Novel Isotopic Biosignatures: Promise & Progress

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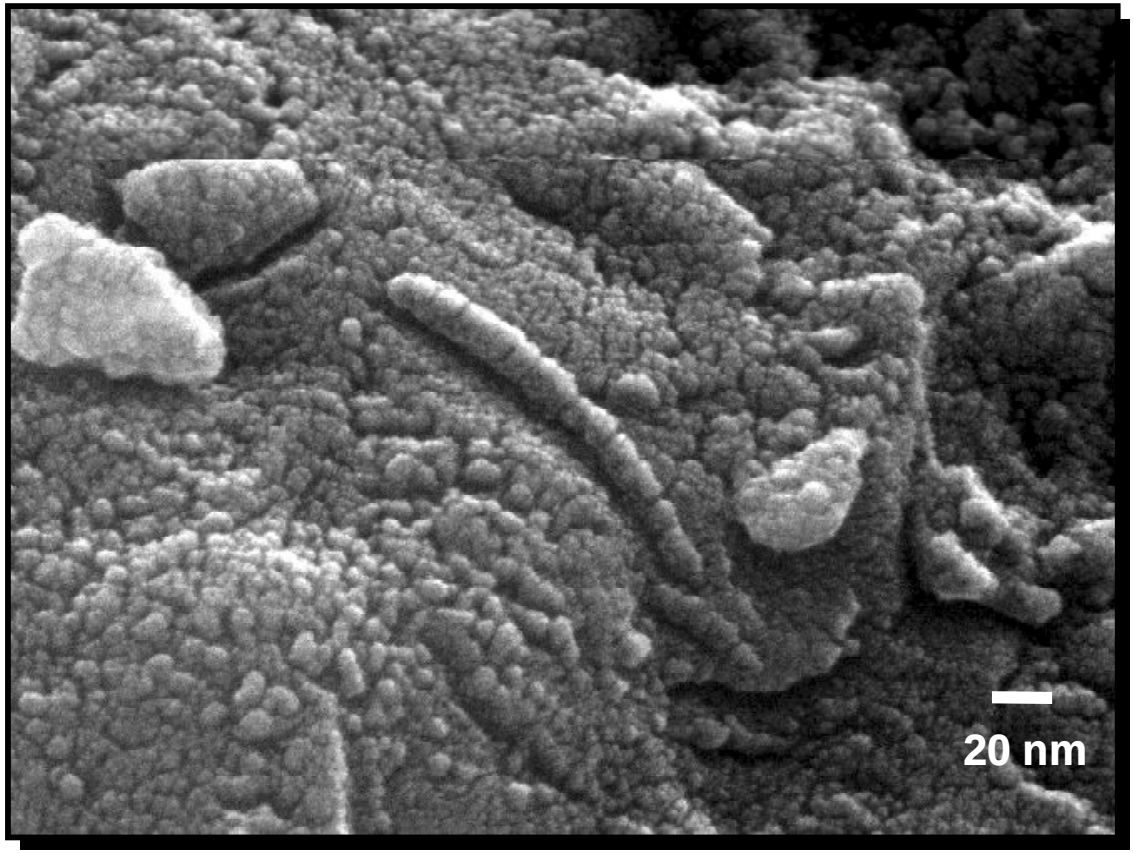
Support: NASA Astrobiology Institute and NSF (LExEn; EAR)

Rubes

"Hey Earl! Look ... I found a microbe!"

Biosignature?



Morphology alone is not enough

Chemical Clues
Isotope Fractionation

Example: Carbon
Two stable isotopes: ^{12}C and ^{13}C

Kinetic Isotope Effect During Photosynthesis:

$$^{12}CO_2 + H_2O \rightarrow {}^{12}CH_2O + O_2 \quad \text{(a)}$$

$$^{13}CO_2 + H_2O \rightarrow {}^{13}CH_2O + O_2 \quad \text{(b)}$$

Rate constant for (a) is *larger* than for (b)

Hence:
$^{13}C/^{12}C$ of biogenic organic C is *smaller* than of inorganic carbon

"δ notation"

Carbon Isotopes

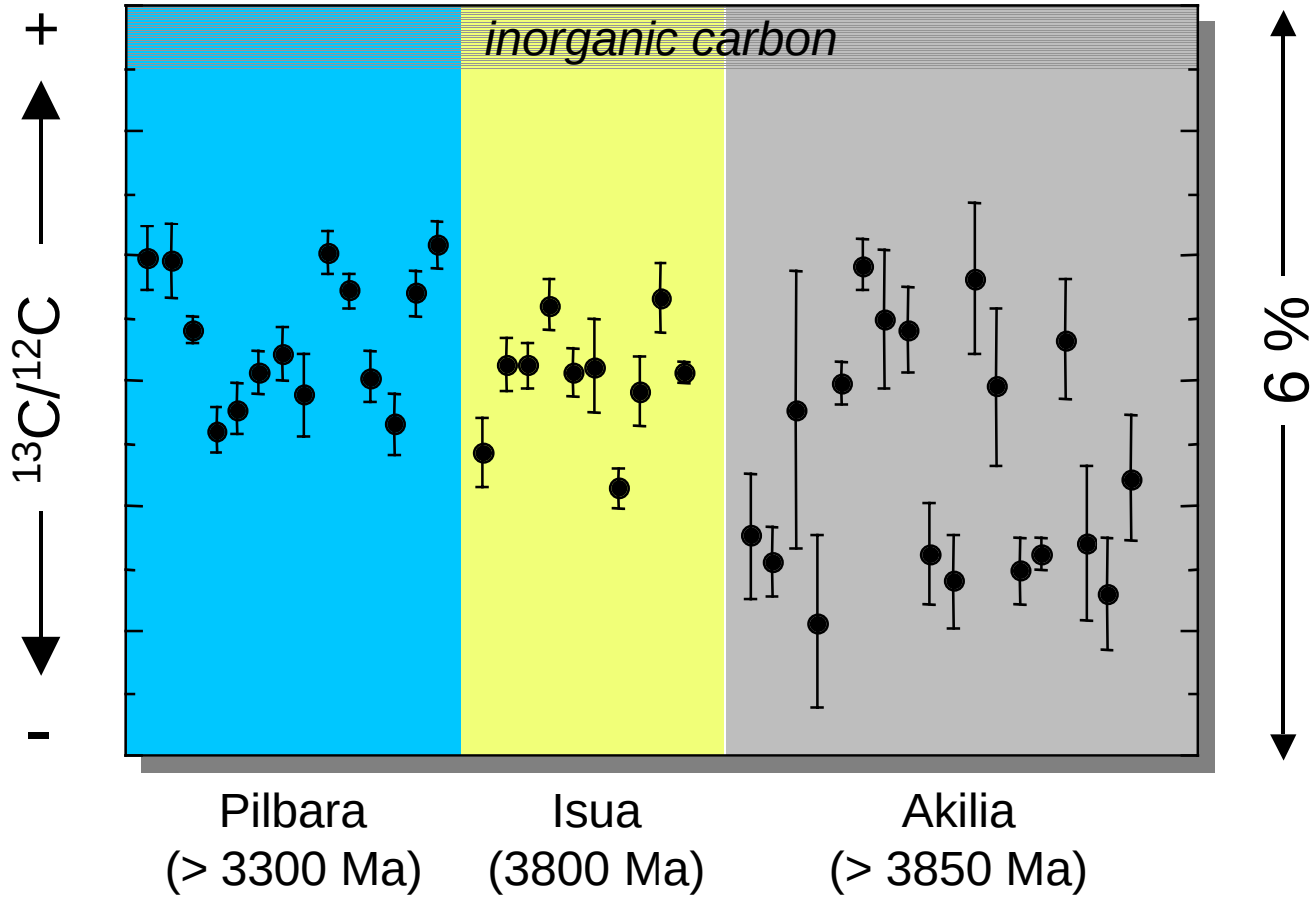
(how geochemists make this seem complicated)

$$\delta^{13}C = \left(\frac{\left({}^{13}C / {}^{12}C \right)_{sample} - \left({}^{13}C / {}^{12}C \right)_{standard}}{\left({}^{13}C / {}^{12}C \right)_{standard}} \right) x1000‰$$

