecting them to multiple investigators and research facilities, we plan to develop a unique assembly of scientists bonded by a common interdisciplinary research them rather than sometimes-anochronistic disciplinary ties.

Water underpins much of the current research of the Astrobiology Institute and our efforts would support these, as well as other NASA efforts, including the Mars Exploration Program and the Search for Origins. One of the products of this research will be an improved understanding of processes that control the frequency and nature of extrasolar planets with aquatic (*i.e.*, habitable) environments, information that is critical to the design of space observatories such as those envisioned by the Terrestrial Planet Finder project. Finally, much of this research will be carried out in Hawaii, a location where a variety of aquatic environments and their conjugate ecosystems, some unique can be studied. It is also a place where the theme of water has an obvious resonance and deep roots. As inhabitants of the most isolated archipelago on Earth, we will contribute a sense of importance to scienctific ties that span great divides of distance and discipline, and, as dwellers in an ocean, an appreciation both scientific and cultural to that great medium from which we all sprang.

This work is well-aligned with NASA OSS programs, closely following the Astrobiology Roadmap (as noted in each section). In addition, the work is closely related to many upcoming and proposed NASA missions, such as Deep Impact, Pluto-Kuiper Express, and most especially the Terrestrial Planet Finder. Our goal, in effect, is to establish the parameters of a "Watery Drake Equation", to better assess the properties which make a world habitable.

2.3 Technical Approach

2.3.1 Infrared Spectroscopy and Sub-mm Interferometry: Young Stars, Circumstellar Disks, and Molecular Clouds

Objective – Identify process and support instrument design for detection and measurements of ice and water biomarkers.

Connections – §2.1.1
Astrobiology Roadmap Links – Goal 3; Objective 3.1
Researchers – Reipurth, Williams, Ceccarelli

Background – We will determine the water ice distribution in molecular clouds by taking infrared spectra of field stars shining through the molecular clouds being investigated. We will obtain high-quality spectra (S/N of 30 or better in the continuum) of a variety of sources located behind the clouds and cloud cores of interest. For the study of water ice distributions in clouds and to explore differences between clouds we will use several telescope/instrument configurations (SPEX on the 3.5 m IRTF, UIST on the 3.9 m UKIRT) to achieve a resolving power of about 1000 in the K plus L bands. About 200 stars will be targetted, requiring about 20 nights, which over a five year period is about 2 nights per semester. For a study of the denser cloud cores we will observe about 20 - 30 stars using larger telescopes (the 8 m Subaru with IRCS, and the 8m

Gemini with NIRI) totalling approximately 10 nights, which is on average one night per semester if spread over 5 years.

For the study of hot water emission towards young stars with massive circumstellar disks we will use high-resolution infrared spectroscopy in the K-band with a resolving power of about 20,000. Since many of our targets are rather bright (K of 8 - 10), we can use medium sized telescopes (*e.g.*, IRTF with CSHELL, UKIRT with CGS4 or its successor), and only for the faintest objects do we need to go to very large telescopes (*e.g.*, Subaru with IRCS in its echelle configuration). Given that this is a largely unexplored research field, it is difficult to estimate the amount of observing time required, but we envisage using a total of around 6 to 7 nights on a 3-4 m class telescope and a few nights on larger telescopes for this study.

We will explore water masers in circumstellar disks by using the Very Large Array (VLA) in New Mexico. This is a general facility which we have used repeatedly in the past. We will employ the A-configuration at 4.86, 8.46, 22.255, and 107.1 GHz. The A-configuration is available approximately every 15 months, and we intend to submit our proposals for each of these deadlines for the coming years.

Through the UH access to telescopes on Mauna Kea, it is possible to dedicate large quantities of time to survey for sub-millimeter line emission from water related species in protoplanetary disks around young stars in nearby star forming environments using the Caltech Submillimeter Observatory (CSO) and James Clerk Maxwell Telescope (JCMT). The resolution of these data will be $\sim 10''$ which is sufficient only to allow abundance measurements to be made. Followup observations of disks with detectable lines will then be made with the SMA to map the different species at a few arcsecond resolution (few hundred AU). In addition to the line observations, the thermal dust emission provides information on the grain mass opacity and thereby the composition of the (icy) mantles. Millimeter interferometric observations of the LkCa 15 disk show interesting morphological differences at this scale[180]. Our proposed observations are at shorter wavelengths than this work and will target warmer gas closer to the protostar where abundances may be higher and line emission stronger. Progress in learning about protostellar disk chemistry will require large amounts of telescope time due to the multitude of species and relative weakness of emission. UH is in a unique position of being able to bring three sub-millimeter telescopes to bear on this problem, including the only one able to image at arcsecond resolution, and this survey can be expected, therefore, to make a signficant contribution to our knowledge of the initial chemical conditions of the solar nebula.

Atmospheric transmission (Fig. 2.3.1a) versus frequency for a range of precipitable water vapor (pwv) levels. The sky offers several "windows" which allow astronomical observations of the line and continuum radiation from the molecules and dust in star forming cores. There are very few telescopes in the world that operate at sub-millimeter wavelengths ($\nu > 300$ GHz) and three are situated on Mauna Kea, including the only one (SMA) that will produce sub-arcsecond resolution images.

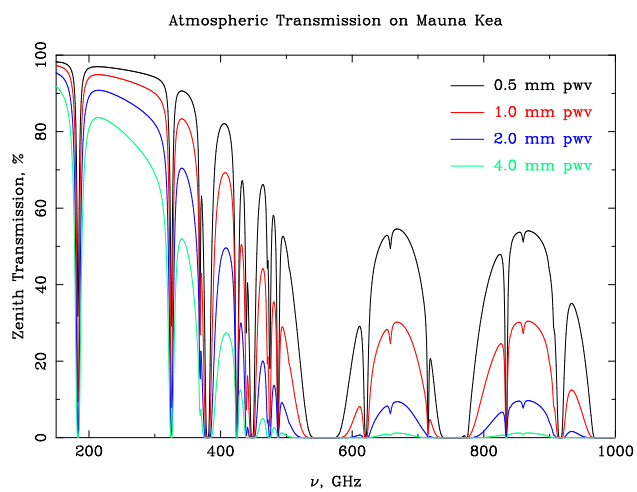


Fig 2.3.1a–Atmospheric transmission in the sub-mm.

Much of the opacity in the atmosphere at these wavelengths is due to water but the extremely dry conditions at the summit of Mauna Kea, pwv$\sim 0.5 - 1$ mm, allow for observations at frequencies as high as 900 GHz. Other sites with sub-millimeter telescopes in Europe and Arizona have much higher water vapor levels, pwv$\sim 2 - 4$ mm, and are consequently rarely able to observe at frequencies greater than 400 GHz.

2.3.2 Extraterrestrial Ice Laboratory Experiments

Connections – §2.1.3
Astrobiology Roadmap Links – Goal 3; Objective 3.1
Researchers – Kaise, Bar-Nun, Ehrenfreund

The Experimental Setup

The experiments on the interaction of charged particles and photons with extraterrestrial ice analog bodies are carried out in vacuum chambers which mimic the interstellar or solar system environments. All experiments will be carried out at the University of Hawaii in an existing oil free ultra high vacuum (UHV) vessel which has the following characteristics:

- Contamination-free operation at extreme ultrahigh vacuum conditions (pressures of about 10^{-11} torr),
- Reproducible preparation of thin frost layers ($<$ 500 nm),
- Target substrates which allow a rapid heat dissipation from the irradiated ices to the cold head thus minimizing surface heating and reaction mechanism changes,
- Temperature monitoring of heated ice surfaces and substrates,
- Preparation of clean charged particle beams and operation of monochromatic photon sources,
- Quantitative on line and in situ analyses of the solid state and the gas phase employing low background detection schemes.

This machine consists of an irradiation unit and a stainless steel chamber which can be pumped down to 3x10^{-11} torr by a magnetically suspended turbopump (Fig. 2.3.2a). A rotatable, two stage closed cycle helium refrigerator is attached to the lid of the machine and holds a polished silver mono crystal. This crystal can be cooled to 9.5 K, serves as a substrate for the ice targets and conducts the heat generated from the impinging photons or particles to the cold head. To allow a selection of any target temperature from 9.5 K to the sublimation point of the samples, a temperature sensor and a programmable heater are attached to the crystal. To assist a quick change of the irradiation source and maintain the UHV conditions in the main chamber, the irradiation unit is placed in a removable side chamber which can be separated from the machine through a gate valve. Three irradiation modules are necessary to simulate the CR and UV effects on interstellar ice mixture: the ion module (shown in Fig. 2.3.2a), the electron module, and a tunable photon source. Continuous ion beams are generated by an ion gun via electron impact ionization of a gas precursor, ion extraction, acceleration up to 5 keV, and mass separation of

contaminants in a Wien filter. The current of the charged particles is monitored by a Faraday cup. Since the gas load inside the main chamber has to be kept to a minimum during the irradiation to avoid any condensation of contaminants on the ice surface, the ion source is triply differentially pumped by magnetically suspended turbomolecular pumps backed by oil free scroll pumps. This pumping scheme reduces the pressure from 10^{-4} mbar via 10^{-6} mbar, 10^{-8} mbar, and 10^{-10} mbar to 3x10^{-11} mbar in the main chamber. Secondly, electrons with kinetic energies up to 5 keV are generated by an electron gun. Due to the modular design of the irradiation unit, the ion source can be simply replaced by the electron gun, which is interfaced then via two differentially pumped regions to the main chamber. Thirdly, photolysis experiments are performed employing a differentially pumped ultraviolet source which is also interfaced together with the differentially pumped monochromator region through two differentially pumped chambers to the main recipient. The advantage of the tunable photon source is the wavelength selectivity. Since experiments are performed at distinct wavelengths, a complete picture of the underlying photochemical processes can be obtained. The main chamber has spare ports, and simultaneous photon/particle irradiation can be also conducted.

To guarantee an identification of the reaction products in the ices and those subliming into the gas phase on line and in situ, two detection schemes are incorporated: (*i*) a Fourier transform infrared spectrometer (FTIR), and (*ii*) a quadrupole mass spectrometer (QMS). The chemical modification of the ice targets are monitored during the irradiation experiments to extract time-dependent concentration profiles and hence production rates of newly formed molecules in the solid state. Since no QMS can distinguish multiple $C_2H_4O_2$ isomers from the raw data alone, the mass spectrometer must be calibrated first for the fragmentation patterns of distinct isomers of interest; hereafter, these raw data are processed via matrix interval arithmetic to extract quantitative information on the isomers formed.

Fig 2.3.2a–Schematic top view of the simulation chamber, ion irradiation unit, quadrupole mass spectrometer (QMS), Fourier transform infrared (FTIR) spectrometer, and the gas condensation unit.

