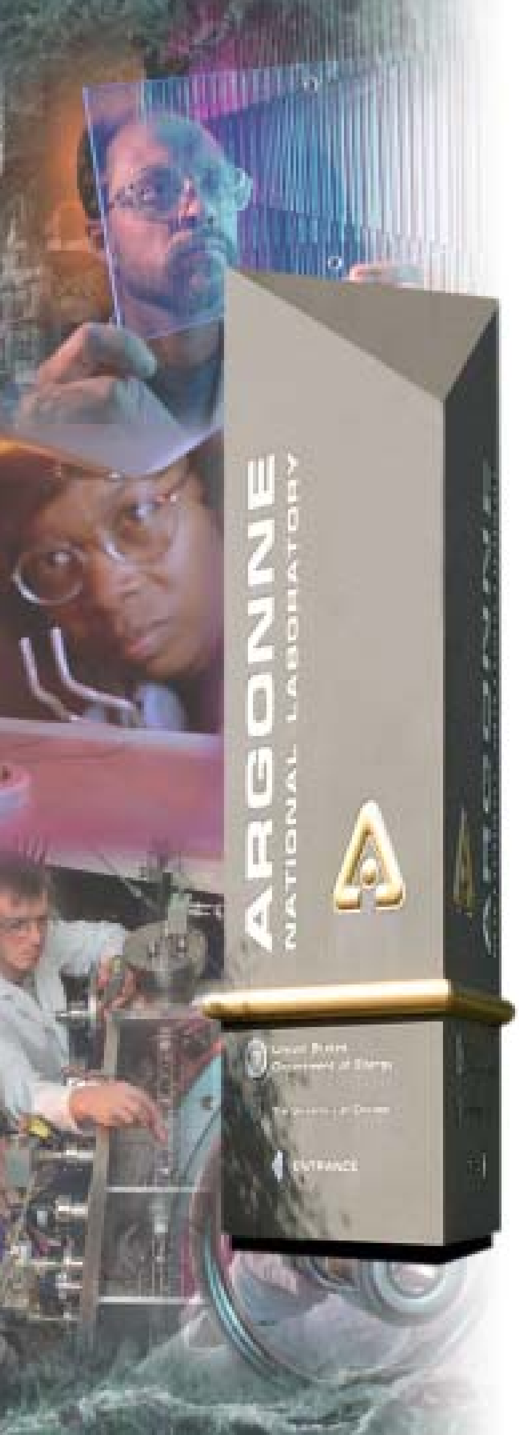


The Advanced Photon Source: How it can Aid in Environmental Studies

Ken Kemner

Molecular Environmental Science Group,
Environmental Research Division

Global Change Education Program Orientation

June 8, 2004

Argonne National Laboratory

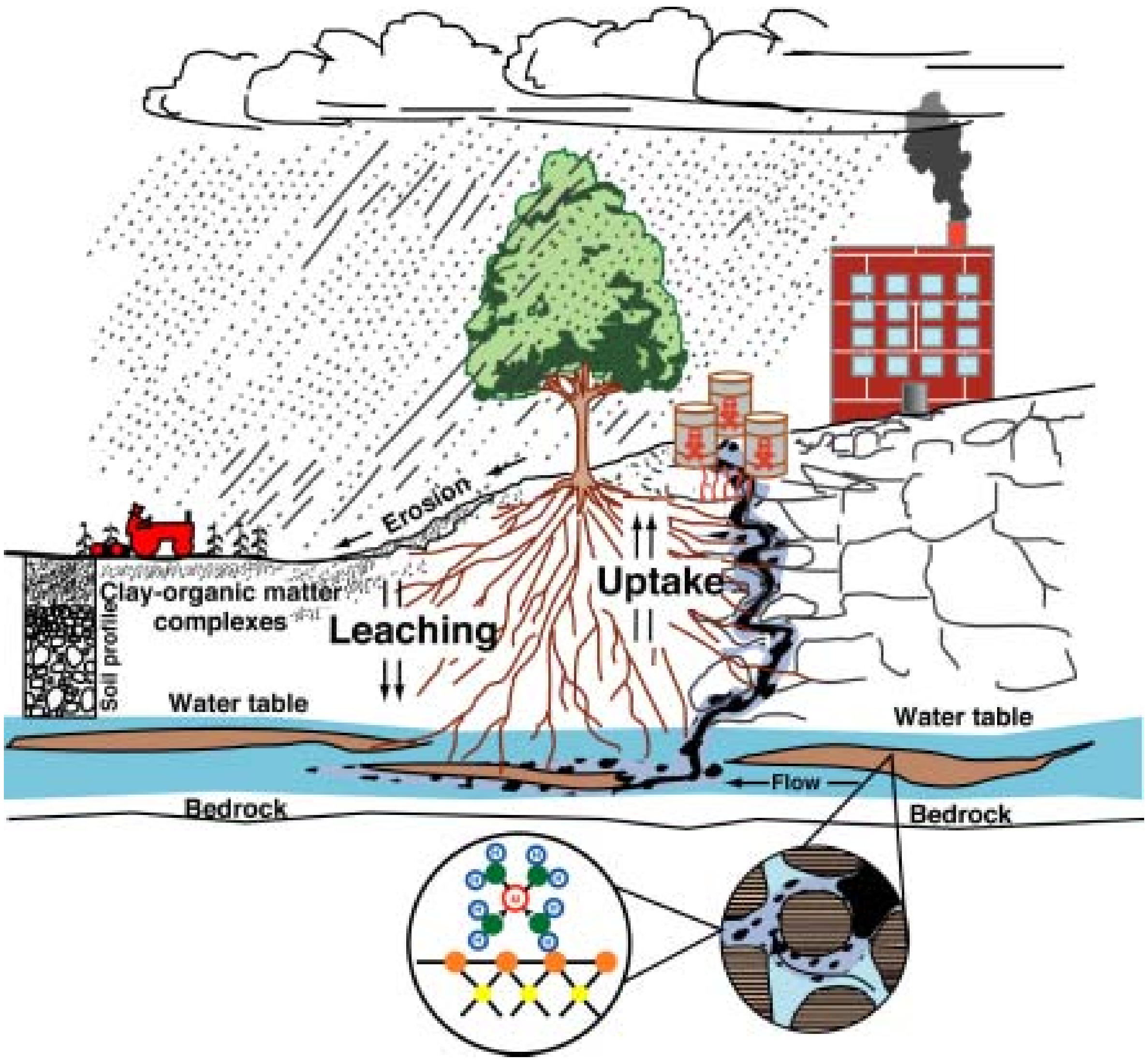
Office of Science
U.S. Department of Energy

A U.S. Department of Energy
Office of Science Laboratory
Operated by The University of Chicago

Outline

- What is Environmental Science?
- Introduction to synchrotron x-ray physics and synchrotron techniques
- Introduction to biogeochemistry
- Examples of the use of hard synchrotron x-rays to investigate biogeochemical systems

What is Environmental Science?



Oceans?
Microbes?