Same nomenclature can be applied to any isotope ratio…

Early Life on Earth

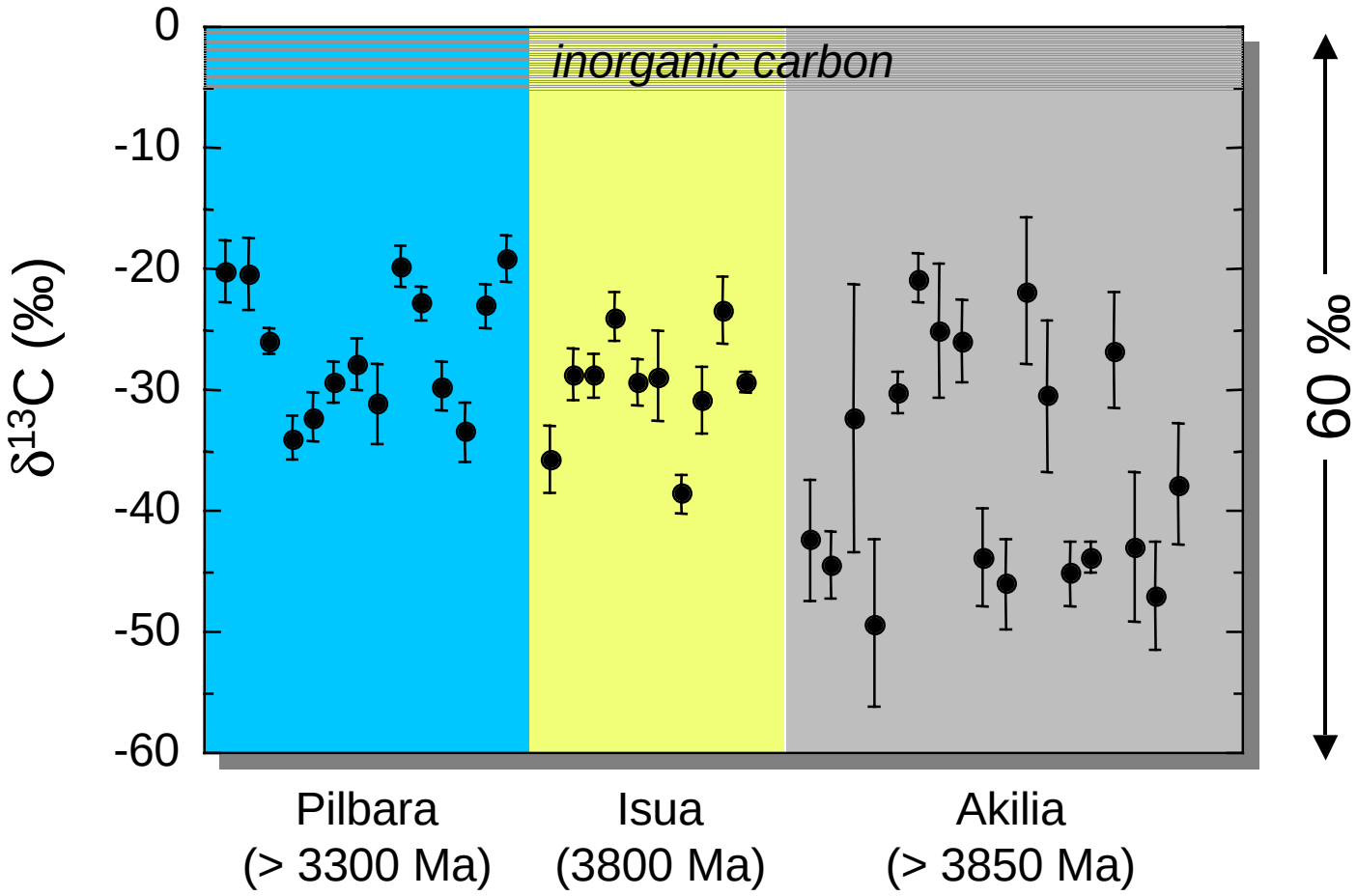
Carbon Isotope Evidence



Modified from: Mojzsis *et al.*, 1996

Early Life on Earth

Carbon Isotope Evidence



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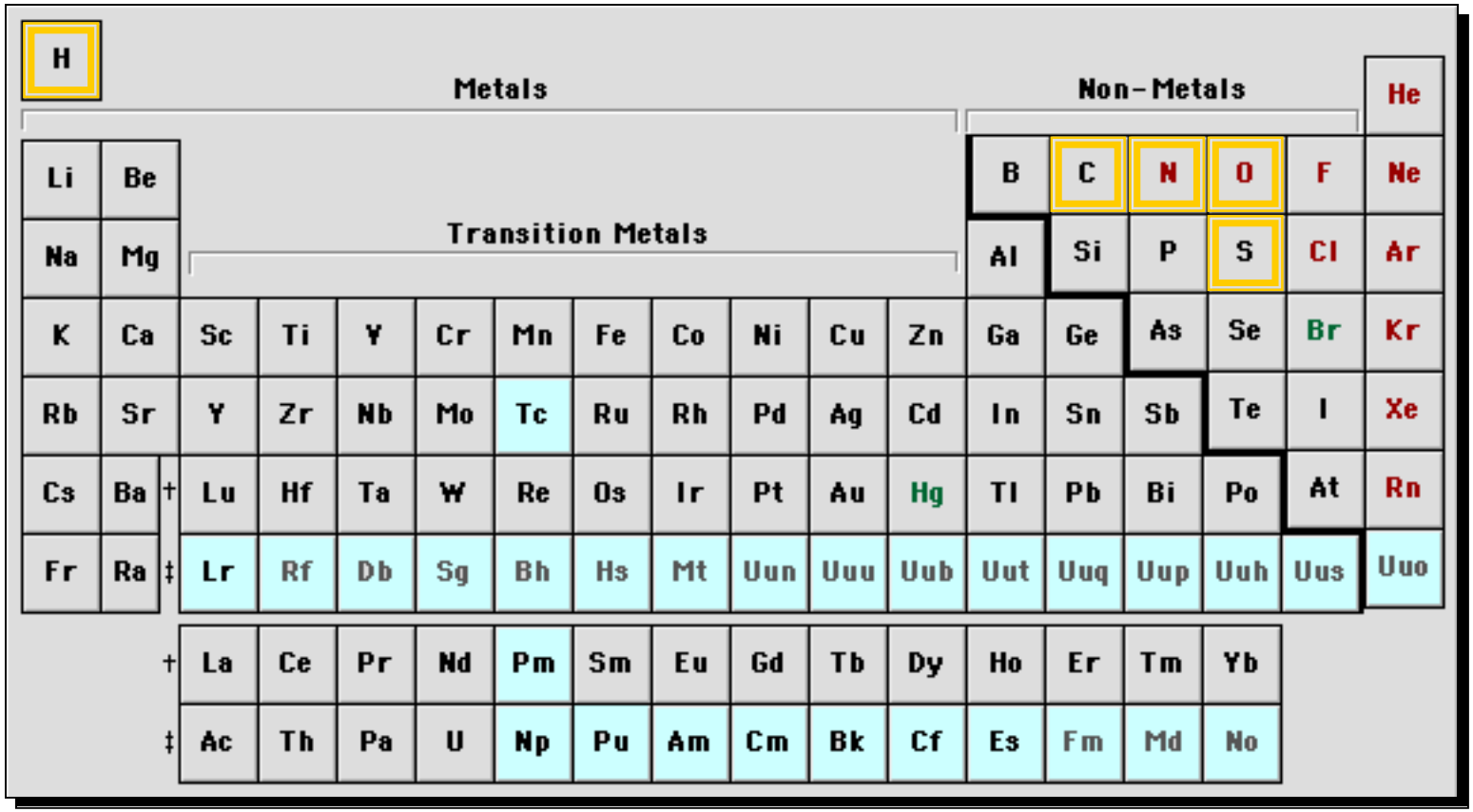
“Traditional” Stable Isotope Systems



Problems: *Preservation of C,H,O,N,S compounds often problematic*
Isotope exchange with C,H,O,N,S in environment

What about other isotope systems?

Why do isotopes fractionate (chemically)?

Mass-dependent differences in zero-point energy…

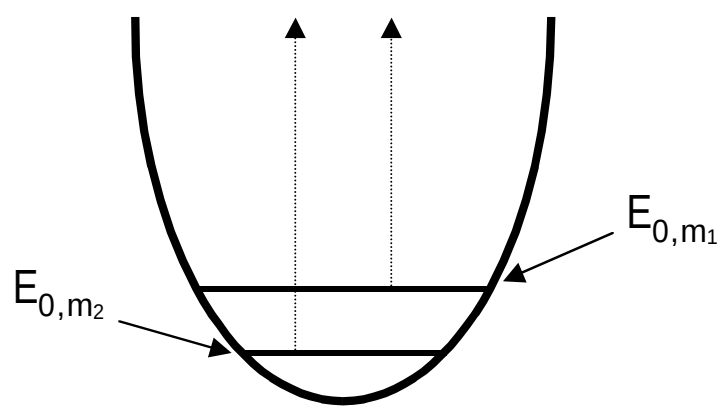
First approximation (SHO model):

$$E_0 = 1/2\ h\nu_0$$

where $\nu_0 = 1/2\ \pi(k/\mu)^{1/2}$; $\mu = (M \times m)/(M + m)$. As $M \rightarrow \infty$, $\mu \rightarrow m$.

Thus: $E_{0,m_1}/E_{0,m_2} \sim (m_2/m_1)^{1/2}$

If $m_2 > m_1$…



∴ *Mass-dependence in equilibrium constants & reaction rates*

Some Elements of Biogeochemical Interest…

Atomic No.	Element	Stable Isotopes[a]	$(m_2/m_1)^{1/2}$
6	Carbon	^{12}C, ^{13}C	1.041
7	Nitrogen	^{14}N, ^{15}N	1.035
16	Sulfur	^{32}S, ^{34}S	1.031
14	Silicon	^{28}Si, ^{29}Si, ^{30}Si	1.035
20	Calcium	^{40}Ca, ^{44}Ca	1.049

[a]Isotopes with average abundance > 1 %.
m_2 is the heaviest and m_1 the lightest stable isotope listed.

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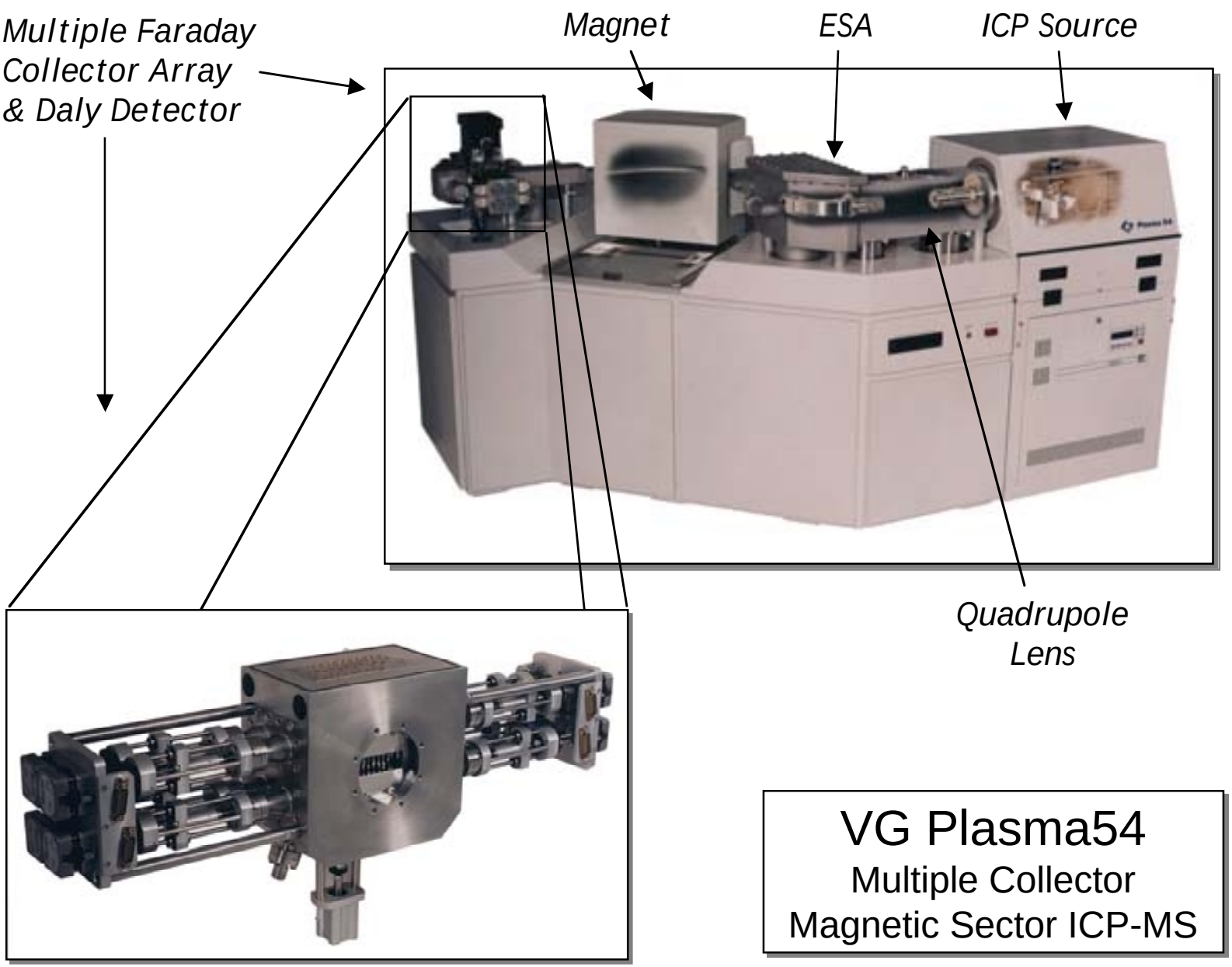
20	Calcium	^{40}Ca, ^{44}Ca	1.049
34	Selenium	^{82}Se, ^{80}Se, ^{78}Se, ^{77}Se, ^{76}Se	1.039
26	Iron	^{54}Fe, ^{56}Fe, ^{57}Fe	1.027
28	Nickel	^{58}Ni, ^{60}Ni, ^{61}Ni, ^{62}Ni	1.034
29	Copper	^{63}Cu, ^{65}Cu	1.016
30	Zinc	^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn	1.031
42	Molybdenum	^{92}Mo, ^{94}Mo, ^{95}Mo, ^{96}Mo, ^{97}Mo, ^{98}Mo, ^{100}Mo	1.043
48	Cadmium	^{106}Cd, ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{113}Cd, ^{114}Cd, ^{116}Cd	1.046
80	Mercury	^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg	1.015

[a]Isotopes with average abundance > 1 %.
m_2 is the heaviest and m_1 the lightest stable isotope listed.