Ice Mixtures–Mechanisms for Formation of $C_2H_4O_2$ Isomers in Interstellar Ices

We elucidate now the mechanisms by which glycolaldehyde, acetic acid, and methyl formate might be formed inside interstellar ices via charged particle bombardment and photolysis. Synthetic routes are derived combining concepts of suprathermal chemistry[102, 187] and photochemistry[212] together with a classical retrosynthetic approach[127]. This will ultimately identify those molecules as potential precursors to the $C_2H_4O_2$ isomers and guide the selection of ice mixtures in our simulation experiments. Note that due to the complexity of the interstellar environments no single experiment can mimic all effects simultaneously, and an understanding of elementary processes

must be based on experiments involving binary systems first before extending them to more complex ice mixtures. As evident from the structures of the $C_2H_4O_2$ isomers, the CO_2-unit can be found in acetic acid and methyl formate, whereas the CO-moiety is present in all isomers (Fig. 2.3.2b). Since CO and CO_2 are common in astrophysical ices, we investigate first if binary ice mixtures CO/CH_3OH (mixture 1) and CO_2/CH_4 (mixture 2) synthesize any $C_2H_4O_2$ structure.

Considering a CH_3OH molecule, a photon or particle can split the CH_3-OH (1.1), CH_3O-H (1.2), or H-CH_2OH bond (1.3) to yield [CH_3 ... OH], [CH_3O ... H], and/or [H ... CH_2OH] radical pairs which could react with a CO molecule to acetic acid, methyl formate, and glycolaldehyde (Fig. 2.3.2b). Similarly, a CH_3-H bond in a CH_4 molecule can be broken to form a [CH_3...H] pair; the radicals/atoms might add to a CO_2 neighboring molecule in the ice matrix to give acetic acid (2.1) or methyl formate (2.2). All routes to $C_2H_4O_2$ investigated so far require only one bond rupture in a single molecule; both reacting radicals/atoms originate from the same precursor. However, reactants could emanate from two different molecules, and two bond ruptures prior to the reaction are required. Hence, ice samples with formaldehyde (H_2CO) are studied, as the formyl group (HCO) is present in methyl formate and glycolaldehyde. Binary mixtures of H_2CO/CH_3OH (mixture 3) could form methyl formate (3.1) and glycolaldehyde (3.2).

After binary mixtures have been investigated comprehensively, ternary mixtures with a radical plus an atom precursor, which requires two bond ruptures, will be also studied. These involve mixtures of CO with CH_4/H_2O, CH_4/CH_3OH, H_2O/CH_3OH, and of CO_2 with CH_4/H_2O, CH_4/CH_3OH, and CH_3OH/H_2O. To get information on the reaction mechanism, isotopic substitutions will be performed. For example irradiation of CH_3OH/ CO/H_2O could give CH_3 (from CH_3OH) and OH radicals (from H_2O) which might react with CO to acetic acid. Since CH_3OH can be cleaved to CH_3 plus OH as well, both CH_3OH and H_2O can form OH. The dominating pathway is unraveled using CH_3OD which produces only OD. CH_3COOH and CH_3COOD have different infrared spectra and can be discriminated experimentally. Note that the radical species formed can recombine either in a radical pair cage inside the solid ice even at temperatures as low as 10 K or - if they are separated inside the ice matrix - after annealing of the ice target in the hot core phase to allow the radicals to diffuse. Since the production rates of the $C_2H_4O_2$ isomers are expected to depend on the initial concentration of the ice components, experiments have to be performed at various concentrations of the ice constituents.

Besides simple bond rupture processes followed by atom/radical additions and recombination reactions, we have to investigate also to what extent insertion processes of suprathermal particles can lead to the $C_2H_4O_2$ isomers. Note that in the following considerations, the spin states of the reacting particles are omitted for clarity. Further, the reader should keep in mind that the reaction of a suprathermal particle with a thermal molecule leads to highly internally excited molecules. In the gas phase, the latter fragments, but inside ices, the surrounding matrix might absorb the excess energy of the newly formed molecule via phonon interaction. Acetaldehyde (CH_3CHO) and formic acid (HCOOH) are identified as the key reactants in this scheme.

Here, suprathermal oxygen atoms, which can be formed as knock on species via interaction of the primary implant with water molecules (CH_3CHO/H_2O; mixture 4) as the dominant constituent of extraterrestrial ices, can insert into the carbon-hydrogen bond of the methyl group of the acetaldehyde molecule to form glycolaldehyde (4.1); an insertion into the carbon-carbon and formylic carbon-hydrogen bond leads to methyl formate (4.2.) and acetic acid (4.3.), respectively.

Fig 2.3.2b–Formation of $C_2H_4O_2$ isomers via simple bond ruptures followed by addition of atomic hydrogen and/or a radial and recombination of radicals (left) and via insertion of suprathermal oxygen atoms and carbene radicals (right).

Alternatively, insertion of carbene radicals (CH_2), which can be generated via interaction of charged particles with methane molecules (CH_4/HCOOH; mixture 5), can undergo three distinct insertion reactions into the carbon-hydrogen (5.1.), oxygen-hydrogen (5.2.), and carbon-oxygen bonds (5.3.) to form acetic acid, methyl formate, and glycolaldehyde, respectively. These considerations make it clear that charged particle irradiation of binary ice mixtures of H_2O/CH_3CHO and CH_4/HCOOH could yield all three $C_2H_4O_2$ isomers.

Note that the single insertion process of a carbine radical can be substituted by an insertion of a carbon atom or methylidene radical (CH) followed by two or one hydrogenation steps, respectively. We like to stress, that after binary mixtures have been exposed to high energy radiation in the laboratory, it is crucial to prepare ternary mixtures of these ices into a water-rich matrix. Water is the dominant component of the interstellar and comatary ice component, and the production rates depend strongly on the nature of the ice matrix.

2.3.3 Comet & Centaur Observations

We will obtain JHK ($1.2 \leq \lambda \leq 2.4$ μm) spectra of a sample of $\sim$50 bright Centaurs and Kuiper belt objects using the Keck, Subaru and Gemini 8-m and 10-m class telescopes. These spectra will be used to determine the surface abundance of water ice from the 1.5 μm and 2.0 μm spectral features, and to search for expected correlations in the water ice abundance with object distance and size. The spectral properties of amorphous and crystalline water ice are measurably different (see reference by John Davies). We will use this fact to place a limit on the abundance of amorphous water ice or to detect it. As noted, this places an upper bound on the temperature experienced by a given body. We will also search for indications of organic and other surface molecules, like those already found in Centaur 5145 Pholus.

The presence of organics is most easily ascertained from the ultra-red optical reflection spectra, of which 5145 Pholus remains the prime example. We will obtain calibrated optical reflection spectra of 100 Centaurs, KBOs and cometary nuclei to examine the incidence of surface organics. We are interested to know whether the organics so prevalent in the outer solar system can survive passage to the hot inner regions. Recent observations, for instance, suggest that they cannot,

based on the relative lack of ultrared objects amongst the comets and dead-comets[?].

While detailed compositional studies of the organics are not possible from low resolution spectra alone, we will, with the aid of laboratory experiments, be able to make useful statements about the degree of hydrogenation of the organics. The ability to take comparable spectral datasets for astronomically remote objects and for laboratory samples designed to model those objects constitutes a powerful aspect of our collaboration. Presently, the literature contains irradiated ice spectra from the laboratory that are difficult to compare with real astronomical data. In some cases this is because the spectral resolutions are mis-matched, in others it is because the relevant spectral range has not been sampled or because astrophysically relevant ice mixtures have not been employed. We will have complete control over all these aspects, and will ensure that the laboratory spectra are fully comparable with those obtained at the telescope.

2.3.4 Comet Isotopic Measurements

Comet Observations

In this proposal, we will use the observational facilities atop Mauna Kea to obtain measurements of cometary D/H in comets having a wider range of dynamical characteristics and origins than the 3 already sampled. The James Clerk Maxwell Telescope (JCMT) has been used to study the $1_{01} - 1_{00}$ line of HDO at 464.92452 GHz in comet Hale-Bopp. This high frequency line is challenging because of telluric absorption: Mauna Kea is probably the only place on Earth where it can be studied. In addition, the new Submillimeter Array Telescope (SMA), will have sensitivity and higher angular resolution at the HDO line. The key is to find comets that are bright enough to permit useful signal-to-noise ratio spectra to be obtained. In practice this means comets must be observed when near the sun and at small solar elongation angles. The optically opaque Gore-Tex screen of the JCMT makes such observations routine.

Here the main emphasis, aside from seeing if there are real variations in D/H among these comets will be to test the pristine nature of the comets. How extensive was the mixing of interstellar material with material in the solar nebula? Can we assume that comets in other systems will resemble ours (little mixing) or will the comets of a given system be dependent on the structure and composition of the local circumstellar nebula (extensive mixing)? The answer will determine to what extent we can generalize the transport of water in our system to other systems. The question can be addressed by investigating D/H and $^{15}N/^{14}N$ in more compounds in more comets, and comparing the results with values measured in interstellar clouds.

For Kuiper belt comets, conceivably the D/H in their water could match that in seawater, but they are too faint to investigate from Earth. However, these are the ones that missions can go to. Owen is Co-I on the Rosina mass spectrometer that will be carried on the Rosetta Spacecraft. This instrument will not only measure D/H in the comet's water, it is capable of measuring the noble gas abundances and isotope ratios, including xenon, in the comet, thereby testing the hypothesis that comets might have brought these particular volatiles to Earth. If they did, they obviously would have brought SOME water as well as organic compounds and other sources of nitrogen. Rosina will also measure the nitrogen isotopes in the comet, providing another test of cometary delivery of volatiles to Earth. We now know that the nitrogen in Hale-Bopp's HCN has an isotope ratio close to the telluric value, but HCN is a minor constituent of comets, so we need to evaluate the total cometary nitrogen, and this Rosina can do.

Mars Observations

As a starting proposition, one might expect that Mars had the same sources of volatiles as the Earth. Support for this assumption comes from a study of noble gases in the Martian atmosphere, which shows that Ar/Kr/Xe and the Xe isotopes are virtually identical on Mars and Earth. We can test the water on Mars by remote observations from Earth and by studies of Martian meteorites (SNCs). We find that water in the Mars atmosphere shows the effect of atmospheric escape: D/H is 5.5 times the value in seawater. Water in minerals in the SNCs shows a range of values of D/H extending to the value measured in atmospheric water, showing that some of this water must have become incorporated in the minerals. But the lowest value of D/H in these minerals is not the value in our oceans, it is the twice higher value found in comets.

Finally, the xenon in the rock that comes from the greatest sub-surface depth shows xenon with solar isotope ratios, rather than the atmospheric values that mimic those on Earth. Thus the stage is set to use Mars to test ideas about the origin of volatiles on Earth. To pursue this investigation, a postdoc supported by the present grant could collaborate to investigate the Mars rocks further to unravel the evolution of the Martian atmosphere and to evaluate the water content of the Martian interior. For example, it will be interesting to compare the total water on Mars with the total on Earth, to see if there is a model for water delivery that is compatible with these results.

2.3.5 Instruments for the Identification of Biomarkers

Objective – Identification of processes and instruments relevant to ice and water bio-markers measurements.