Acknowledgements

- **ANL-Environmental Research Division (Molecular Environmental Science Group)**
  - E. O'Loughlin (Environmental Chemist)
  - S. Kelly (X-ray Physicists)
  - K. Orlandini (Environmental Radiolimnologist)
  - M. Boyanov (X-ray Physicists)
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  - R. Csencsits, R. Cook (Electron Microscopist)
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- **U. of Southern California**
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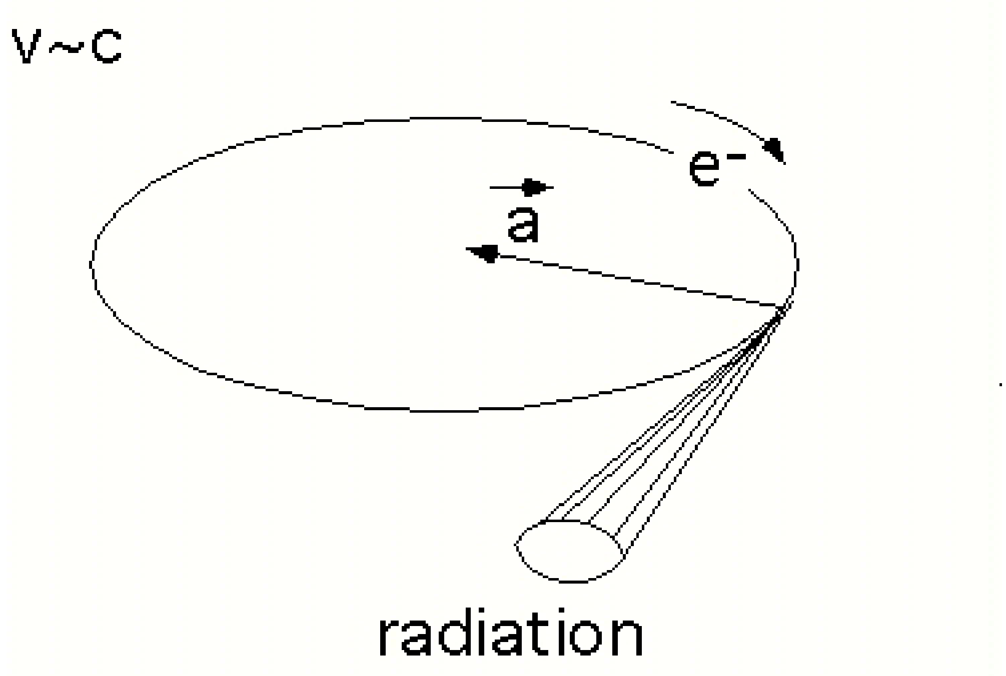
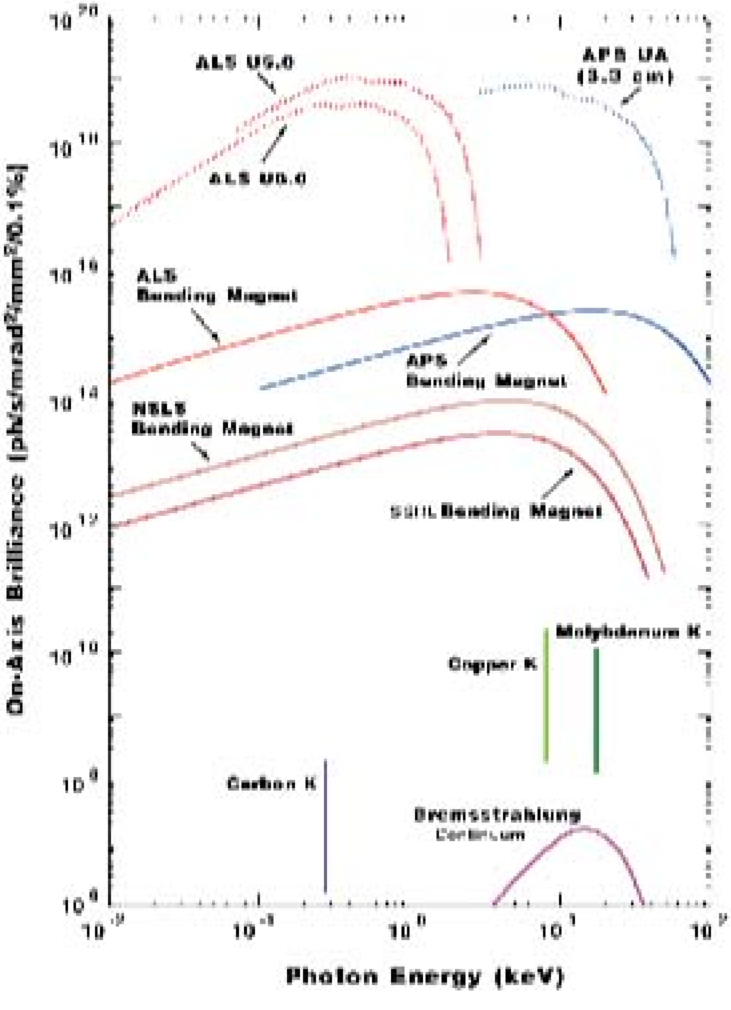
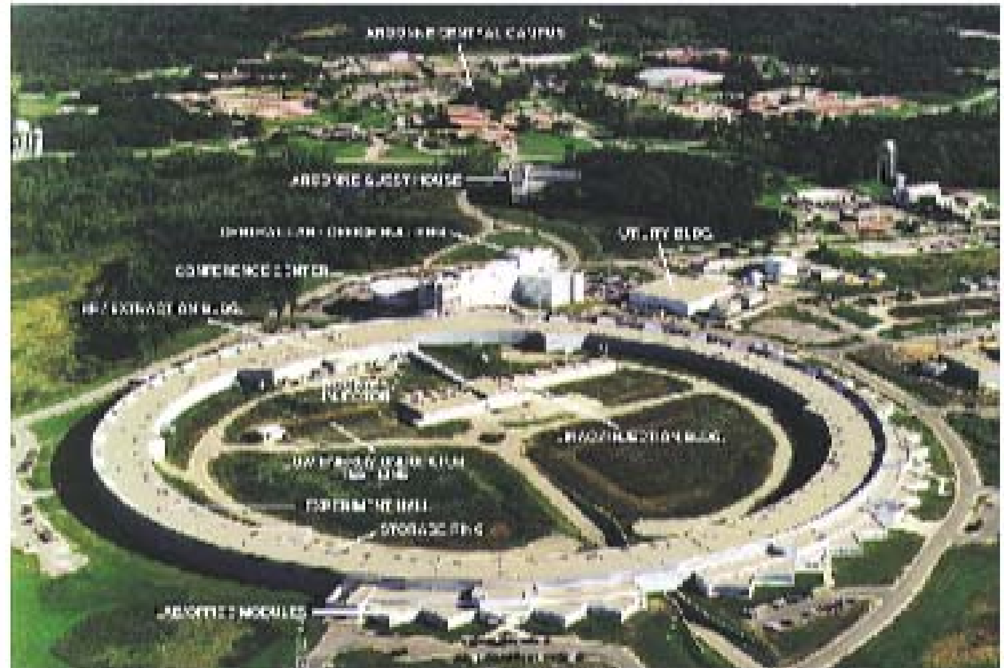
- **U. of California, Berkeley**
  - J. Banfield (Mineralogist, Electron Microscopist, Geomicrobiologist,)
- **U. of Guelph**
  - S. Glasauer, T. Beveridge (Microbiologists, Geomicrobiologists)