Measurable effects are possible for many transition metals

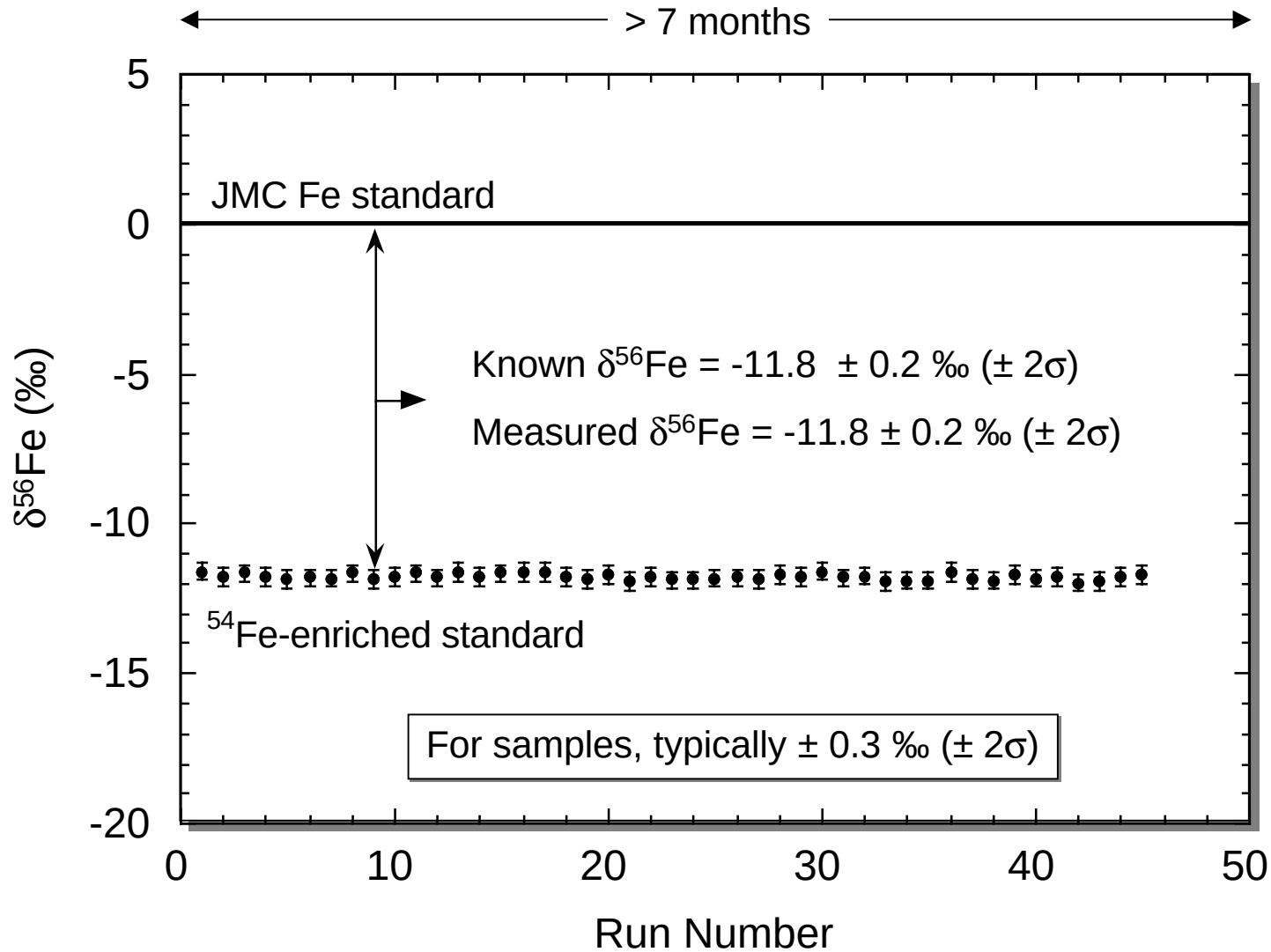
Recent progress made possible by analytical advances



VG Plasma54
Multiple Collector
Magnetic Sector ICP-MS

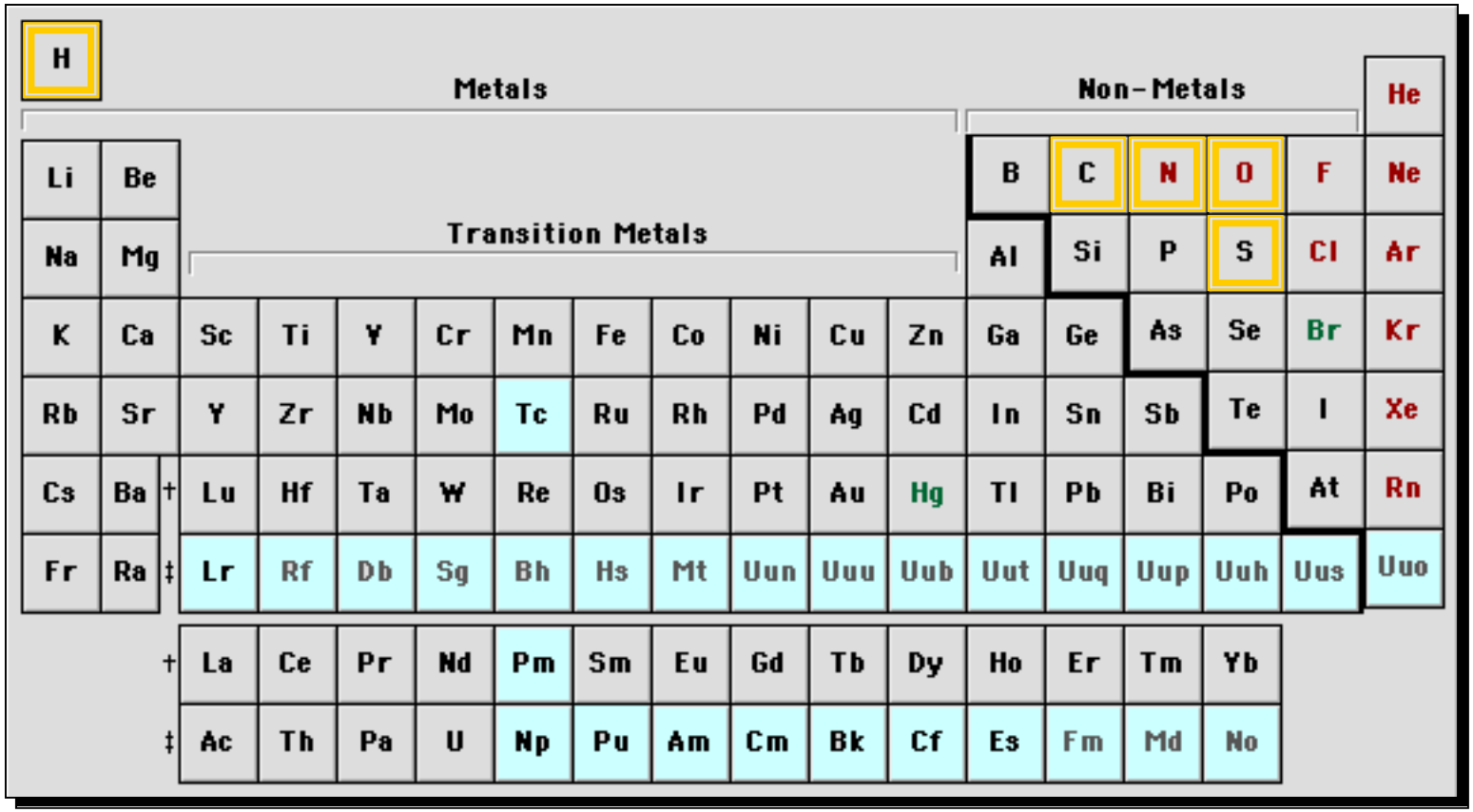
Example: Fe isotopes

Long-Term Accuracy and External Precision



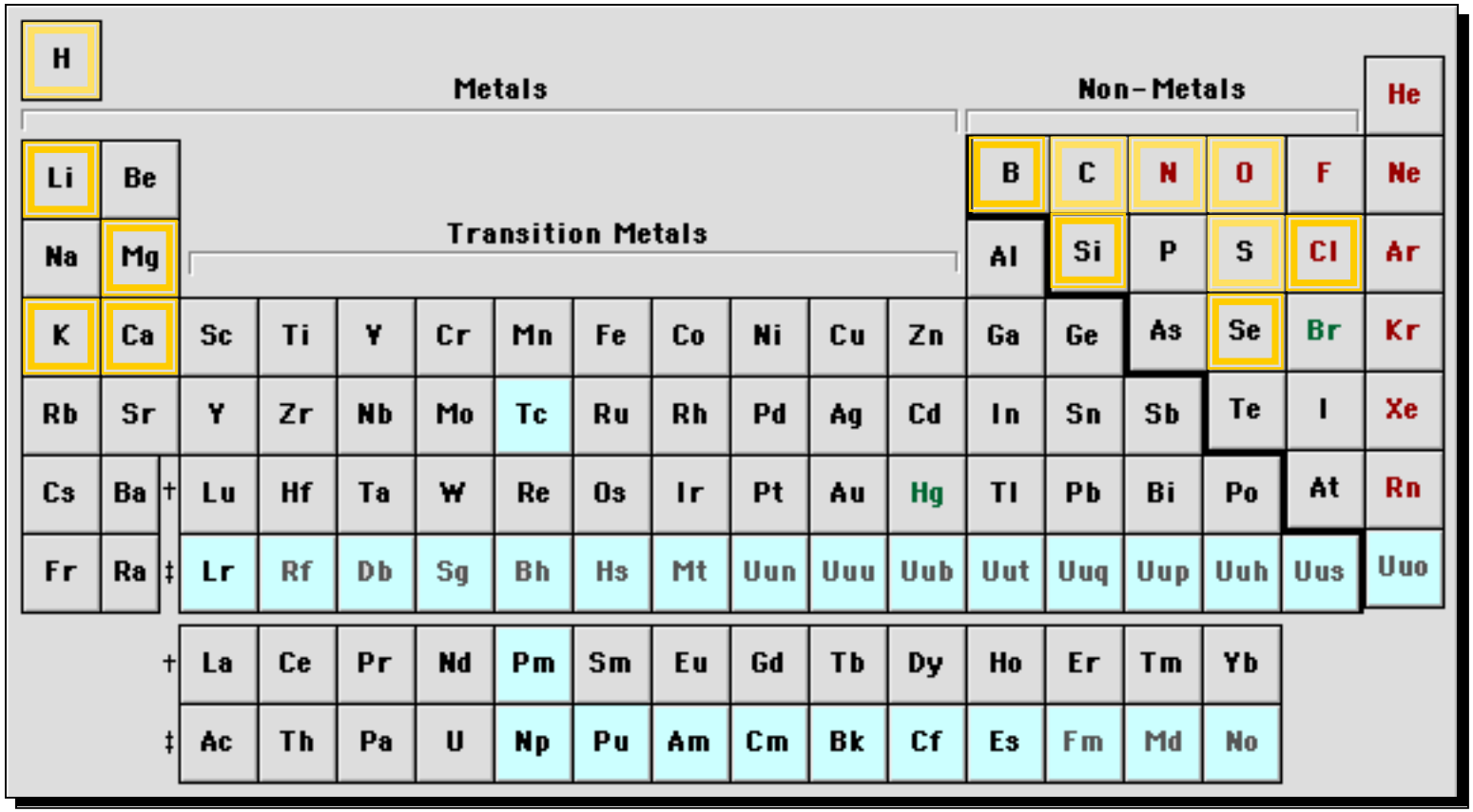
Anbar *et al.*, unpublished

“Traditional” Stable Isotope Systems



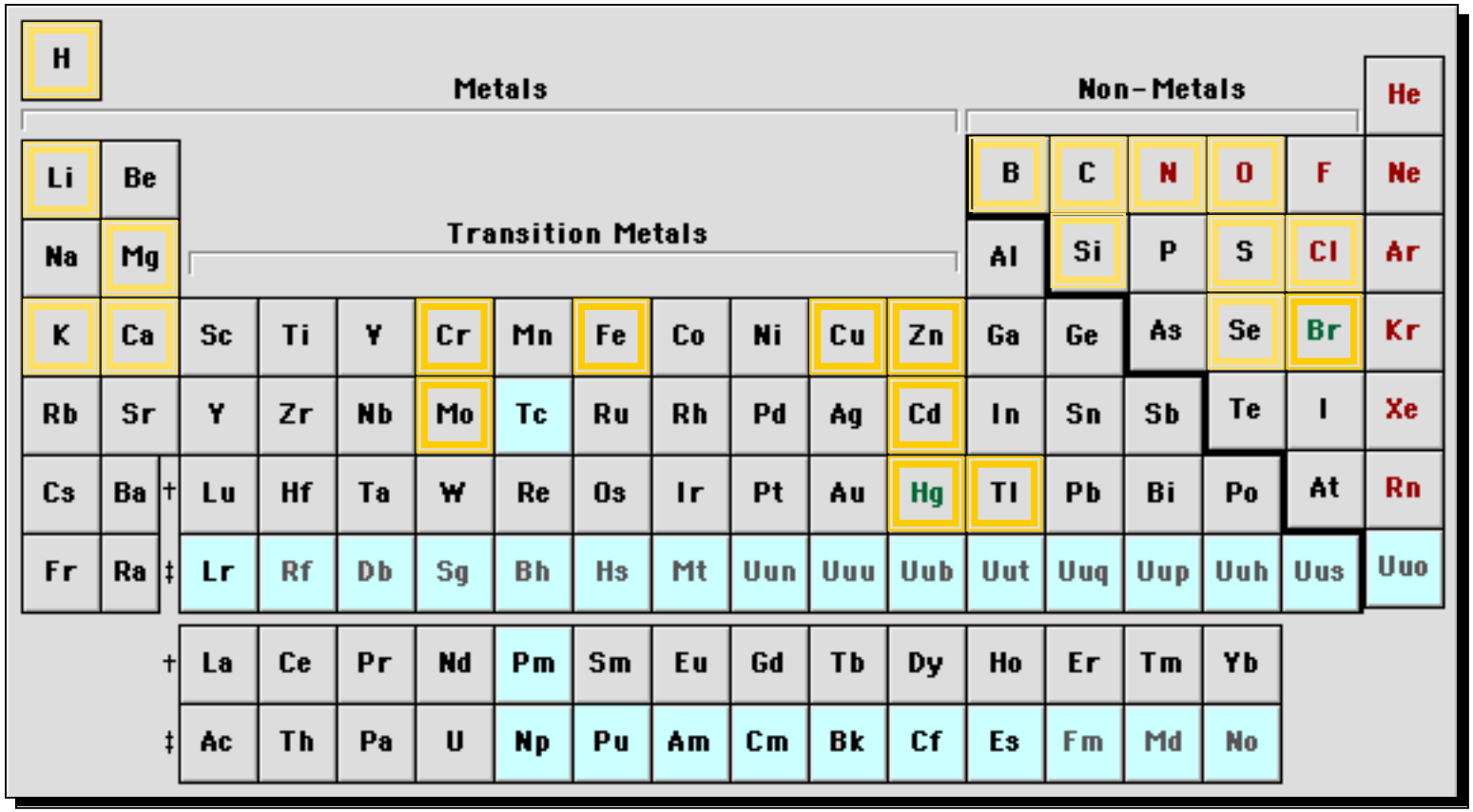
Geoscience applications beginning in 1950’s