Connections – §2.1.6, 2.1.7
Astrobiology Roadmap Links – Goal 7; Objective 7.2
Researchers – Anderson, Gaidos

Background – Detection and identification of biomarkers and bio-molecules in extraterrestrial aquatic environments remains a key challenge for future exploration of planetary bodies[143], *e.g.*, the posited sub ice oceans of Europa[202, 112], the icy polar caps of Earth, Mars, Callisto, and the icy surface of Europa. Our approach will use knowledge of these types of water and ice locales as the basis for testing both vehicles and instruments required to find bio-habitats, as well as the specific measurements that will most rapidly assess the presence of biomarkers in that environment. Our proposal specifically seeks funding to support continued operational testing of a unique Cryobot vehicle for in-situ analysis of ice and water environments, in conjunction with sample handling and biomarker detection studies using simple definitive instruments tested in both the laboratory and in analog sites.

The Cryobot is a tethered vehicle capable of penetrating ice to kilometers of depth using a combination of passive heating and active hot water jetting that has been developed by partners at both the University of Hawaii and the Jet Propulsion Laboratory (Fig. 2.3.5a, b). It has the advantage of being small in size, mass, and power, while providing the capabilities of a hot water drill, while considerably simplifying ice contamination issues from both dangerous drilling fluids

and surface biology. The onboard avionics allow the Cryobot to steer, cut side channels to store debris, and transmit real time data to the surface via a tether that spools from the back of the vehicle, allowing it to continue descending following refreeze of the bore hole. The tether supports gigabit communication speeds, enabling realtime high resolution video, mass spectroscopy, and sample handling.

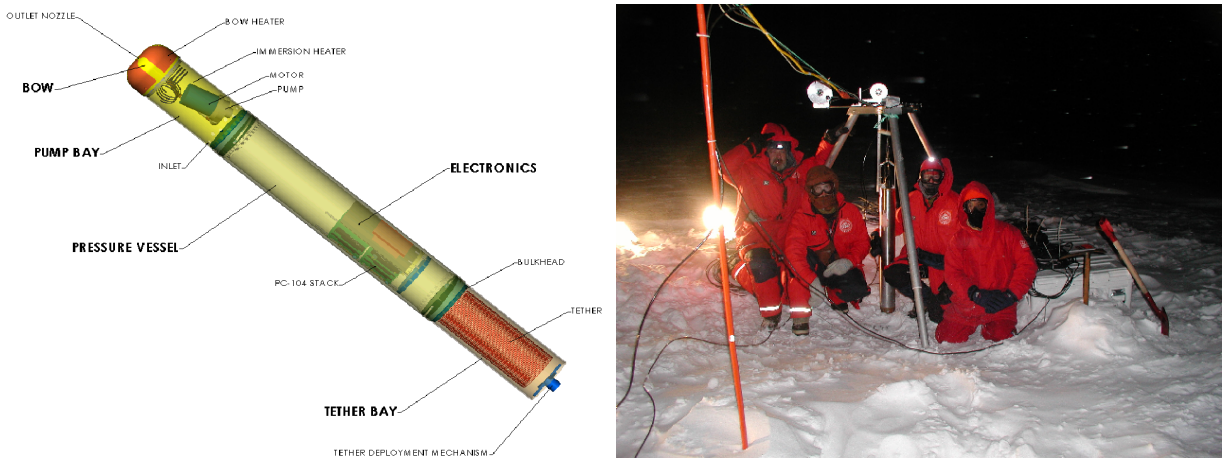


Fig 2.3.5–*a)* Funding from this proposal will aid in linking technology development, such as the ice drilling Cryobot vehicle shown above with astrobiology instruments appropriate for watery and icy environments. The Cryobot is a fully independent robotic vehicle, including: 1) a nose that heats and jets hot water, 2) an instrument and avionics bay containing an advanced computer and control system, including directional control and fault tolerance, as well as on-board analytical capability, and 3) a fiber optic tether capable of transmitting video and gigabits of instrument data in real time. *b)* The Cryobot has been tested in extreme environments, including the northern polar island of Svalbard, Norway, in winter. Additional funding will be used to bridge the existing design with our ongoing science efforts in Iceland and in the laboratory. The system does not use environmentally harmful chemical like many ice drilling methods, protecting the biosignatures that the science effort seeks to measure.

The vehicle has been tested in ice sheets during the harsh winter in Svalbard, Norway, and is ready for integration with instruments, as well as testing of sampling and sterilization technology. It can also be used in the deep ocean to study hydrothermal vents, and other astrobiologically significant targets. Though not selected, the vehicle was one of the most highly rated Scout proposals for exploring Mars, and is under consideration for the ice penetrating studies of other icy bodies in the solar system. Further development of this system by terrestrial analog studies is supports the long term astrobiology infrastructure of searching for life associated with water. Our proposal seeks money to support laboratory tests of the existing vehicle, as well as funds to allow us to use the vehicle in ice and lakes in collaboration with our other projects in Iceland, Antarctica, Kauhako Lake crater on Molokai, and Lake Waiau near the summit of Mauna Kea.

Identification of processes and instruments relevant to ice and water bio-marker measurements is ongoing, and is one of the weakest links in the existing astrobiology infrastructure. While numerous astrobiology detection concepts have been suggested[7, 160, 161, 86, 56], no definitive flight ready astrobiology instrument exists today. To that end our NAI proposal will focus on the provenance and association of life with water to the design and selection of appropriate instruments for planetary use. We will both partially support new instrument designs as well as survey the astrobiology

instrument field, as a wide range of possible instruments are planned or are in partial stages of completion throughout the planetary science community. This effort will focus on identifying the most relevant instruments for the ice and water environment, as well as the basic technology hurdles to their broader acceptance within the ASTEP or ASTID programs. Successful interpretation of complex natural environments will require detailed laboratory study of the physical processes that produce a measurable astrobiology signature in water and ice, which we will carry out at the University of Hawaii in our existing labs, as well as in partnership with the National Ice Core Laboratory (NICL), in Denver Colorado. Our proposal effort will focus on processes and related measurement techniques by using samples obtained from terrestrial analogs, including the dry deserts of Antarctica, the relatively wet environments of the desert southwest, and the unique water environments in Hawaii. Hence, the research effort we are proposing has specific strengths in that it builds on:

- The Astronomy and Laboratory portions of this proposal, including the sources, sinks, and history of astrobiologically relevant water.
- The rich history of design, construction, and field use of instruments in the harshest environments by the University of Hawaii, including a range of spectrometers and wet chemistry systems. For example, the University has recently deployed mass spectral systems to 4000 m in hydrothermal vents, and demonstrated stand-off Raman instruments.
- Integrates previous work for identifying biomolecules and markers, both at the University[12], and in the literature.
- Integrates new measurements using instruments currently being proposed by the University of Hawaii for astrobiology by testing them at terrestrial analog sites, as well as supports a inclusive effort to identify the physical processes underlying biologic processes that will produce the definitive instruments of the future.

Definition of Biomarkers

There are two broad classes of environmental signatures that can be interpreted as evidence for life: geochemical fractionation and direct bio-molecule identification. One task of this proposal will support the ongoing development of astrobiology instruments for the direct detection of biomolecules in water or ice enivronmnets. A second will be a feasibility analysis to use the basis for new ASTID, ASTEP, PIDDP, and MIDP proposals. We will demonstrate the practicality of using of a rotating field mass spectrometer (RFMS) currently in our possession at the University of Hawaii to search for biosignatures such as stratified geochemistry both using the Cryobot and samples from hydrothermal vents, Antarctic ice cores, and field-testing in Iceland. What makes the RFMS truly unique is its rotating field approach, which allows us to measure molecules with weights from 1-100,000 daltons, far more than comparable systems, with a length of $\sim$6 cm and no precision parts (Fig. 2.3.5c). Because RFMS uses a semi-soft-ionization technique, this range is sufficient to measure elemental abundance, ions, and large organic molecules. We will calibrate the instrument by measuring the inorganic, organic, and isotopic chemistries of a variety of liquid samples containing biosignatures. These instruments are currently operational, and demonstrated at high-pressures and extreme temperatures (Fig. 2.3.5d).

In addition to supporting lab testing of the instrument, we propose to use funds from this proposal to enable us to test this instrument on currently proposed NSF and future ASTEP ice projects. These bridging funds will allow us to bring along the existing instrument on these additional field proposals to constrain real world sensitivity to biosignatures. The proposed effort will not only return useful science data on the range of environments conducive to life, but provide real world in situ testing of advanced instrumentation that could be used to study oceans, ice sheets, and hydrothermal vents on Europa, or the icy poles of Mars. These technologies enable the science goals of MEPAG, ASTEP, and the Europa Focus Groups, and represent a convergence of science objectives, enabling technologies, and field deployment experience. This technology will enable NASA to directly search for biomarkers, measure the metabolic potential of biota should they be present, and characterize the geologic and climatalogic environment whether or not biosignatures are present.

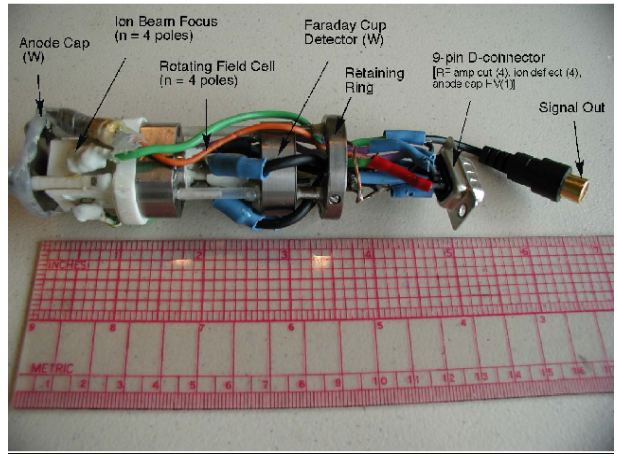


Fig 2.3.5c–The core of the RFMS instrument shown here is small and tolerant of the space-flight environment. Funds from this proposal will be used to test the sensitivity of the existing instruments using lab standard biomolecules from the American Type Culture Collection (ATCC), and bridge the existing technology with ongoing science efforts.

Fig 2.3.5d–The RFMS instrument, shown here in a very large pressure chamber, has been used in high pressure aqueous environments similar to those that may be analogs for life on Mars or on Europa. While the instrument casing shown here is not suitable for ultimate space-flight, we are interested in lab testing, as well as ultimate modification for Cryobot use.

Cellular Membranes (Lipids)

Biosignatures in organic lipids include specific biomarker compounds and other features (stereochemistry, non-random distributions) of the lipid fingerprint that cannot be explained by abiogenetic synthesis[149]. Both lipid distributions and the occurrence of complex molecular structures requiring biosynthesis (biomarkers) are important when screening for organic biosignatures. In screening for biomarkers it may be possible to recognize whether any of the three extant domains, Bacteria, Archaea and Eucarya, were present by including hopanes, head-to-head isoprenoids and steranes, respectively, in the search protocol used to interrogate the mass spectra. Distributions of non-biomarker compounds can amount to a biosignature, *e.g.*, a normal alkane distribution that

shows predominance of the even (or odd) carbon numbered compounds, reflecting biosynthetic pathways that homologate by two-carbon increments.