“X-ray Physics 101”

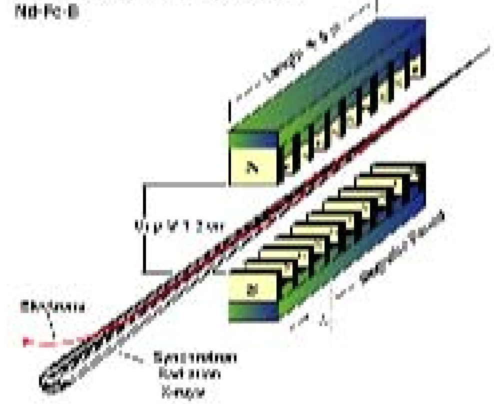


The Advanced Photon Source, Argonne National laboratory





INSERTION DEVICE (WIGGLER OR UNDULATOR)
PERMANENT MAGNETIC MATERIAL
Nd-Fe-B

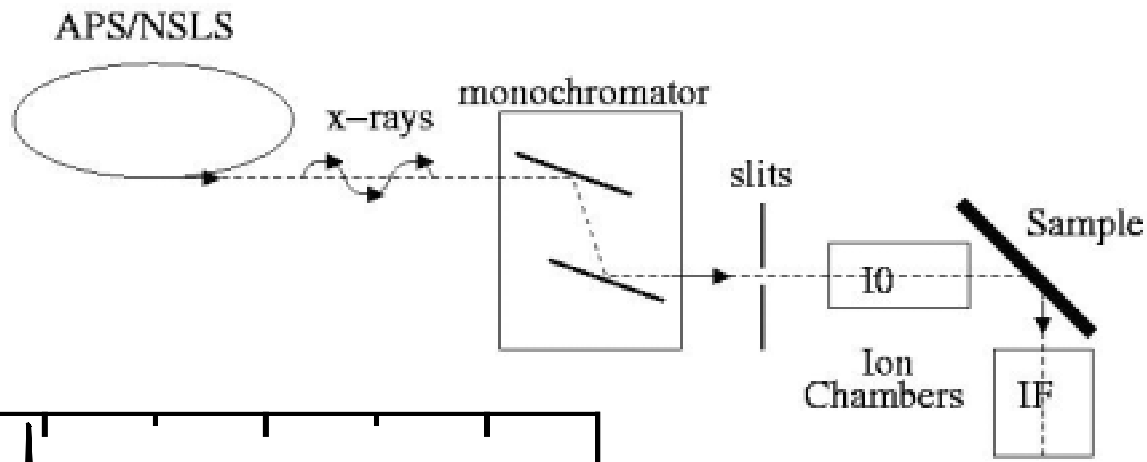


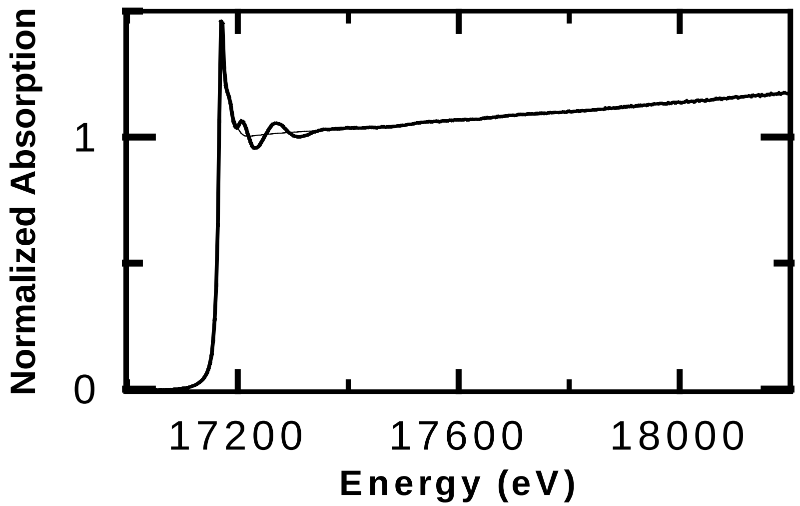
Why use hard x-rays for investigating environmental systems?

- **Hard x-rays (i.e. greater than ~2 keV) interact "weakly" with matter (relative to charge particle probes) and enable the investigation of hydrated and/or buried samples.**
- **Hard x-rays enable highly sensitive elemental analysis on extremely small objects.**
- **High sensitivity of x-rays enables x-ray absorption spectroscopy (i.e. interrogation of chemistry)**

- ***Examples in this presentation will span 9-12 orders of magnitude in length.***

X-ray-Absorption Fine Structure (XAFS)