“Nontraditional” Stable Isotope Systems



Geoscience applications beginning in 1960’s - 1990’s
Renaissance beginning in mid-late 1990’s (esp. B, Ca, Cl, Se)

“Emerging” Stable Isotope Systems



Geoscience applications beginning in late 1990’s and 2000’s
State of research in 2002: Similar to light stable isotopes in the 1950’s

Biosignatures: Why Iron Isotopes?

- Fe has rich role in biochemistry; undergoes changes in redox and coordination that could drive isotope effects.
- Magnetite (Fe_3O_4) is a well-known “biomineral”, produced both intracellularly and extracellularly by bacteria.
- The presence of magnetite of possible biogenic origin in the Alan Hills meteorite has been cited as evidence of life; arguably, the strongest line of evidence.
- Fe minerals, such as magnetite, are more robust than organic carbon- so could be better “time capsules” to preserve evidence of ancient or extraterrestrial life.

Iron Isotope Systematics

^{54}Fe: 5.8 %

^{56}Fe: 91.7 %

^{57}Fe: 2.2 %

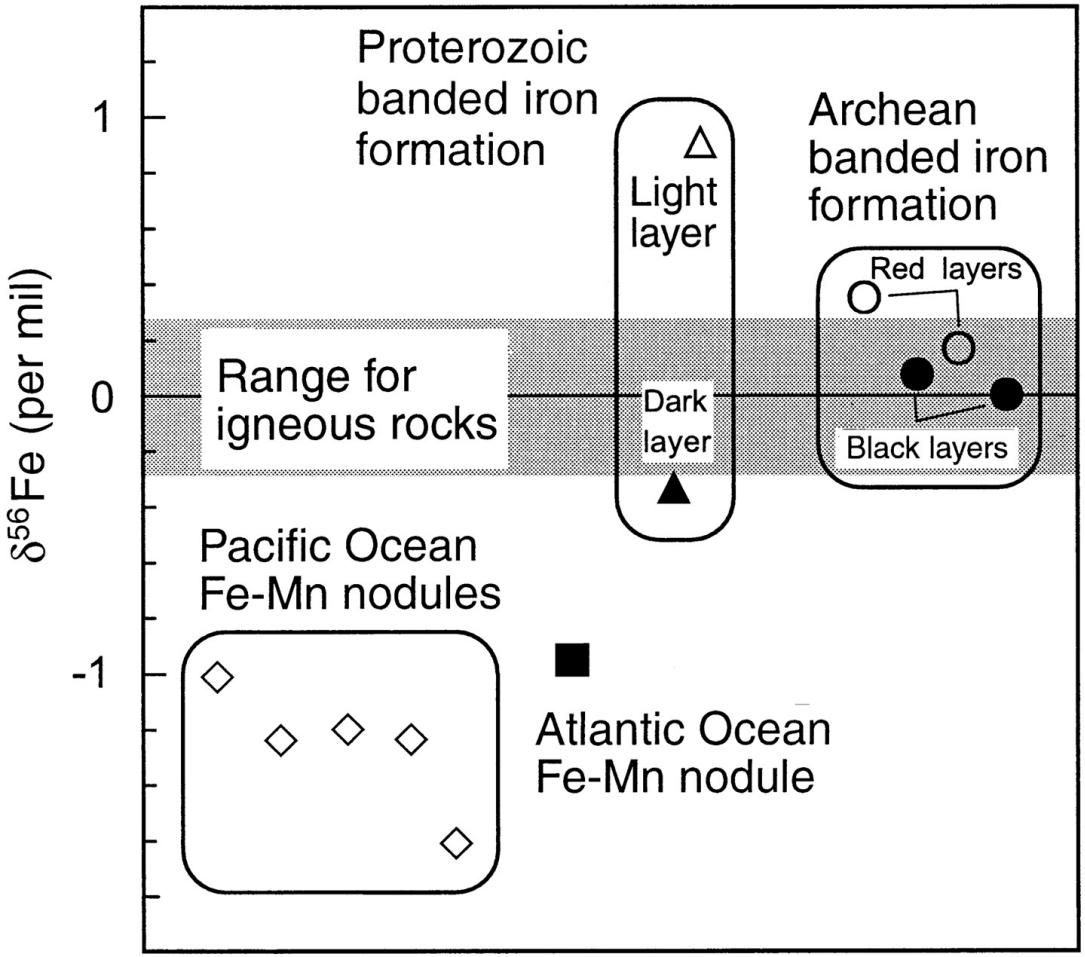
^{58}Fe: 0.3 %

$$\delta^{56}Fe = (^{56}Fe/^{54}Fe)_{sample}/(^{56}Fe/^{54}Fe)_{std} - 1) \times 1{,}000\ ‰$$

- or -

$$\varepsilon^{56}Fe = (^{56}Fe/^{54}Fe)_{sample}/(^{56}Fe/^{54}Fe)_{std} - 1) \times 10{,}000\ ‰$$

Fe Isotopes: Natural Variations

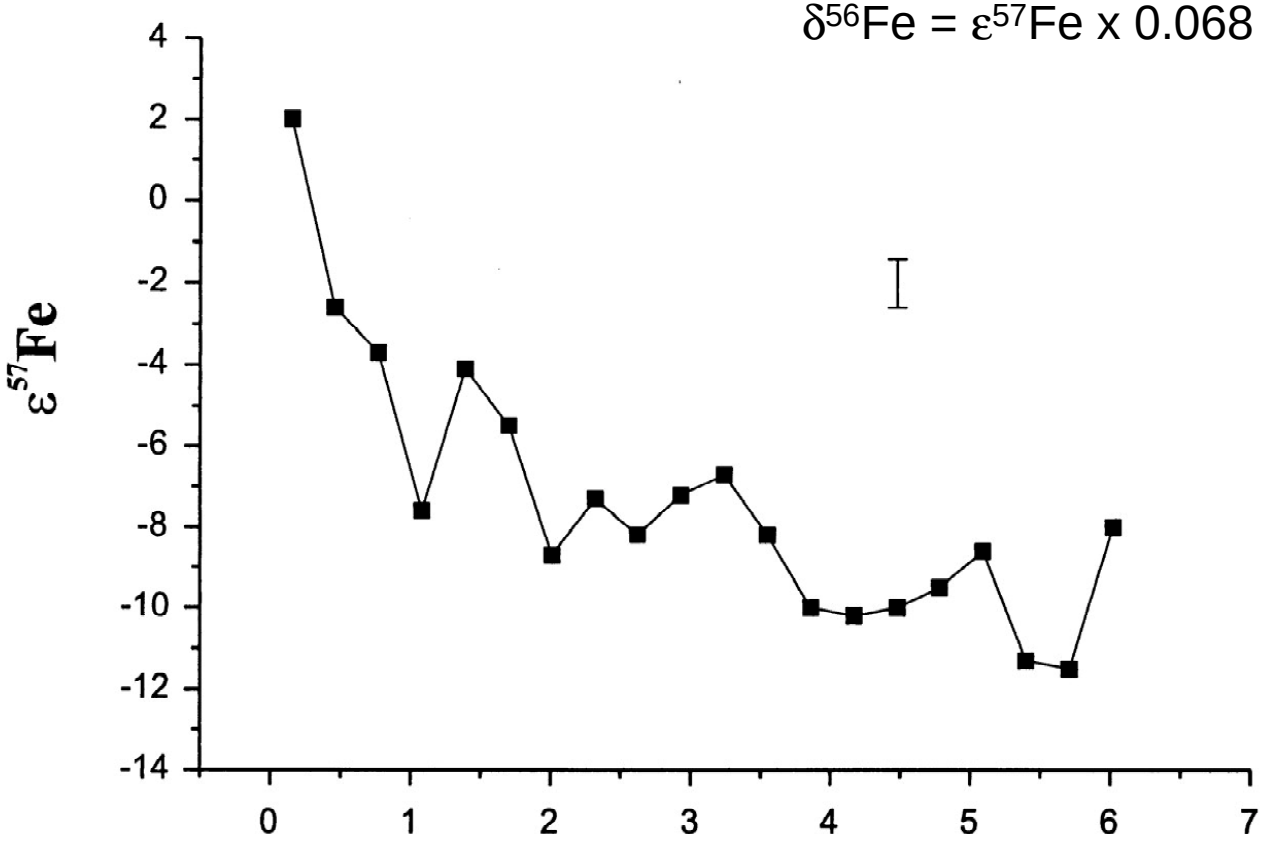


Beard *et al.*, *Science* (1999)

Natural Variations

North Atlantic Fe-Mn crust

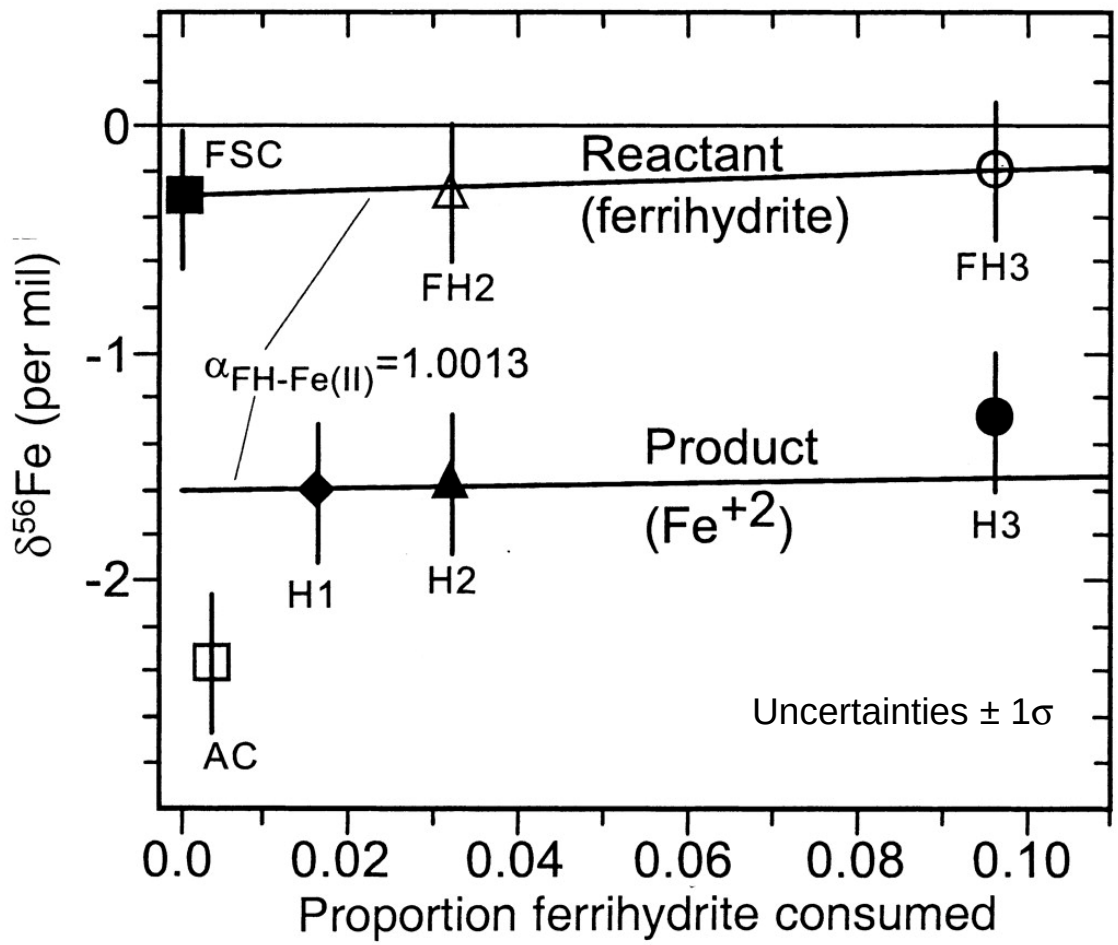
$\delta^{56}Fe = \varepsilon^{57}Fe \times 0.068$