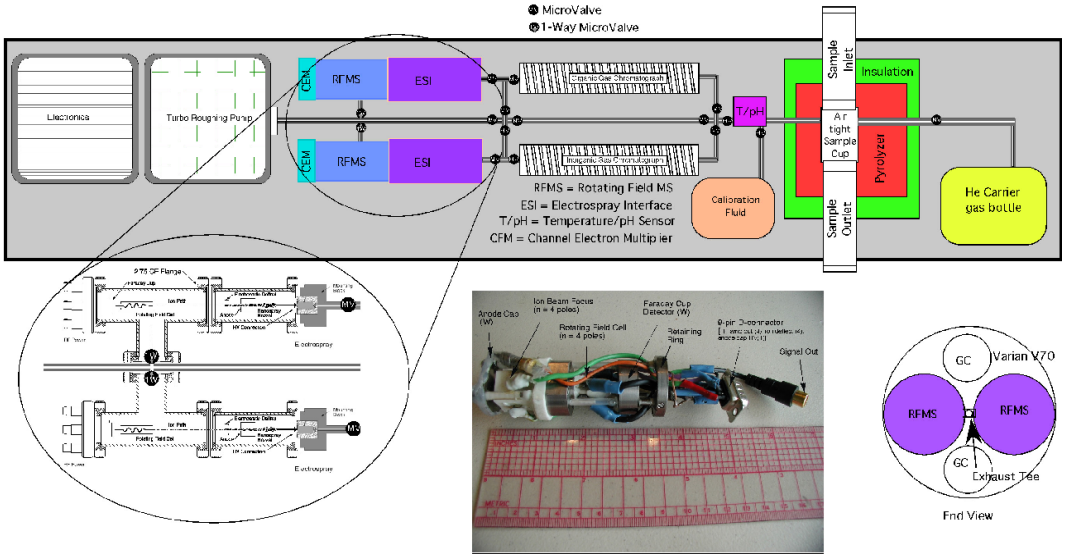


Fig 2.3.5e–New designs for incorporating pyrolysis and gas chromatography into a RFMS system are currently under consideration. We will use funds from this proposal to use the ATCC samples as a test suite for designs like the one shown here, to determine their sensitivity to bio-molecules.

In general, lipids are found as complex mixtures. We can recognize biosignatures in these mixtures when their composition is out of balance with what could be formed from abiotic synthesis alone. Characteristics of a biosignature may include (1) excesses of some structural isomers or homologues over others outside the realm of possible random (abiotic) synthesis, (2) repeating structural sub-units in a molecule, for example, the C_5 isopentene sub-units in isoprenoids, and (3) enantiomeric or other stereochemical excesses that do not reflect relative thermal stability.

The most abundant large lipids produced either by abiogenetic processes or by enzymatic synthesis are the normal alkanes. A large component of these are biosynthetically produced as functionalized compounds, such as fatty acids. Fischer-Tropsch synthesis, an abiotic process, also produces these straight hydrocarbon chains, again mostly with functionality. The smooth distribution (by gas chromatography or gas chromatography-mass spectrometry) of fatty acids or n-alkanes in a Fischer-Tropsch product typifies what would be expected from abiotic synthesis[129]. This distribution of homologs is controlled by conditions of the synthesis, such as temperature, pressure, and catalyses. In contrast, biogenic distributions typically show predominance of the even carbon numbered homologues unless they are obscured by thermal alteration or diagenesis. For example, diagenetic effects of clay can result in decarboxylation and produce an n-alkane distribution with an odd C-number predominance. But the biosignature, though altered, remains legible because it differs from the smooth distribution characteristic of an abiogenic origin. Simple methyl-branched alkanes whose methyl group shows a positional preference along the carbon chain may be a key biosignature, because of the link between these lipids and primitive organisms[110].

Many biomarkers carry taxon specificity in which they show input from various kingdoms, families and even individual species. Examples of taxon specific biomarkers[149] from the three

domains, Bacteria, Archaea, Eucarya, are hopanes, head-to-head linked isoprenoids and steranes, respectively. Squalene is a widely used isoprenoid intermediate in the biosynthesis of many polycyclic biomarkers, but the cyclization varies in different domains[172]. Bacteria cyclize squalene to make hopanes while in Eucaryas squalene lies on the pathway that eventually leads to sterols. Archaea have a unique way of joining isoprenoids to form a head-to-head linkage.

Analysis of samples from water or ice environments composed of living or recently living organisms in complex mixtures, *e.g.*, from natural ecosystems, presents a challenge. The challenge is to recognize key biomarkers and biosignatures against a large background of organic debris that is un-interpretable as a biosignature. A primary task is to determine the biomarker detection limits of the RFMS instrument and to recognize any biomarkers or biosignatures by in situ analysis. This ability will be demonstrated by lab tests under various conditions that simulate the kinds of primitive extraterrestrial ecosystems that might exist now or in the past. We further propose to optimize our RFMS system for biosignature detection by integrating a laboratory gas chromatograph and pyrolyzer system, allowing us to determine sensitivity for measurements of key biomarker information such as lipid compounds and isotopic biosignatures, amongst a wide range of other biomarkers (Fig. 2.3.5e).

Other Approaches: Amino Acids & ATP

Amino acids are the building blocks of proteins and enzymes required for life in terrestrial organisms. A capability to engage in direct detection of amines, amino acids, and poly-aromatic hydrocarbons would provide a useful tool for distinguishing between biotic and abiotic organic compounds. For example, homochirality of amino acids provides an unambiguous indicator of a potential biogenesis[8]; D-amino acids would suggest a non-terrestrial origin, while the detection of non-racemic amino acids would favor a biotic origin. ATP is used as an intermediate energy-storage molecule by ll life on Earth. A number of ideas exist for the in-situ identification of ATP, including UV fluorescence, and the labeling of phosphates for uptake and respiration of organisms. These labeled phosphates can then be identified via mass spectroscopy or tunable diode laser spectroscopy (TDLS). While we currently do not have active programs supporting in-situ analysis of these types of biosignatures, we will provide a comparative analysis of the strengths of these types of instruments of use in ice or water as part of our study. Specifically, we propose to study: 1) microchip capillary electrophoresis systems capable of identifying both any amino acids and enantiomeric composition of a compound, following the work of[96], and 2) ATP detection using UV florescence, mass spectroscopy, or TDLS.

2.4 Outline of Work Plan

The proposed research framework will support a group of postdoctoral scholars who will carry out independent, interdisciplinary research spanning two or more of the investigators' (or affiliated investigators') research specialties. The funding for an NAI does not equate to a substantial amount of money per Co-I, and is not intended to fund major new instrumentation initiatives, but is rather a seed to foster collaborations among existing groups. We are proposing an innovative model to maximize the collaborative science output. By supporting a group of postdoctoral scholars we intend both to influence young scientists at a formative phase of their careers, and to provide them with a circle of expert faculty advisors and collaborators from diverse but intercon-

nected research backgrounds.

2.4.1 Management Approach

Since interaction between scientists working in different areas is the key to the success of our astrobiology institute, we will employ a management style that heavily promotes interaction. The PI will take overall responsibility for ensuring the vigor and success of the effort. She will hold bi-weekly meetings with the co-investigators to discuss research direction and progress, financial/management issues and to ensure an active E/PO effort with broad participation.

The co-Is are widely scattered over the University of Hawaii campus and beyond, meaning that face-to-face contacts must be engineered in order to provide a microcosm of the distributed researchers' network which is intrinsic to NASA's astrobiology institutes. We will use 2000 sq. ft. of office space provided to this project by the University President to provide a focal point and venue for regular meetings. These will include weekly meetings at which current research results of the Oo-I's and astrobiology postdocs will be presented and discussed. In addition, the UH-NAI will offer an astrobiology seminar series in which broad university and community involvement will be sought. A sophisticated software tool will be implemented to facilitate scientific interaction between members of the group, building on the capabilities of the NAI Science Organizer. The outside collaborators will be involved in this through the Visiting Scholars Program and frequent communications.

2.4.2 Timelines & Deliverables

With a collaborative framework of this magnitude, there will undoubtedly be a wealth of new ideas, and research directions initiated. However, we do have a set of well-defined deliverables which involve our collaborative efforts.

1. *The Origin of Water and the Formation of Stars and Planetary Systems –*

§	Time	Investigators	Task
2.1.1	5.0 yr	Reipurth, Williams	IR spectra of ∼230 background stars, 20 young stars
2.1.1	5.0 yr	Reipurth, Williams	Water maser maps toward 10 stars
2.1.1	1.0 yr	Williams	Submm interferometry, 20 disks (chemistry), 2 mapped in detail

2. *Obs. and experimental investigation of interstellar and cometary water ice chemistry –*

§	Time	Investigators	Task
2.3.2	5.0 yr	Kaiser	120 ice irradiation experiments
2.1.2	0.5 yr	Kaiser, Bar-Nun, Ehren.	Ne/Ar/Kr/Xe ratios trapped in amorphous ice in the presence of excess CO, N_2
2.1.2	1.0 yr	Kaiser, Bar-Nun, Ehren.	N_2/CO in cometary ices
2.1.2	1.0 yr	Kaiser, Bar-Nun, Ehren.	Gas trapping in CHON particles
2.1.2	4.0 yr	Kaiser, Bar-Nun, Ehren.	Mini-enzymes formation in aqueous media

Note: Typically, one experiment takes 2 weeks (ultra high vacuum generation, preparing ice mixtures, carrying out the irradiation). Including maintenance time (1 mo/yr), 24 irradiation

experiments can be conducted per year. Each mixture has to be investigated at different temperatures, irradiation sources, and particle/photon energies. Realistically, we investigate then 10 distinct ice mixtures each at 10 different parameter sets (T, energy, flux).