Zhu *et al.*, *Science* (2000)

Biosignature Potential: Biological Fractionation

Microbially Mediated Reduction of Fe^{3+}



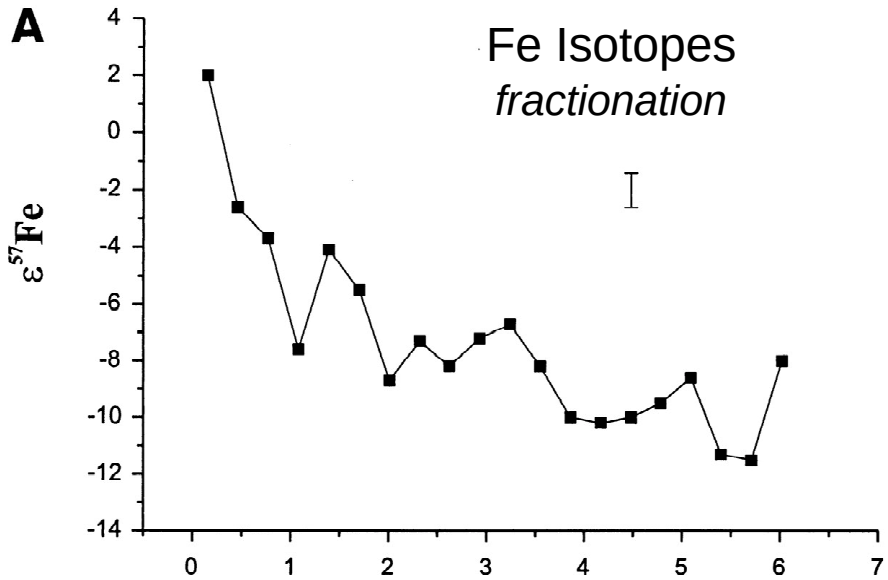
Beard *et al.*, *Science* (1999)

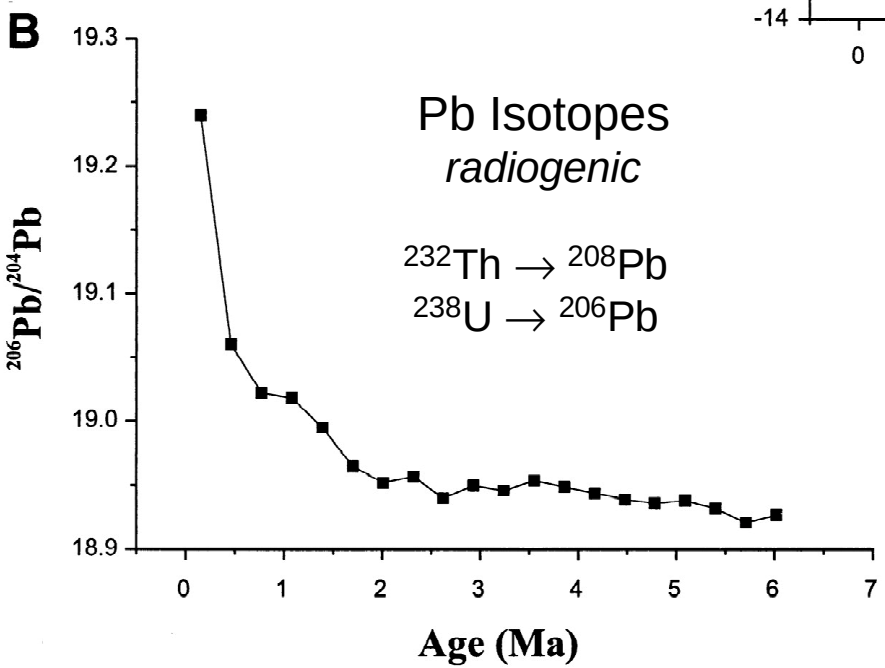
Complications:

It's never as simple as it first appears…

Natural Variations

North Atlantic Fe-Mn crust





Correlation of radiogenic Pb isotope variations and Fe isotopes suggests that Fe isotopes vary due to change in Fe sources, rather than *in situ* isotope fractionation

Zhu *et al.*, *Science* (2000)

Test for Nonbiological Fractionation Effects...

Anion Exchange Column

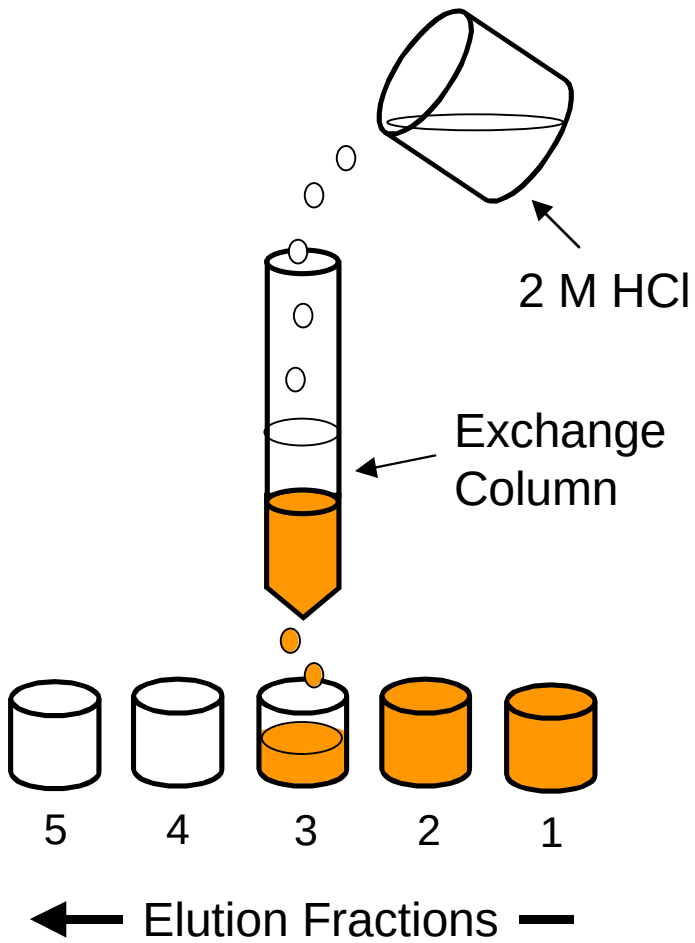
Biorad AG MP-1 Resin

Functional group: $[R\text{-}CH_2N(CH_3)_3]^+$
Solution: Fe dissolved in HCl
Separation: $FeCl_4^-$ binds to resin

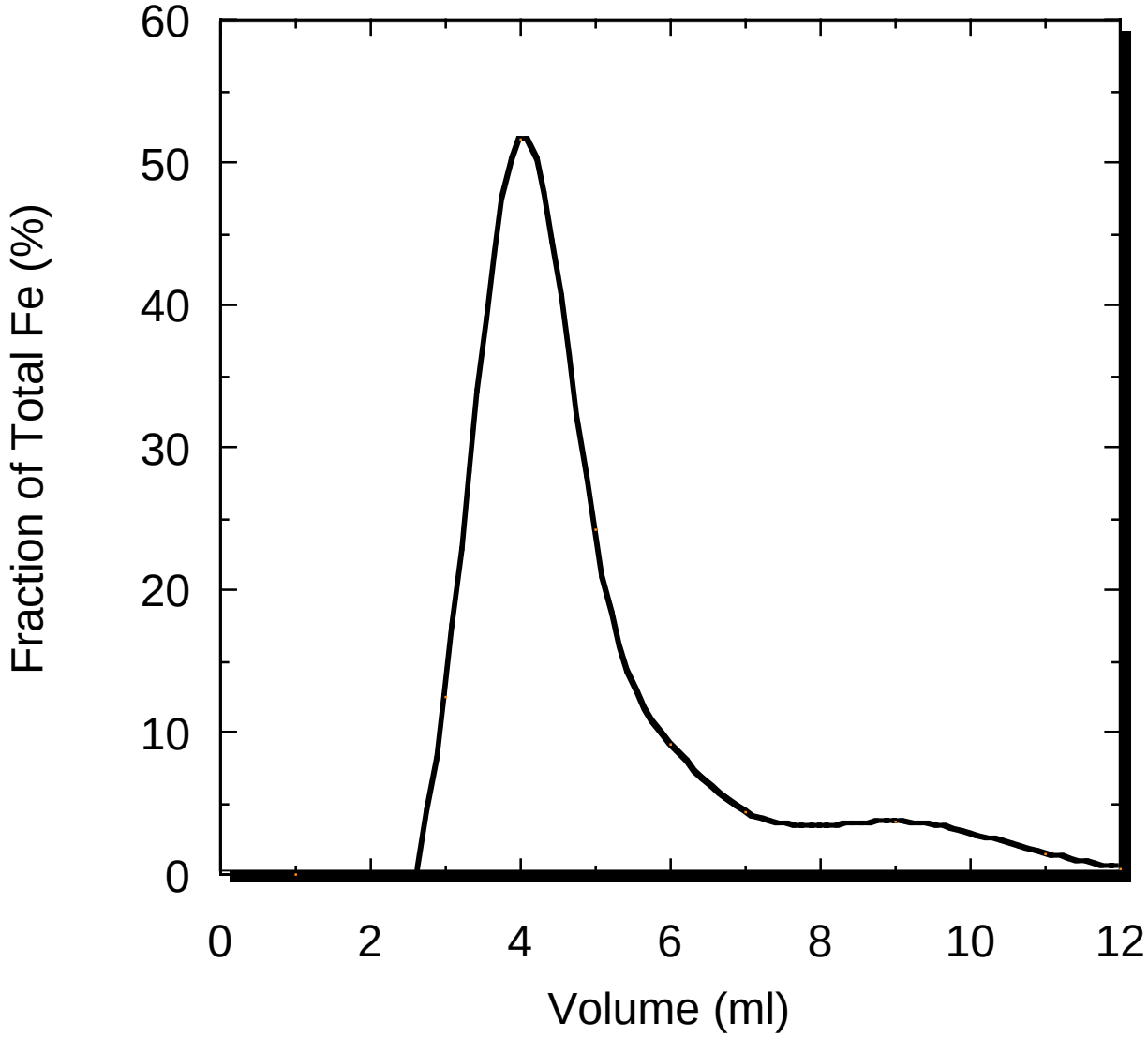
Equilibrium binding model:

$$K_D = [Fe]_{resin}/[Fe]_{dissolved}$$

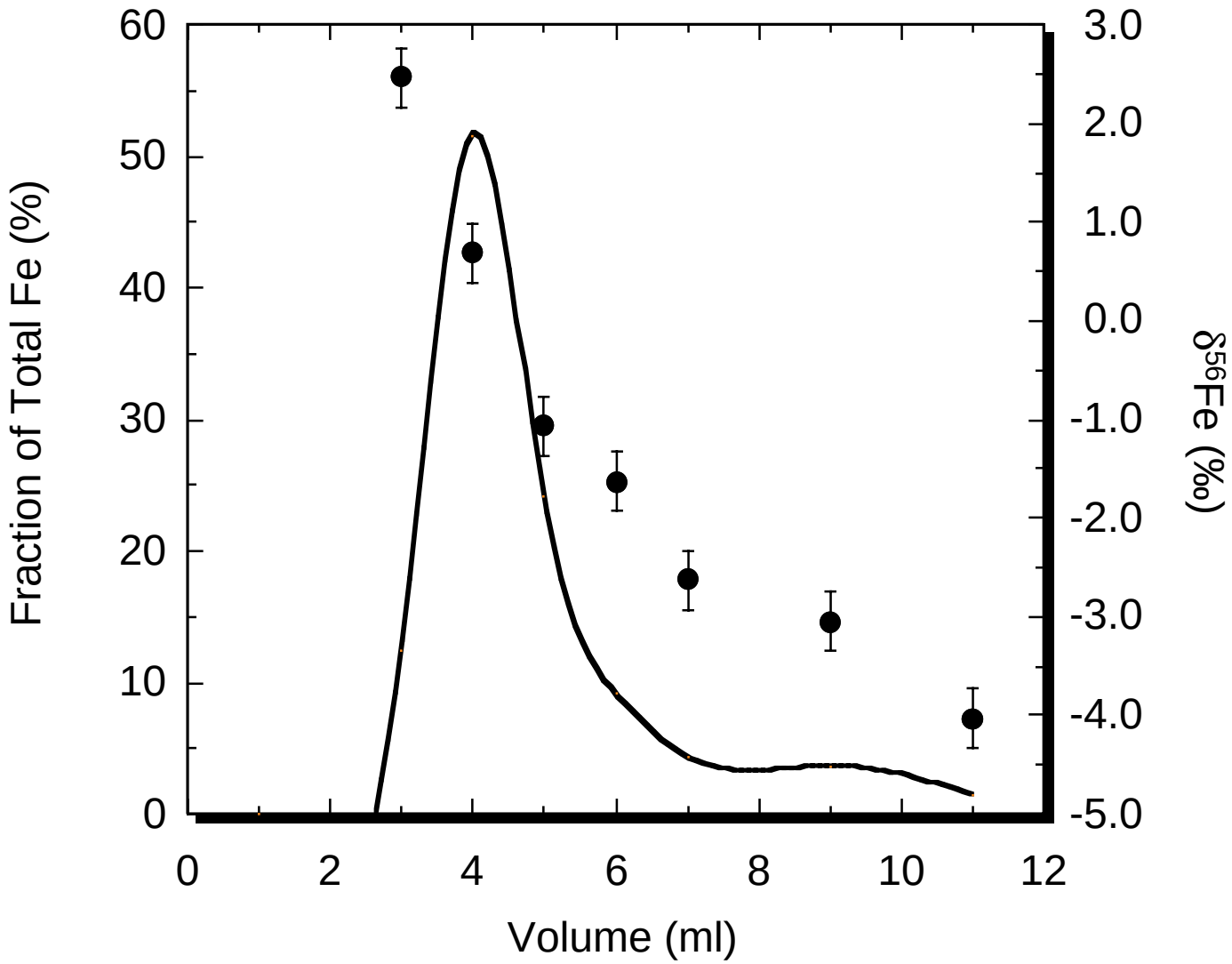
$$^{54}K_D/^{56}K_D \stackrel{?}{=} 1$$



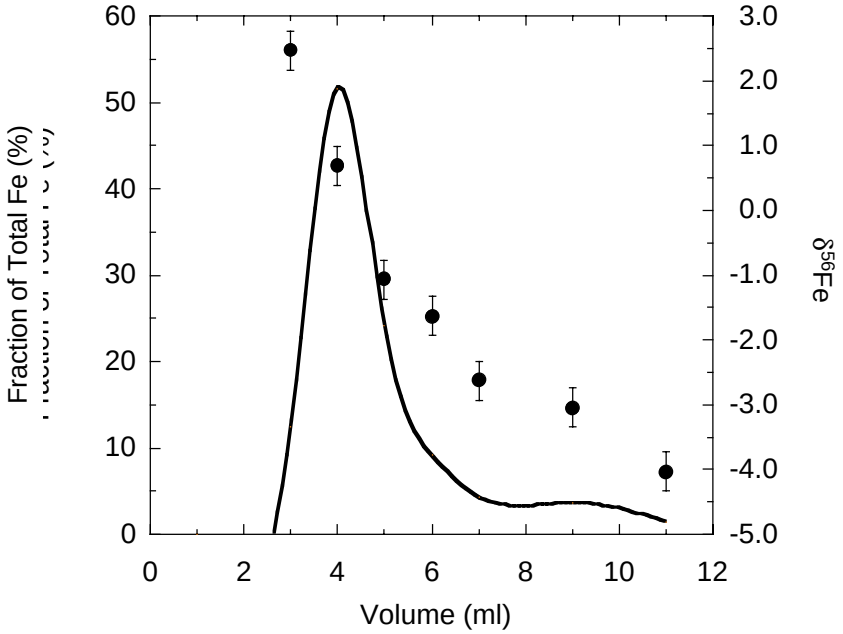
Fe Elution Curve



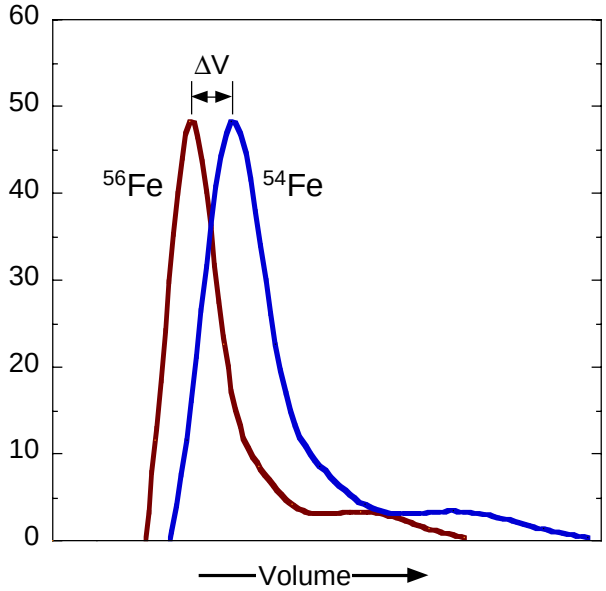
Elution Curve & $\delta^{56}Fe$



Anbar, Roe, Barling & Nealson, *Science* (2000)



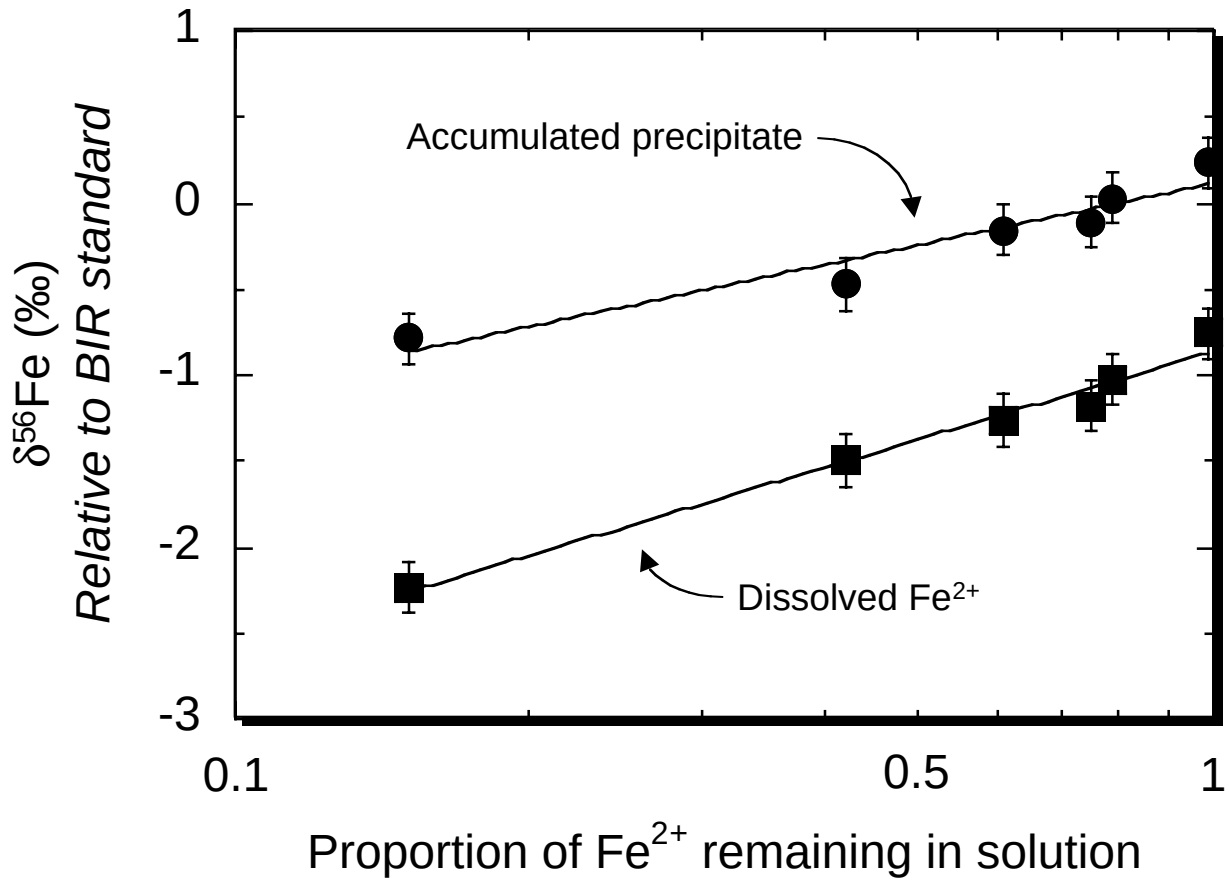
Simplest interpretation:
$1.0001 < {}^{54}K_D/{}^{56}K_D < 1.001$



Evidence of nonbiological effect
But how relevant to nature?

Nonbiological Fractionation

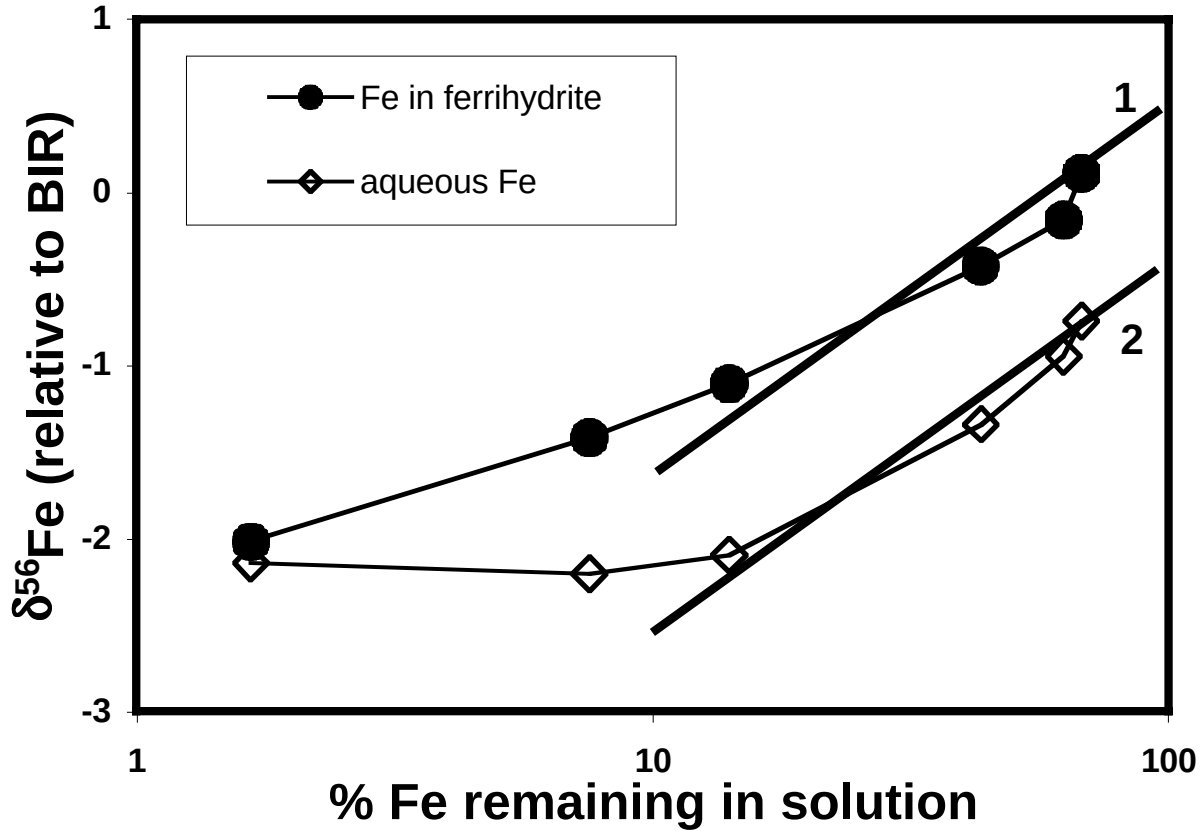
Oxidative precipitation of ferrihydrite



Bullen *et al.*, *Geology* (2001)

Nonbiological Fractionation in Nature?