3. *Origin and Distribution of Planetary Water –*

§	Time	Investigators	Task
2.1.4	1.0 mo	Keil, Krot, Scott	X-ray mapping of 2-5 thin sections of 6 chondrites, backscattered electron studies and electron microprobe studies

4. *Water and Aqueous Alteration on Mars –*

§	Time	Investigators	Task
2.1.5	1.0 yr	Taylor, Gaidos	Develop model of melting ice fields and hydrothermal systems on Mars

5. *Water-rock Chemistry and Habitats for Life –*

§	Time	Investigators	Task
2.1.6	3.0 yr	Cowen, Mottl, Gaidos	Assay biological activity carry out survey of microbial community in lava-seawater plumes
2.1.6	5.0 yr	Cowen, Mottl	Microbial diversity & physiology in low water/rock oceanic crusts
2.1.6	2.0 yr	Mottl	Geochem char of unusual low T, high pH/alkalinity fluids emanating from mud volcanoes at Maiana forearc
2.1.6	3.0 yr	Mottl, Cowen	Char microbial communities of low T high pH/alkalinity fluids at Maiana forearc

6. *Extreme Aquatic Environments & Their Analogy to Potential Habitats in the Solar System –*

§	Time	Investigators	Task
2.1.7	5 yr	Thorsteinsson, Gaidos	Survey biological activity and community composition in Iceland subglacial environments
2.1.7	5 yr	Gaidos	Survey biological activity and community composition in high-altitude fumaroles

7. *An Integrated Model of the Planetary Water Cycle –*

§	Time	Investigators	Task
2.1.8	2 yr	Hammer, Keil, Gaidos	Develop an integrated model of the geologic water cycle and explore the parameter space based on plausible models of planet formation and composition

8. *Signatures of Water-Bearing Exoplanets –*

§	Time	Investigators	Task
2.1.9	5 yr	Meech, Gaidos	Conduct long-term monitoring of earthshine spectrum with particular attention to signatures of water and model its diurnal, season, and interannual variations. Develop model of water loss from planets and giant comets around young stars and assess its detectability

2.4.3 Role of Co-Investigators and Collaborators

All of the project Co-Investigators are taking an enthusiastic and active role in the proposed UH-NAI, regardless of financial support. The core team of Co-Is are those who have been involved in the discussion and planning of the UH-NAI, some since the last Cooperative Agreement Notice. The core team represents 1-2 lead scientists in each of the major water-related disciplines of our research theme. Collaborators , in general, are providing invaluable (non-funded) contributions in more specialized areas of science. This group also includes our international colleagues. All of the Co-Is will be involved in the weekly meetings discussed above, and the local collaborators will be encouraged to join. In addition, we plan an active visiting program with our international collaborators (3.2.2)

- **Meech** – As PI of the program, Meech will be responsible for the overall management of the group. In addition, she will actively conduct research in the areas of cometary studies outlined above. In addition, because of her experiences with running large outreach programs, she will contribute to the E/PO component of the NAI.

- **Anderson** – Anderson will supervise a series of lab based life detection experiments in laboratory ice using technologies like the unique ice penetrating probe, the cryobot, and instruments such as the rotating field mass spectrometer. In addition, these experiments will be extended to ice cores at the National Ice Core Laboratory, which houses cores from around the world. The instruments will also be tested in the glacial lake on the summit of Mauna Kea.

- **Binsted** – Binsted will be responsible for designing, implementing, testing and evaluating a software tool to aid interdisciplinary scientific collaboration. She will also conduct research in scientific visualization and collaborative systems. Because the resulting network of astrobiological concepts, hypotheses and results may well be useful in introducing non-experts to the field, Binsted will also participate in the E/PO component of the NAI.

- **Cowen** – Cowen will actively conduct field research in microbial geochemistry of hydrothermal systems at mid-ocean ridges and in ridge flank crusts. He will also lead response expeditions to study seafloor eruptions for geochemical and microbial indicators of subsurface communities. He will participate in outreach activities, especially in science teacher enhancement projects (*e.g.* teacher at sea).

- **Gaidos** – Gaidos will lead expeditions to subglacial volcanoes in Iceland and high-altitude fumarolic environments in South America to assess the presence and adaptation of microbial communities in these environments and to explore the possibility that life could exist

outside the range of stable liquid water. He will also participate in research on extreme aquatic lake environments in Hawaii. He will develop models of liquid water production and transport on the surface of Mars and will be responsible for developing models of the planetary water cycle and the detectability of extrasolar cometary and planetary comae. He will lead a program of observations of the earthshine spectrum from Mauna Kea.

- **Jewitt** – Jewitt will be in charge of studying Kuiper Belt objects and comets and determining their physical properties, and icy compositions. Jewitt is also involved in the Pan Starrs telescope project, and is chair of its Science Working Group. His role will be to help determine the mass spectrum of all NEAs, and to enable further compositional study.

- **Kaiser** – Kaiser will perform all of the ultra-vacuum lab experiments in his capacity as the lead of the Reaction Dynamics group. In addition Kaiser will take an active lead in the development of a graduate program of astrobiology courses.

- **Karl** – Karl will supervise and participate in field and laboratory studies related to comparative aquatic ecosystem analysis and in the evaluation of life detection systems, especially ATP analysis. He will also supervise a postdoc who will conduct laboratory studies of Antarctic ice cores and experimental studies using ice mesocosms.

- **Keil** – Keil collaborates with NAI colleagues Sasha Krot and Ed Scott to conduct research into the aqueous and weathering history of asteroidal meteorites. Keil will synthesize the information to derive an understanding of the aqueous evolution and activities on small, asteroidal-sized planetesimals.

- **Kudritzki** – Kudritzki will help provide the astronomical infrastructure for the proposal by ensuring the future development of Mauna Kea and Haleakala. In addition, he will assist with the management of the NAI. He is keen to take an active role in all of the science of the NAI, including regular science meetings, infusing astrophysical perspectives.

- **Mottl** – Mottl will conduct field research in the geochemistry of microbial habitats, including cold springs on serpentinite mud volcanoes in the Mariana forearc and hot springs in the Lau (back-arc) Basin, where he has NSF-funded programs. He will participate in the studies of Hawaiian lakes and coastal areas where lava is flowing into the sea, as described in the proposal. He will provide chemical analyses and will collaborate with microbiologists in characterizing unusual microbial habitats. He will participate in outreach activites, especially in science teacher enhancement projects, including taking teachers to sea.

- **Owen** – Owen will investigate the D/H composition of comets in order to establish how much isotopic equilibration took place in the solar nebula between H_2O and H_2. In addition he will investigate the ability of water ice to trap noble gases to determine how much cometary water was contributed to Earth.

- **Reipurth** – Reipurth will carry out the research on water observations in the interstellar medium. In addition, he will develop and run the Astrobiology winter school, and will be significantly involved in the outreach component of the UH-NAI.

- **Taylor** – Taylor will have overall responsibility for studies relating to aqueous processes on Mars. In addition, Taylor is the lead on the E/PO program discussed herein. †Jeff Taylor will spend at least one month of his time on this his Hawaii Space Grant activities. His participation University of Hawaii as part of the state's matching Grant.

Table 1: UH NAI Proposal Collaborators

Personnel	**Location & Role**	**Expertise**
Akiva Bar-Nun	Tel-Aviv University	• Comet Impacts; Chem of Emergence of Life
Cecilia Ceccarelli	Obs. Grenoble	• Astrochemistry, Water in Circumstellar Disks
Pascale Ehrenfreund	Leiden Observatory	• Astrochemistry; Interstellar Ices & Organics
Julia Hammer	University of Hawaii	• Volatile/Mineral Interactions at Mod. Pressures
James Heasley	University of Hawaii	• Faulkes Telescope Outreach
Mary Kadooka	University of Hawaii	• Educational Outreach; Physics Resource Agent
Alexander Krot	University of Hawaii	• Meteoritics
Gary McMurtry	University of Hawaii	• Methods for in-situ Biomolecule Identification
Dina Prialnik	Tel Aviv University	• Comet Thermal models; Ice Physics
Ed Scott	University of Hawaii	• Meteoritics & Cosmochemistry; Petrology
Thor. Thorsteinsson	Nat. Energy Auth.	• Glaciology
Jonathan Williams	University of Hawaii	• ISM; Submm Interferometry

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3 Plan for Strengthening the Astrobiology Community

3.1 Education and Public Outreach

3.1.1 Introduction

Our group has a strong record of education and public outreach, and we intend to capitalize on that experience by providing innovative laboratory-based learning opportunities for teachers and by engaging the public in our research. We will leverage our efforts funded under this proposal with other programs in our state, particularly with the Hawaii Space Grant Consortium, Space Science Network Northwest (OSS Broker/Facilitator), and the Pacific Regional Planetary Imaging Facility. We will also use our extensive network of teachers throughout the state and our active involvement in the Hawaii Science Teachers Association, the American Association of Physics Teachers and other professional organizations to help disseminate the products we create. For the first time, due to the Hawaii Teachers Standards Board, teachers need to be re-certified by 2005 and this requires professional development. This provides the perfect opportunity for our proposed programs to assist in Hawaii teacher re-certification. Our outreach programs will be funded by several sources.

1. We will be supporting outreach at a level of ∼$50K / year (5% of the total) from the UH-NAI budget ($10K for workshops, and $40K for staffing (1 summer month of graduate student, 2 months for a web programmer, and 2 months of an Education specialist);

2. The University of Hawaii is committing $50K/year to UH-NAI outreach (see 3.4.1);

3. The Pacific Resources for Education and Learning group is commiting ∼4K per year for teacher travel from Micronesia (2 trips per year).

3.1.2 Astrobiology Laboratory Institute for Instructors (Alii)

The multidisciplinary nature of astrobiology lends itself to broad scientific training of teachers. It can revitalize a teacher's approach to whatever science subjects they teach, including biology, chemistry, physics, earth science, and astronomy. Because the typical secondary science teacher reaches about 150 students a year, a focus on teacher training leverages our resources effectively. We will develop a one week summer program to have grade 7-12 teachers learn about astrobiology and how to incorporate activities into their courses. activities into their courses. Exemplary activities such as those in SETI's Voyages Through Time modules will be used. Pacific Island teachers will be recruited with support from the Pacific Resources for Education and Learning (see 3.4.2). (PREL is one of 10 educational laboratories that serve geographic regions across the nation to improve education, and is supported by the U.S. Dept. of Ed.)

This program, Astrobiology Laboratory Institute for Instructors, or acronym, Alii, means royalty in Hawaiian (actually spelled Ali'i and pronounced "ah-lee-ee"), a fitting name for important people like teachers. Experience in doing teacher workshops (TOPS-Towards Other Planetary Systems; and Exploring Planets in the Classroom) has taught us the most effective teaching strategies. Begun in 1993 with NASA funding, with a student component funded by a private donor, the TOPS program has trained over 120 secondary science and mathematics teachers from

Hawaii, Micronesia, and other Pacific Islands. The TOPS team and the Hawaii Space Grant Consortium (sponsor of Exploring Planets in the Classroom) are committed to helping implement our teacher-training program. Our plans focus on integrating laboratory experiences with delivery of content materials in alignment with state and national science standards.

3.1.3 Summer Workshop and Research Experience

The focal point of our teacher-training program will be a semester-long professional development program to do cutting-edge research in astrobiology,. We will collaborate with the school district to support this program by allowing teachers to take a semester sabbatical leave to minimize our cost. Teachers from the Alii summer workshops with their basic astrobiology background will be recruited for a rich research experience. Each semester several teachers will conduct research in a focus area of this proposal (protostar formation, comets and the interstellar medium, use of spacecraft data for Mars, meteorite studies, extreme aquatic habitats,

This research component will be patterned after the Research Experiences for Undergraduates and Research Experiences for Teachers program that has been run at the Institute for Astronomy (NSF). Besides doing research, the teachers will explore hands-on astrobiology activities, attend talks by our Astrobiology Institute, and develop skills mentoring students for astrobiology science projects. These teachers will make presentations on their research at local and national science teachers conferences. (For those who have only lived in Hawaii, this could be their first experience since the school district does not subsidize teachers.