Fe isotopes in Fe-rich groundwater spring Tongariro National Park (New Zealand)

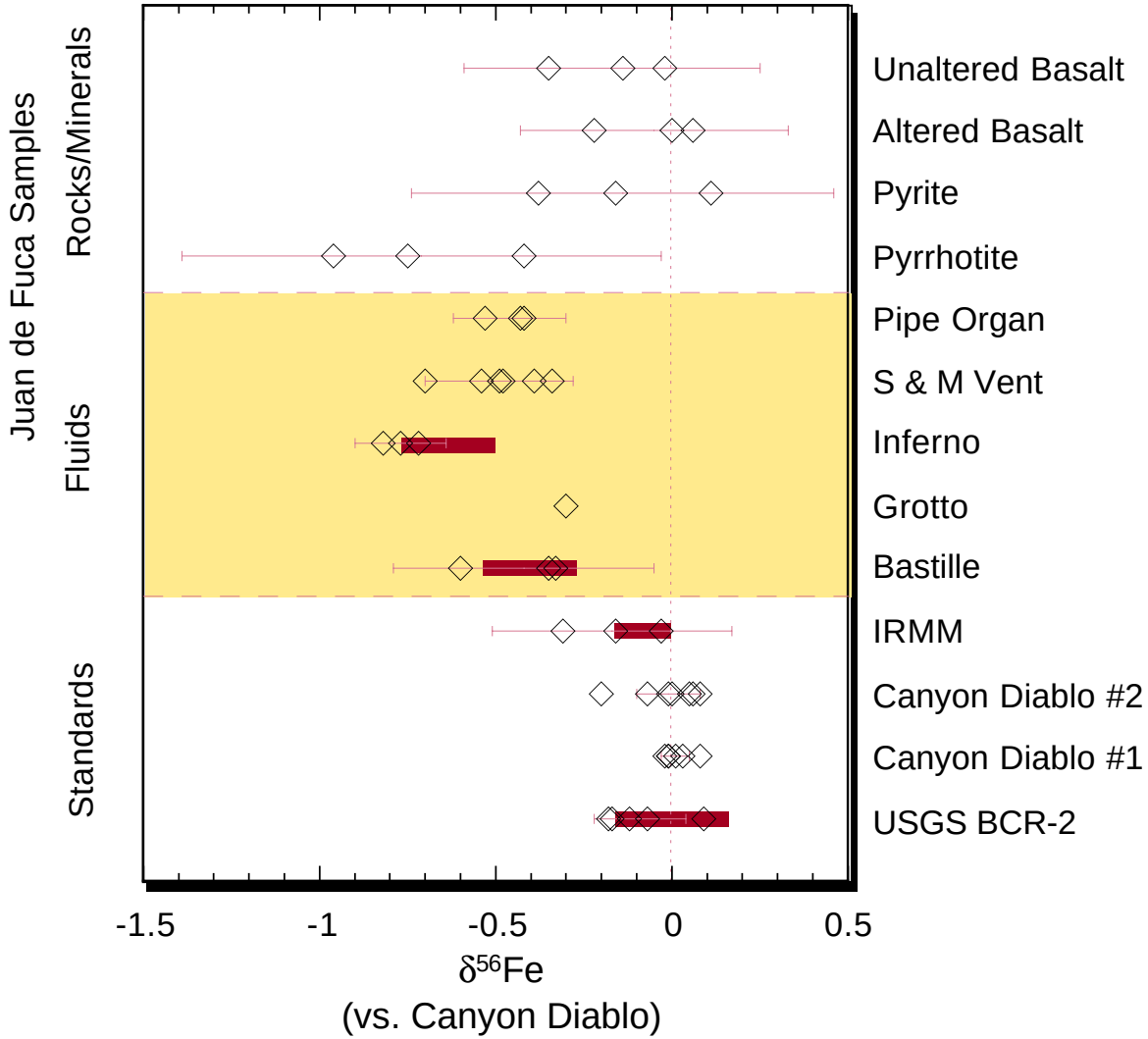


Bullen *et al.*, *Geology* (2001)

Nonbiological Fractionation in Nature?

Fe isotopes in deep sea, high-T hydrothermal fluids

Juan de Fuca Ridge



Sharma, Polizzotto & Anbar, *EPSL* (2001)

Fe Isotope Fractionation…

…occurs during dissimilatory reduction of Fe^{3+}

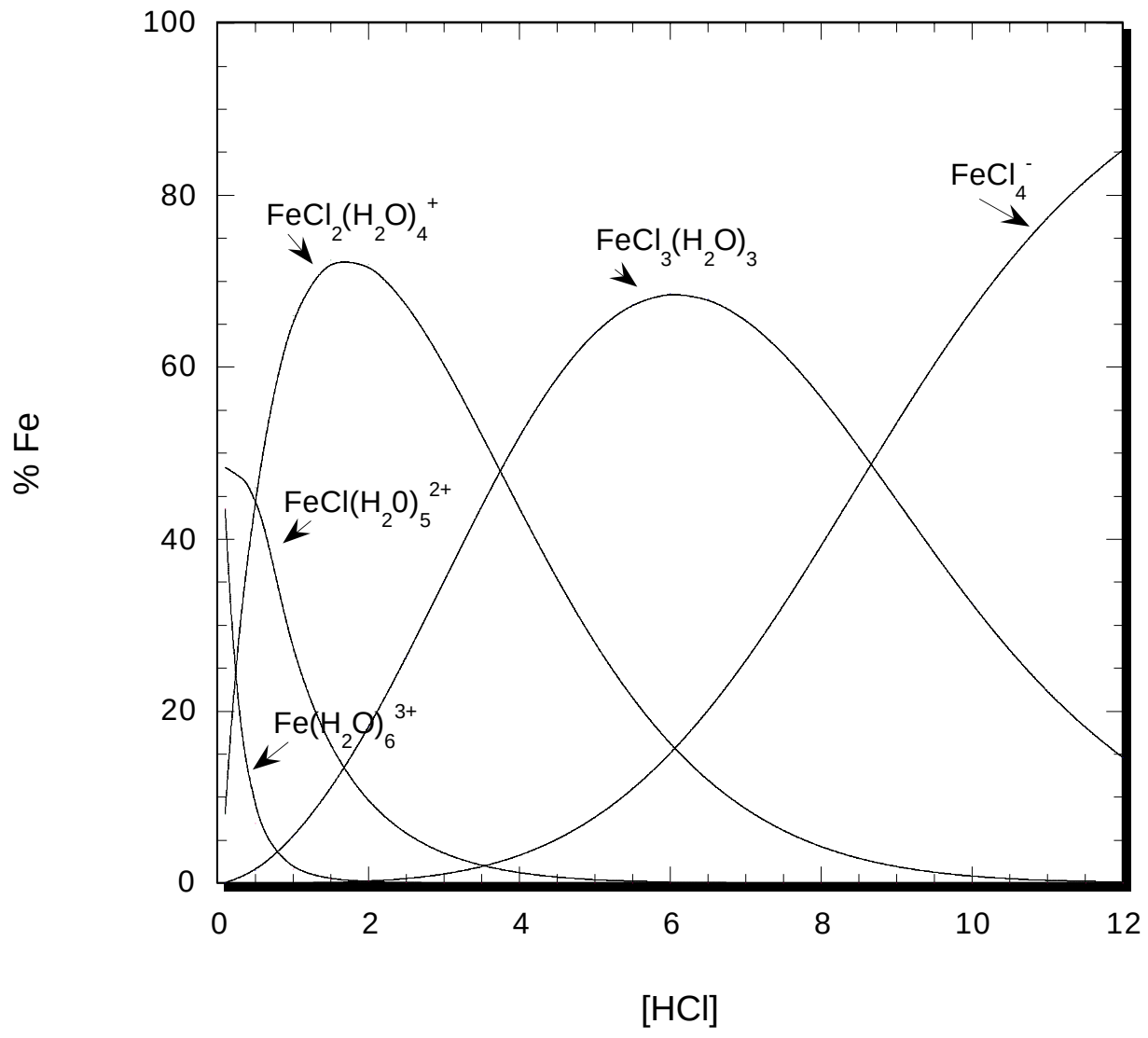
…occurs during nonbiological laboratory chemistry

…apparently occurs nonbiologically in nature

Where do we go from here?

Consider fractionation mechanisms…

Fe Speciation in HCl Experiments



Fractionation may result from changes in Fe coordination:

Species	*Coordination*
$Fe(H_2O)_6^{3+}$	*octahedral*
$FeCl(H_2O)_5^{2+}$	*octahedral*
$FeCl_2(H_2O)_4^{+}$	*octahedral*
$FeCl_3(H_2O)_3^{o}$	*octahedral**
$FeCl_4^{-}$	*tetrahedral*
$FeCl_4$-R	*tetrahedral*

Proposed equilibrium fractionation during exchange reaction:

$$^{54}FeCl_3(H_2O)_3 + {}^{56}FeCl_4^{-} \leftrightarrow {}^{56}FeCl_3(H_2O)_3 + {}^{54}FeCl_4^{-}$$

Fractionation driven by differences in Fe bonding environment

(similar effects are well-known for C and B isotopes)

*Geometry not certain

Anbar, Roe, Barling & Nealson, *Science* (2000)

Calculated Equilibrium Fractionation Factors
Inorganic Fe Complexes

(reported as 1000 ln $\beta(^{56}Fe\text{-}^{54}Fe)$ factors)

	Schauble *et al.* (2001)	This work	Difference
$FeCl_4^-$	7.2	6.9	0.3
$FeCl_4^{2-}$	4.0	3.7	0.3
$FeBr_4^{2-}$	6.4	6.3	0.1
$FeCl_6^{3-}$	3.8	3.7	0.1
$Fe(H_2O)_6^{2+}$	6.2	6.1	0.1
$Fe(H_2O)_6^{3+}$	11.6	11.9	-0.3

"Classical" G/F matrix calculations using the Urey-Bradley force field model and published spectroscopic data.