For six hours each day, teachers will work on well-defined projects aligned with the NAI Roadmap objectives and under the supervision of NAI-affiliated scientists. For example, teachers could experience the 13,000 ft altitude of Mauna Kea while participating in observing. This corresponds to the Roadmap Goal 3, “Understanding how life emerges from cosmic and planetary precursors”. Or teachers could utilize the educational Faulkes Telescope Facility (see 3.1.5). Co-I Cowen will continue his “Teacher-at-Sea” program, where a teacher is involved in a research project by being assimilated into the planning and staging of the research cruise, participates in the cruise as a scientist in training, and writes a report or paper on his/her experiences. The teacher helps his/her class or school set up an active web page and arrange for ship-to-shore email communications to integrate others into the research project. Also, the teacher will carry out experiments at sea based on questions posed by the students prior to or during the cruise. For the UH-NAI program, Cowen plans to mentor the teacher during studies of geochemical and microbial indicators of subsurface communities. This project aligns with NAI Roadmap Goal 5, objective 5.3, “Biochemical adaptation to extreme environments”. The real challenge is getting the teacher/school to build on their opportunity; the goal is to have a sustained program by which the school reaches out to their new contacts and instill in the school personnel sufficient boldness to ask familiar and potential educational partners for help. With the contributed funding from the University of Hawaii (see 3.4.1) we will have a person to help coordinate the followup.

With each year, the number of astrobiology teachers will multiply and promote astrobiology education in Hawaii’s schools. This model replicates our existing cadre of TOPS teachers who conduct workshops, give presentations at national conventions and continue to implement astronomy activities in their classrooms.

3.1.4 Astrobiology in a Course for Pre-Service Teachers

Using the summer workshop and research experience as a base, we will also enhance an existing course for pre-service teachers. Geology & Geophysics 168 is designed both to fulfill a science requirement for elementary teachers and to introduce planetary science to future science teachers. It is a combined lecture/laboratory course, with emphasis on learning from hands-on, inquiry-based classroom activities. We will greatly increase the astrobiology content in the course through collaboration with others in our branch of the NAI. The course content may include some of the following topics: the processes operating on the surface of Mars, extremophiles, searching for and recognizing fossilized microorganisms, Mars site selection for sample return, debate about life in Martian meteorite ALH8400. The class will also include some exposure to research, such as mini-internships in which students help in research laboratories for a few hours a week for part of the semester. This course will also be offered to experienced science teachers to introduced them to astrobiology. We hope to establish a a wide network of teachers who will promote astrobiology topics in their course curriculum.

3.1.5 The Faulkes Telescope Facility

The Faulkes Telescope is a joint project between the Faulkes Telscope Corporation and the University of Hawaii Institute for Astronomy. The telescope, located on Haleakala, Maui, to be completed in late 2003, will be the largest professional grade telescope in the world dedicated to education and public outreach. The 2.2 m diameter telescope will be used for astronomy education with students in Hawaii and the U.K. being mentored by teachers and professional astronomers. Research projects with astronomers searching for extra-solar planets and Kuiper Belt Objects will give our students first-hand experience on cutting-edge research relating to the water theme of our NAI proposal.

Under the auspices of a broad Hawaii NAI outreach program, we will have a teacher training program for use of this telescope. TOPS 2003 summer workshop is piloting an experimental program in preparation for this proposed Faulkes Telescope NAI workshop. TOPS teachers will learn how to conduct telescope observations, *e.g.*, target selection, filters to be used, exposure time dependent upon magnitude, and how images are used for analysis of data. At the end of three weeks, they will be expected to write a research report. With proper training the teachers will be able to mentor students for science fair projects. This will serve as a model to be used for the NAI workshop. Teachers will also be mentored by project scientists, their post-docs and graduate students. A survey completed, whereby IFA astronomers and HIGP scientists indicated interest in mentoring teachers, will be used. Hawaii's team, NASA OSS Space Science Network Northwest, conducted this survey. This exemplifies how Hawaii's NAI education and public outreach effort will be integrated with other entities to play a a major role in promoting science and mathematics education.

3.1.6 Engaging the Public

Discoveries in Astrobiology: a Science Magazine on the Internet

We will manage and edit a web magazine modeled after PSRD, whose central theme is "planetary scientists sharing ideas and discoveries". (PSRD can be found at www.psrd.hawaii.edu, and is

managed by G. Jeffrey Taylor and Linda M. V. Martel.) The audience for PSRD is the public, teachers, high school and college students, and government decision makers. Discoveries in Astrobiology (DNA) would be similar: "astrobiologists sharing ideas and discoveries." We do not intend this online magazine to duplicate the excellent Astrobiology Magazine produced by the Astrobiology Institute. Instead, our site would focus on reporting recently published peer-reviewed articles by the scientists associated with our branch to be responsible for translating articles for the public. The magazine would also contain summary articles about various central topics in astrobiology, profiles of scientists involved, and virtual tours of our laboratories. We envision having a board of editors composed of scientists involved in NAI to review articles for content and clarity, including ease of navigation and quality of graphics. We would be responsible for html coding and production of graphics not supplied by our scientific colleagues.

Taylor and Martel began PSRD because they feel strongly that scientific research is not finished until the results are communicated to the general public, which pays for it. The articles in DNA will tell the public what their dollars discovered, and do so in understandable English. In addition, it is an excellent format for keeping policy makers at NASA Headquarters and in the Congress informed about the accomplishments of the Institute.

Other Public Engagement Activities

It is crucial to share our discoveries with the public, in order to help raise the level of science literacy both in Hawaii and in the nation. We plan to do this in three major ways. First, we will host a regular series of public lectures about astrobiology. These will be advertised widely to attract a large audience. Second, we will organize a speaker's bureau, allowing local organizations (*e.g.*, Rotary Club, Downtown Club of Honolulu, etc.) to request a researcher to talk about the wonders of some aspect of astrobiology. Initial contacts with these organizations are already in place. Third, we will make effective use of our local press to publicize our most interesting research findings. The press is a vital partner in public engagement and we have an experienced and talented group of local science reporters.

3.1.7 Evaluation

Our plans for evaluation is that we will use the effective partnerships we have already developed with teachers. They will help us in the development and testing of classroom activities. Testing will be done partly during teacher workshops, via an evaluation form for each activity. Testing will also be done in the classrooms of cooperating teachers. We will rely on their expert opinion as to the effectiveness of each new activity. Teacher workshops themselves will also be evaluated through questionnaires filled out by the participants and through follow-on surveys designed to determine if teachers are using the materials and, if not, why not. We will also adapt the rigorous evaluation techniques developed for the TOPS program, which involved a continuous, action-oriented, decision-making evaluation model. This involved planning, formative and summative assessment phases. We will work with PREL to develop and execute a formal evaluation of our outreach programs.

3.2 Professional Community

3.2.1 Winter Astrobiology school

A fundamental aspect of astrobiology is its cross-disciplinary nature. The network of Astrobiology Institutes is successfully creating an environment in which researchers from widely different backgrounds can interact and break down the barriers between subjects erected during their different paths of training. However, if astrobiology is going to be an increasingly vibrant discipline, we must prepare the young researchers who, a generation from now, will be leading the future efforts. We should foster a cross disciplinary attitude in young people *before* they develop the myopic science view that arises from traditional specialization. This is possible if we reach out to students well before they finish their PhD. Young people have a remarkable ability to connect easily to each other, and the strong personal connections that are established in youth tend to last the longest. By bringing young students together across the astrobiology spectrum, we are helping to build the network of cross disciplinary contacts that will be part of the foundation of astrobiology for the next generations.

We propose to establish the Hawaii Astrobiology Winter School to be held yearly in the Hawaiian islands. It will be open to all graduate students in the course of doing a PhD in astronomy, biology, biochemistry, chemistry, or geology, and with an interest in astrobiology. We envisage a school with about 20 students lasting two, or ideally three, weeks, with 4 to 6 courses taught by experts in various fields. The students will, in small teams, write the lecture notes themselves under supervision of the lecturers, and the lectures will be made available at a dedicated web site. It will be easier to engage busy senior scientists if they do not have to do a time consuming write-up afterwards. The idea is not that each years school should be covering all of astrobiology, rather each years school will have its distinct character, determined by the subjects offered by the lecturers.

Young people move around a lot after their PhD until they eventually get tenure somewhere, so there is reason to be concerned that the contacts established during the school will die out merely for practical reasons. To help counter this we will establish the Astrobiology Quarterly (AQ), an internet - bazed forum for dialogy and interaction amongst past participants in the Winter School. The AQ will contain abstracts of papers written by past and present school participants, summaries of PhD theses by school participants, and address changes so contacts can be maintained.

3.2.2 Visiting Faculty Scholars Program

The UH-NAI is proposing a Visiting Faculty Scholars program which will bring external collaborators to work with local researchers, to participate in the Astrobiology Winter School (3.2.1), and help with the E/PO (3.1). Having a strong visitor program will inject a breadth of innovative ideas to the UH-NAI, and maintain and strengthen our interdisciplinary research. Because the University of Hawaii has made a concession to the usual Faculty Housing policy (3.4.1) we will be able to house our visitors for longer-term visits to engage in meaningful research that is fully integrated into all aspects of our proposed NAI.

3.3 Teaming with Minority Institutions

The University of Hawaii is a Minority Institution, with only 19% of the student body Caucasian, 14% Hawaiian or part Hawaiian, and another 12% of mixed ethnicity, the remainder being Asian. In addition, our unique geographical location places us in an ideal position to interact with communities in Micronesia (90% minority groups). Through our previous E/PO programs, we have considerable experience and have developed contacts with the Micronesian educational community in the US-Affiliated pacific entities: (American Samoa, Chuuk, CNMI, Guam, Kosrae, Pohnpei, Palu, Marshall Islands and Yap), and we will actively integrate teachers from these regions into our E/PO program (3.1.2).

3.4 Institutional Commitment

3.4.1 University of Hawaii

The University of Hawaii fully supports this project and, to this end, is contributing a substantial matching component to the program.

- **Staff Salaries** – First, we will be utilizing the bulk of the funds to recruit high-caliber postdoctoral fellows to work with the team and foster the collaborative interactions, only a small component of salary support is requested by the team. The in-kind value of the state-funded salary for the team amounts to $1,545K.

- **Tenure Track Positions** – The Institute for Astronomy will committ a senior tenure-track professorship in the area of Star and Planet Formation to the NAI, at an anticipated level of $140K/year (plus fringe and overheads), and in addition will contribute a junior tenure-track position at a level of $77K/year. This will be continued beyond the 5 year NAI program. The Institute sees the NAI goals as being an area of science where Hawaii is especially well-qualified to become a world leader in research because of our unique astronomical facilities and is therefore making this substantial commitment.

- **Center for Star and Planet Formation** – The Institute for Astronomy will provide $20,000 in funds per year to establish the Center for Star and Planet Formation. The goal of the Center is to develop closer ties between researchers studying star and planet formation, primitive planetary bodies (including the Kuiper belt objects and comets) and meteorites and the early solar system. The funding will be used to bring visiting scientists to the Center.

- **EPO Staffing** – The University of Hawaii will fund outreach staffing at a level of $50,000 (loaded) per year to help orchestrate our outreach program.

- **Office Space** – The University of Hawaii will give the program 2000 sq. feet of space for our postdoctoral community, and an additional $20,000 of funds to renovate the space, purchase furniture and set up wireless networking.

- **Faculty Housing** – In order to support our strong visitor program (see 3.2.2), President Dobelle of the University of Hawaii has agreed to place us on a high priority list for rented faculty housing. Hawaii is expensive, and even at the state per diem level of $80 / day (which cannot begin to cover hotel costs in Honolulu), a visitor program would be costly.

Short-term apartments are difficult, if not impossible to find. The UH faculty housing has a long waiting list (1-2 years) for space. Eligibility for priority for faculty housing is determined by the Board of Regents Policy, which only the President can override. Visiting Faculty Scholars, as a group, are at number 12 on the list, which means, in effect, that an apartment would be impossible to acquire. In response to our request, the President will place the UH-NAI at a high priority on this list, and extend the term of lease to the duration of the UH-NAI, if funded (normally it is 1-3 years maximum). Our project proposes a 3 month period per year, and the Institute for Astronomy Director will pick up the remaining 9 months of the year. Apartment rents range from $600-$1800 per month.

3.4.2 Outside Contributions

The director of the Pacific Eisenhower Mathematics and Science Regional Consortium at Pacific Resources for Education and Learning (PREL) will support the cost of airfare for 2 Pacific Island teachers each year to attend our summer Astrobiology programs. (Est. value: $4K/year).

Table 2: Summary of Cost-Sharing and In-Kind Contributions Amounts Correspond to Fully Loaded Salaries

Contribution	**Amount [$K]**
Faculty Salaries	1,545
1 Tenure track senior position ($140K/yr)	1,639
1 Tenure track junior position ($70K/yr)	934
Center for Star & Planet Formation	100
Outreach Staffing	250
2000 s.f. office space	N/V
Office rennovation	20
PREL Teacher Contribution	20
Access to R/V Kilo Moana	
Total Matching	**$4,508**

3.5 Flight Missions

Karen Meech

- Meech is a co-Investigator on NASA's 8th Discovery mission, *Deep Impact*, which will explore the interior of a comet. One of her roles in the mission is to be in charge of all the ground-based observing support both for pre-mission target characterization, and during the encounter. She is also heavily involved in the mission outreach.

Scott Anderson

- Anderson helped to run the Mars Orbiting Laser Altimeter (MOLA) on the Mars Global Surveyor and the Gamma Ray Spectrometer (GRS/NS/HEND) on 2001 Mars Odyssey.

- Member of the 2003 MER rover landing site working group.

Tobias Owen

- Interdisciplinary Scientist & member of Galileo Mission Probe Mass Spectrometer Team ('97-'03).
- Interdisciplinary Scientist & member of the Spectrometer and Aerosol Collector Cassini-Huygens Teams.
- Associate investigator on Rosetta, which will be the first good chance to get noble gas abundances for a comet.
- Member of the Neutral Mass Spectrometer Team on the Japanese Mission NOZOMI.
- Owen will also be joining Discovery Proposal teams for two comet missions, one mission to search for water on Jupiter (the greatest unknown for that planet right now), and a Venus mission designed to study atmospheric abundances and isotopes, again to investigate the origin of the Earth's volatiles and the possible comet (=ice) connection.

Jeffrey Taylor

- Member of the 2001 Mars Odyssey Gamma Ray Spectrometer Team.

3.6 Information Technology

We plan to design, implement and evaluate a software tool to aid interdisciplinary scientific collaboration, tentatively called HypMaT (Hypothesis Management Tool). It would be aimed at groups of researchers working in different fields, possibly remotely, but towards a common goal or theme.

NAI already uses ScienceOrganizer[109], a knowledge management tool that allows distributed NASA teams to organize and navigate through project information. Like ScienceOrganizer, HypMaT would be a combination of a semantic network and a relational database. However, it would represent the knowledge at a higher level, and provide an intuitive graphical interface, allowing users to visualize relations between hypotheses, concepts, experimental results, and speculations across scientific fields. We plan to make HypMaT compatible with ScienceOrganizer, so that users can move easily between the two tools.

One of the problems of interdisciplinary scientific collaboration is how to allow a non-expert in some particular field to successfully interpret and use the high-level expertise of an expert in that field. For this reason, HypMaT would allow users to assign Confidence Factors (CFs), ranging from -1 to 1, to both the nodes in the network and to the relationships between them. A CF value captures the expert user's confidence in the information represented by the node. These CF values would percolate through the network, allowing a non-expert to access, at a high level, an expert's deep understanding of how concepts in his or her field of expertise interrelate.

For example, a biologist might want to know which of two theories of planetary formation are favored by the astronomers in the collaborating community. Using HypMaT, the biologist could see at a glance that Theory A (CF=3D0.8) is strongly preferred over the Theory B (CF=3D-0.1).

If she were to explore the network further, she could see that, although most observations support both theories equally, one recent observation would seem to disconfirm Theory B. That is, there is a relationship between the observation (CF=3D0.8) and Theory B, and this relationship contributes negatively to the CF of Theory B. Finally, the biologist could choose to access (perhaps via ScienceDesk) detailed information regarding that observation, including published papers, web pages, and data archives.

HypMaT is intended to be used by distributed teams of scientists. HypMaT users in a collaborative group would be able to synchronize their versions of the knowledge network over the internet. HypMaT would also maintain a record of past and alternate versions of the knowledge network, so that users can revert to earlier versions, or maintain different versions in cases of disagreement.

As an aid to collaborative composition, HypMaT would be able to transform a knowledge network (or sub-network) into a text outline, which could serve as a starting point for a scientific paper. The text outline would include any bibliographic data that were included in the network, saving time and reducing cross-referencing errors.

Although we envisage HypMaT as primarily for collaboration, it could also be used as a pedagogical tool. Students could explore the networks that result from various NAI projects, and thus learn more about the various disciplines involved in astrobiology and how they interrelate.

Kim Binsted, the co-investigator in charge of HypMaT, is a specialist in artificial intelligence and human-computer interaction. She is currently designing and building a prototype of a collaborative system similar to that described above. Because such systems are best developed in the context in which they will be used, Co-I Binsted is eager to use the distributed, interdisciplinary team associated with this proposal as a prototypical set of users for the system. Likewise, the team is eager to explore a tool that will allow them to collaborate more effectively.

3.7 Other Astrobiology Commitments

Karen Meech

- **Towards Other Planetary Systems Outreach Program** – Meech has run a major NSF- and NASA-funded teacher enhancement program in Hawaii since 1993. The program is designed to help high school math and science teachers integrate math and science standards into their classes by teaching astronomy. The Astrobiology content of this summer program also teaches about cutting edge astronomical research that is ongoing in Hawaii. A privately funded student component also exists for this 3-week summer program. This program was funded as a pilot by NASA from 1993-1995, and then by the NSF for 1999-2003.

- **Bioastronomy 1999 Meeting, Hawaii** – Meech was the Local Organizing chair for this meeting held on the Kona Coast in Hawaii, and in addition she facilitated a large educational outreach component at the meeting.

- **Bioastronomy 2004 Meeting, Iceland** – The local organizing chair of the meeting is Thorstein Thorsteinnson, one of our NAI collaborators. PI Meech is also a member of the local organizing committee, and the scientific organizing committee (SOC), and Gaidos and collaborator Ehrenfreund are members of the SOC. Many of the UH-NAI members

will take an active role in this meeting (and 4 of the 15 SOC members are UH-NAI affiliates).

- **IAU Commission 51 on Bioastronomy** – Meech is a member of the Organizing committee, and the incoming President of Commission 51 of the International Astronomical Union as of the summer of 2003, and is currently the Vice President of the Division.

- **WGESP** – Meech is a member of the IAU Working Group on Extra-Solar Planets. The working group is charged with acting as a focal point for research on extra solar planets and organizing IAU activities in the field, including reviewing techniques and possibly maintaining a list of identified planets.

- **PSRD** – Meech is a member of the Board of Editors of the University of Hawaii on-line magazine, Planetary Science Research Discoveries.

- **COMPLEX** – Meech is a member of the National REsearch Council's Committee on Planetary and Lunar Exploration and has taken an active role in the development of several studies, including: *The Quarantine and Certification of Martian Samples*, *Organic Environments in the Solar System* and the *Assessment of Mars Science and Mission Priorities*.

James Cowen

- **Ridge2000 Program** – Cowen is a Steering and Executive Committee member of NSF's Ridge2000 program. The program is focused on the complex linkages between life and planetary processes at mid-ocean ridges. There are strong linkages between Ridge2000 and NASA's Astrobiology programs.

- **LExEn** – Cowen has been an invited participant to numerous planning workshops including those for LExEn (Life in Extreme environments) and NEPTUNE (ambitious plan for seafloor observatory based on fiber optic cable/instrument node/AUV system).

- **Teacher at Sea** – Cowen has developed and actively supports a Teacher at Sea program (see 3.1.3).

Eric Gaidos

- **Terrestrial Planet Finder** – Gaidos is a member of the Terrestrial Planet Finder Working Group (2002-2006)

- **Planetary Ecosystems and Biosystems Lab** – Gaidos has set up this laboratory and curriculum at the University of Hawaii.
http://www.soest.hawaii.edu/GG/FACULTY/GAIDOS/pebl.html

Ralf Kaiser

- **UK Astrobiology Network** – The Co-I is member of the UK Astrobiology Network and also an advisor to the UK Astrobiology Forum highlighting future research initiatives in astrobiology.

- **Centre for Astrobiology, The Open University (UK)** – The Co-I holds an adjunct professorship at the Department of Physics and Astronomy, Centre for Astrobiology, at The Open University, Milton Keynes (UK). In collaboration with Prof. Nigel J. Mason, one experiment is currently being setup at the synchrotron to investigate the synthetic routes and destruction rates of aminoacids and carbon hydrates in astrobiologically important ices.

- **Course Development** – Kaiser developed a graduate course at the University of Hawaii in Astrochemistry and Astrobiology.

- **Textbook** – Cambridge University Press commissioned Kaiser to write a textbook for graduate students entitled "The Chemical Evolution of the Interstellar Medium: From Astrochemistry to Astrobiology".

- **Graduate Program** – In collaboration with faculty members from Department of Chemistry, Department of Physics & Astronomy, the Institute for Astronomy (IfA), and the Hawaii Institute of Geophysics and Planetology (HIGP), Kaiser organized a new graduate program at the University of Hawaii. Astrobiology is a key component of this endeavor.

David Karl

- **1998 Lake Vostok Workshop** – Washington DC, Karl was co-chair of this international workshop that led to a series of recommendations on future research in Antarctic subglacial lakes.

- **Polar Research Board, National Research Council** – Karl is currently a member of this polar sciences advisory board.