Recent Experimental Data

Equilibrium Fractionation

Fe(III) vs. Fe(II)

$$^{54}Fe(H_2O)_6^{3+} + {}^{56}Fe(H_2O)_6^{2+} \leftrightarrow {}^{56}Fe(H_2O)_6^{3+} + {}^{54}Fe(H_2O)_6^{2+}$$

$$K_{eq} \equiv \alpha \sim 1.0025$$

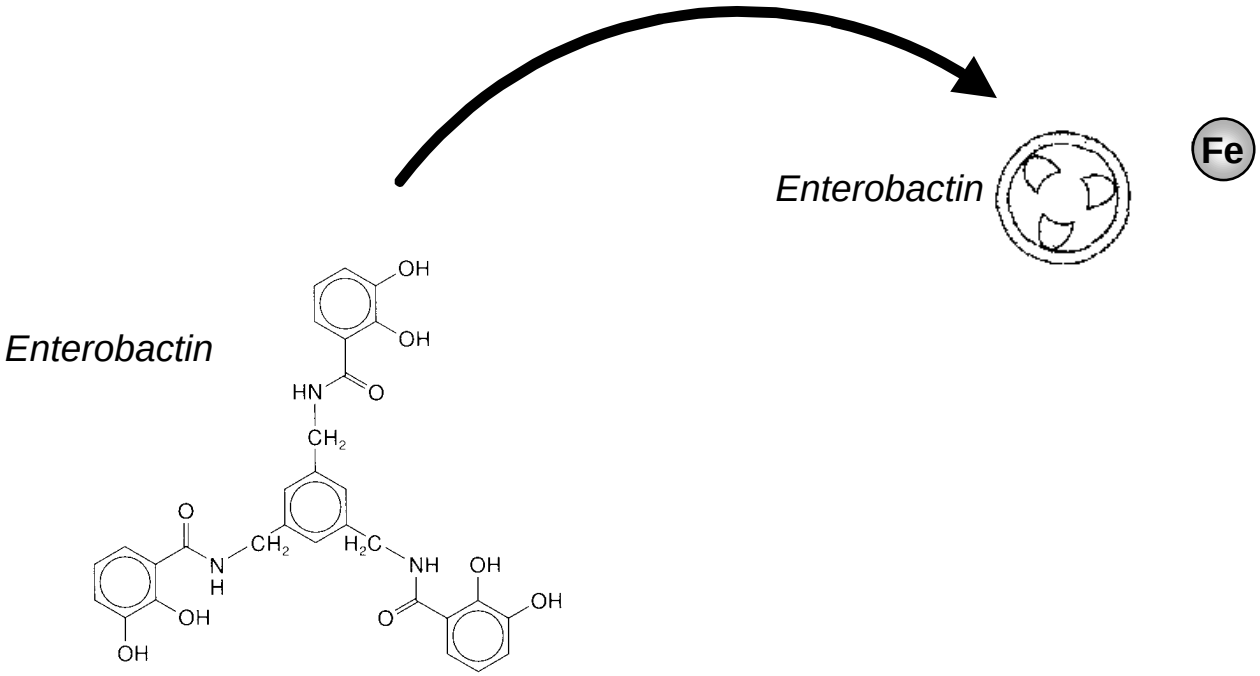
(Compare to theoretical prediction ~ 1.005)

Bottom Line: Changes in bonding environment are key!

Johnson *et al.*, *EPSL* (2001)

Biological Possibilities: Fractionation During Fe Uptake

"Siderophores"



Lippard & Berg, *Intro. to Bioinorganic Chemistry* (1994)

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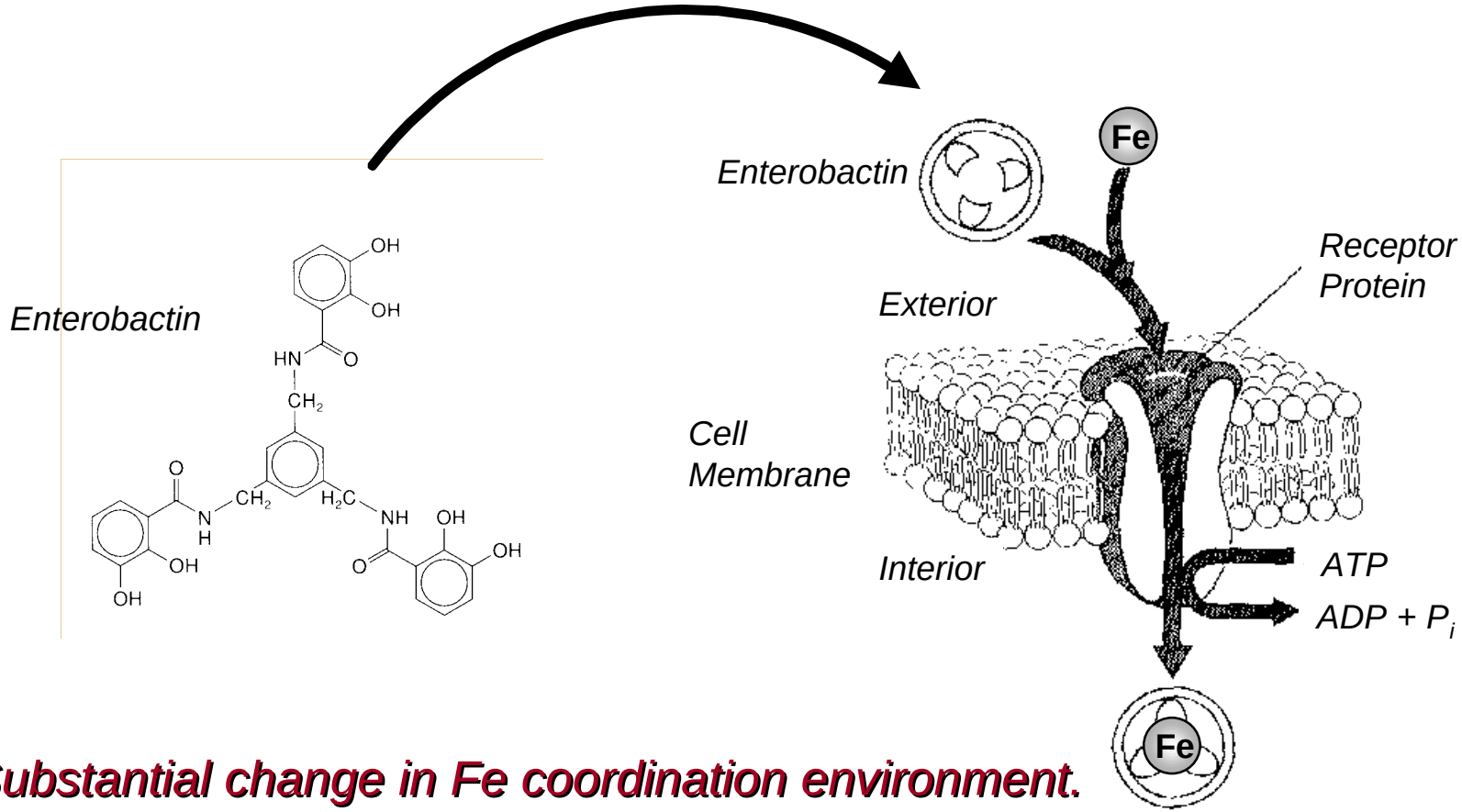


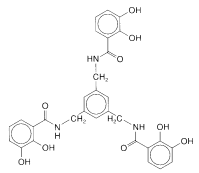
Substantial change in Fe coordination environment. Can we use Fe isotopes to detect the effects of such biologically-produced ligands? Fe isotope biosignatures?

Lippard & Berg, *Intro. to Bioinorganic Chemistry* (1994)

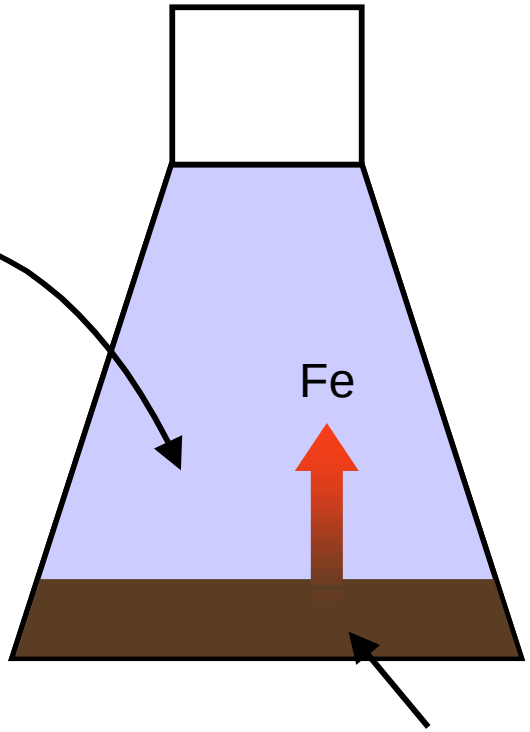
Fe Isotope Fractionation During Mineral Weathering

Ligand

e.g.:



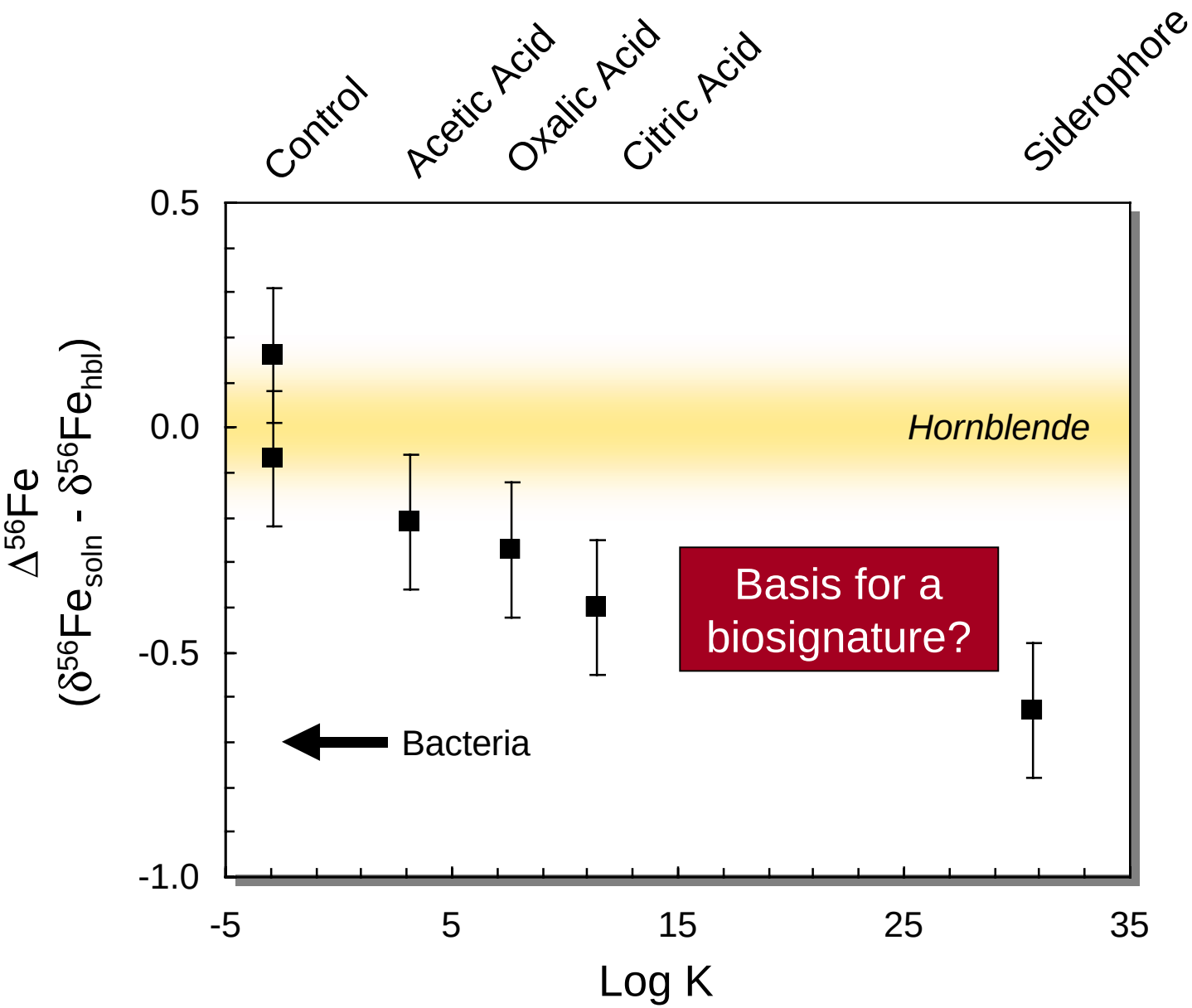
or bacteria
(bacillus, streptomyces)



Measure δ^{56}Fe of dissolved Fe…

Mineral powder
(hornblende)

Modified after R. Guynn et al., AGU '01



Brantley, Liermann & Bullen, *Geology* (2001); Guynn, Anbar, Barling, Liermann & Brantley, GCA *in revision* (2002)