- **Meeting Planning Committee** – Karl is currently co-chair of an American Academy of Microbiology planning committee for a future international colloquium with the tentative title, "Marine Microbial Diversity: The Key to Earth's Habitability"

Klaus Keil

- **SScAC** – Keil served on the Space Science Advisory Committee (SScAC), NASA Headquarters, Washington, D.C. from 1993-2000, during which time discussions took place regarding the creation of the Astrobiology Program.

- **Planetary Protection** – Keil served on the Planetary Protection Task Force, NASA Headquarters, in 1999.

- **Chondrule and CAI Formation** – Krot, Reipurth, Scott and Keil are organizing this interdisciplinary workshop in October 2004, in Kauai which aims at bringing meteoriticists and astronomers together to explore formation mechanisms for chondrules and CAI's.

Tobias Owen

- **Astrobiology Text** – Owen has written a well-used Astrobiology textbook with D. Goldmith: *The Search for Life in the Universe*, (University Science Books, Sausalito) 573 pp. (2002).

- **Astrobiology Conference** – Co-Director, 7th Trieste Conference on Chemical Evolution and the Origin of Life 15-19 Sep. 2003.

Bo Reipurth

- **IAU Division IV – Interstellar Medium** – President: B. Reipurth.
- **Protostars and Planets V Meeting, 2005** – Reipurth has spearheaded getting this meeting to come to Hawaii during Oct 24-29, 2005. Team members Reipurth, Keil and Jewitt constitute the Scientific Organizing Committee of the meeting and Meech is the Local Organizing Chair. This will be a highly visible meeting with Proceedings published through the University of Arizona series.
- **Outreach** – Reipurth is very active in outreach, having conducted over 150 radio broadcasts, participated in various TV programs, and has written over 30 newspaper articles on popular astronomy, some related to Astrobiology.
- **NAI Affiliation** – Reipurth was an affiliate of the Colorado Astrobiology group (1998-2001).

Jeffrey Taylor

- **Planetary Science Research Discoveries** – Manager and editor of a web magazine called Planetary Science Research Discoveries (www.psrd.hawaii.edu) which translates recently published articles to the level appropriate for teachers, high school students and the public.
- **Hawaii Space Grant Consortium** – Taylor is the Associate Director for Space Science for the Hawaii Space Grant Consortium. He has been instrumental in helping to develop on-line classroom activities which are related to exobiology.
http://www.spacegrant.hawaii.edu/class_acts/index.html

4 Facilities & Equipment

- **The Mauna Kea Observatories** – The 4,200 meter high summit of Mauna Kea in Hawaii houses the world's largest observatory for optical, infrared, and submillimeter astronomy. The University of Hawaii staff have priviledged access on a competitive basis to all of the facilities on Mauna Kea which are shown in the Table below, thus all of our UH NAI team members will have the opportunity to use these facilities. Through collaborations with our local NAI team, members of the national NAI will also have access. The UH gets 10-15% of the time on all of the facilities listed, and 100% of the time on the University of Hawaii 2.2m telescope.

Table 3: Facilities on Mauna Kea, Hawaii

Telescope	**Diam [m]**	**Partners**	λ **Regime**
Keck	2×10	Caltech, USC, NASA	optical, IR
Subaru	8	Japan	optical, IR
Gemini	8	Internat. consortium	optical, IR
UKIRT	3.8	United Kingdom	IR
CFHT	3.6	Canada, France, Hawaii	optical, IR
IRTF	3.0	NASA	IR
UH	2.2	University of Hawaii	optical, IR
JCMT	15.0	UK, Netherlands, Canada	submillimeter
CSO	10.0	Caltech	submillimeter
SMA	8×6	Smithsonian, Taiwan	submm interferometer

The Submillimeter Array (SMA), is nearing completion on Mauna Kea, and will be optimized for high angular aresolution observations at λ = 1.3-0.3mm, ideal for looking at thermal continuum emission, rotational lines of light molecules, and atomic fine structure lines, which arise in compact regions such as the vicintiy of young stars, protoplanetary disks and solar system bodies. This will be a unique tool for astrobiology.

- **The Haleakala Observatories** – The Faulkes Telescope is a joint project between the Faulkes Telescope Corporation and the University of Hawaii's Institute for Astronomy. The 2-meter facility is under construction and scheduled for completion this year. When complete, this will be the largest professional grade telescope in the world dedicated to education and public outreach.

- **Ultra-High Vacuum Surface Scattering Machine** – All experiments will be carried out in an extreme ultrahigh vacuum (10^{-11} mbar) surface scattering machine in which frozen, astrobiologically relevant ice samples (10K-293K) are irradiated with charged particles (electrons, protons, helium nuclei) and photons. The analysis will be carried out quantitatively on line and in situ via Fourier transform spectroscopy in absorption-reflection (solid state) and through a calibrated quadrupole mass spectrometer (gas phase) to determine temperature dependent production rates of astrobiologically relevant molecules such as sugars, aminoacids, and phosphates. This machine presents the only setup world wide in which an extreme ultra high vacua and low temperatures can be reached and the solid state as well

as the gas phase can be monitored simultaneously. This setup can be operated as a user facility for qualified NAI members.

- **Meteorite Instrumentation** – At the present time at the University of Hawaii, we have the following equipment available to our group in HIGP and SOEST: Three Nikon research optical microscopes (two with automated photography attachments, including a digital camera attached to a computer, and Swift point counter); two Nikon stereomicroscopes (one with automated photography attachments); a state-of-the-art, fully automated 5-spectrometer Cameca SX-50 electron microprobe; a Hitachi H-600 scanning transmission electron microscope (STEM) and sample preparation laboratories including plasma etcher, carbon and gold-palladium coating devices and ultramicrotomes; a JEOL-5900LV scanning electron microscope (SEM); a Princeton Gamma-Tech 4-Plus energy dispersive X-ray spectrometer and digital image analysis systems, both of which are interfaced with the STEM and TEM; atomic absorption and ICP laboratories; a fully automated Siemens SRS303-AS-X-ray fluorescence facility; a fully automated Scintag Pad V X-ray diffractometer; experimental petrology apparatus; extensive computer facilities; laboratory for the preparation of polished thin sections; photography and drafting facilities; and others. In situ isotopic studies of secondary minerals resulted from aqueous activity will be performed in collaboration with Dr. K. D. McKeegan at the University of California, Los Angeles using the UCLA ims 1270 ion microprobe and in collaboration with Dr. I. D. Hutcheon at Lawrence Livermore National Laboratory using the modified Cameca ims-3f and NanoSIMS 50 ion microprobes.

- **Cryobot Access** – In addition to state of the art computational support, Anderson has a 200 square foot laboratory, two rotating field mass spectrometers (RFMS), and access to a Cryobot as part of a shared agreement with JPL (as he is a team member). One of the RFMS systems is housed in a casing rated to 2000m for deep sea deployment, and the other is a benchtop unit suitable for life detection experiments. The Cryobot is a prototype hot water ice drill capable of carrying instruments to hundreds of meters depth in Iceland or Antarctica, as well as deployment in the ocean. The Cryobot can steer, make measurements, process data, and produce realtime video of the subsurface ice. The data is sent through a tether to the surface. Our lab space is suitable for maintenance on the Cryobot and RFMS, as well as experimentation with the RFMS instrument.

- **BioLab – BSL2** – Gaidos operates an 825 sq. ft. complex that includes a Biosafety Level 2 clean laboratory for manipulation of environmental samples and culturing organisms. This laboratory includes a BSL-2 laminar-flow clean bench, PCR cabinet, compound epifluorescence microscope, stereo field microscope, two (2) water-jacketed controlled atmosphere incubators, an anaerobic chamber with airlock, several refrigerators and freezers, centrifuges, autoclave, dishwasher, ultrapure water system, and miscellaneous equipment to treat samples and extract DNA. The facility also includes a computer lab work area for data analysis and modeling with 3 PC computers equipped with the Linux operating system. In addition, he manages a 1200 sq. ft. shared molecular biology facility that includes a multiple capillary DNA sequencer, laser-scanning blot reader, spectrophotometer, centrifuges, complete denaturing gradient gel electrophoresis (DGGE) system, a laser flow cytometer system, and miscellaneous equipment for molecular biology.

- **Kilo Moana Oceanographic Research Ship** – The R/V *Kilo Moana* is the latest member of the University of Hawaii's research fleet. Owned by the Office of Naval Research and operated by SOEST (School of Ocean and Earth Science and Technology), the twin hull research ship has been called the best in the U.S. academic fleet. The modern vessel is the first Auxiliary General Oceanographic Research ship to be constructed on a Small Waterplane Area Twin Hull design. This provides a comfortable, stable platform for multi-disciplinary marine research. The overall length of 186 feet and 88-foot beam provide ample exterior deck space and 3,000 s.f of dedicated science space. The vessel is equipped with both a deep-water (Simrad EM 120) and a shallow-water (Simrad EM 1002) multi-beam echo sounder capable of accurate sea-floor mapping at any ocean depth, dynamic positioning, and a complement of winches, cranes and other handling gear. The ship has an endurance of 50 days at sea, and a range of 10,000 nautical miles at 11 knots. The ship missions include water sampling, equipment launch, towing, recovery, and shipboard sample analysis and data processing. The *Kilo Moana* can accommodate 31 scientists and 17 crew. Facilities include library and conference rooms and hydrographic, computers, chemistry, wet and meteorology laboratories.

- **Mid-ocean ridge (magmatic/tectonic) Event Detection and Response** – MOR eruptions are unpredictable and short-lived. Scientific exploitation of these fundamental phenomena requires an extensive, well-coordinated infrastructure involving essential equipment, extensive planning and established communication, and dedicated personnel. The Northeast Pacific ED&R team consists of a partnership between researchers at NOAA- PMELs VENTS Program and NSF-RIDGE2000 funded Universities. NOAAs T-Phase Monitoring Program is responsible for event detection: the remote detection of T-Phase seismic waves via the U.S. Navys Sound Surveilance System. The University research team, led by the University of Hawaii (NAI-HI co-PI Cowen), is funded by NSF to develop and maintain readiness to rapidly respond to significant seafloor eruptive events along the Northeast Pacific MOR system; they will be joined by NOAA response colleagues. Communication is facilitated by the RIDGE2000 Program, overseen by the Time-Critical Studies Committee (chaired by Cowen).

- **Hawaii Undersea Research Laboratory** – The Hawaii Undersea Research Laboratory (HURL) was established by the National Oceanic and Atmospheric Administration (NOAA) and the University of Hawaii. HURL's facilities include two deep-diving (2000 m) submersibles Pisces V and Pisces IV, a remotely operated vehicle RCV-150, and the support ship R/V Kaimikai-o-Kanaloa. Its mission is to study deep water marine processes in the Pacific Ocean, including a research focus on submarine volcanology: the geology, geophysics, geochemistry, and biology of volcanic processes. Loihi Volcano, on the flanks of the island of Hawaii, is a prime site for this work, which includes monitoring submarine geophysical, geological and geochemical processes using an Ocean Bottom Observatory.