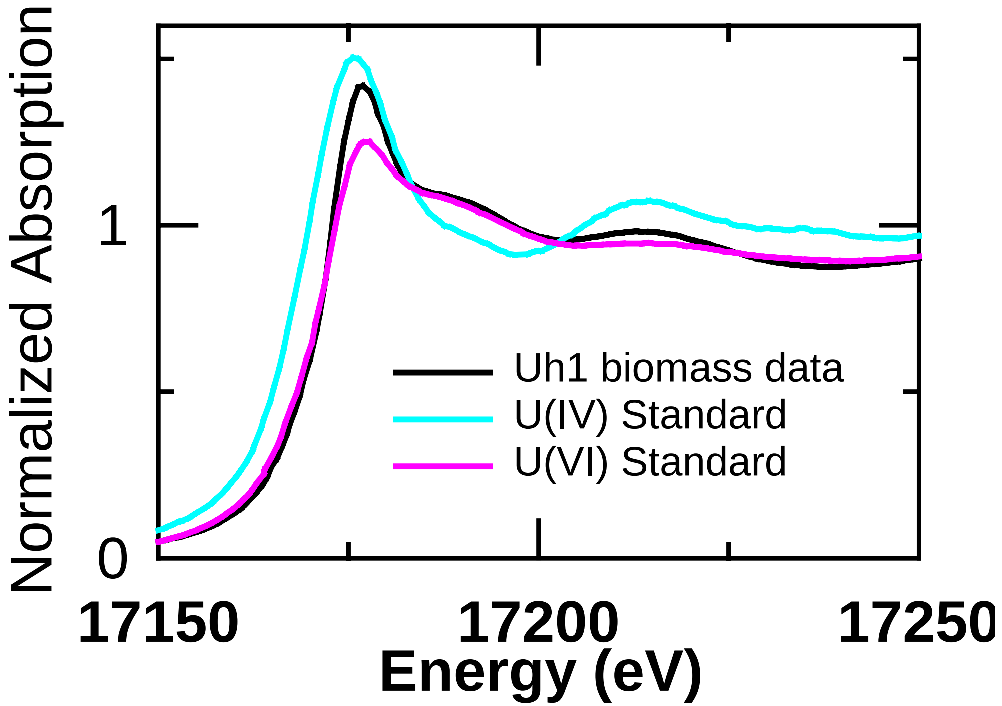
- **Attenuation of x-rays**

  $$I_t = I_0 e^{-\mu(E)\cdot x}$$

- **Absorption coefficient**

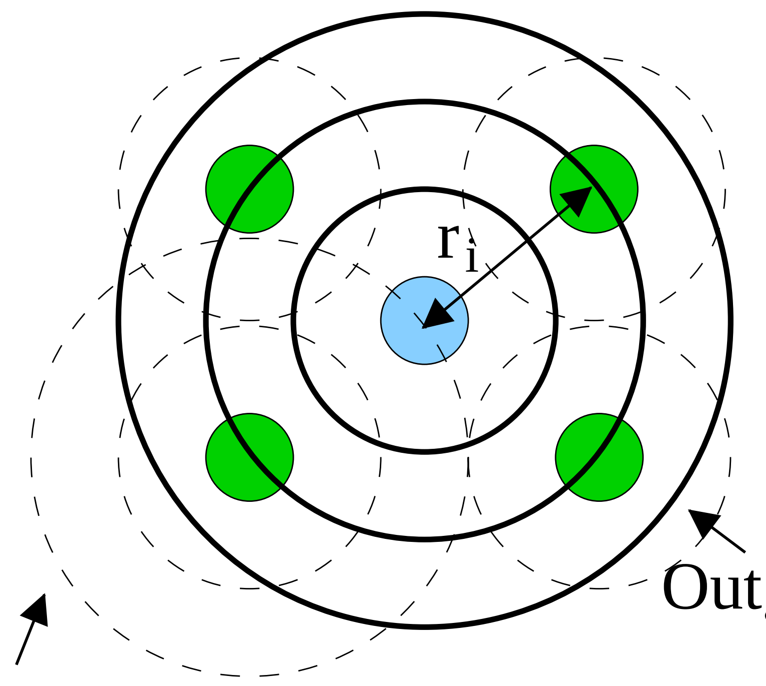
  $$\mu(E) \propto I_f/I_0$$

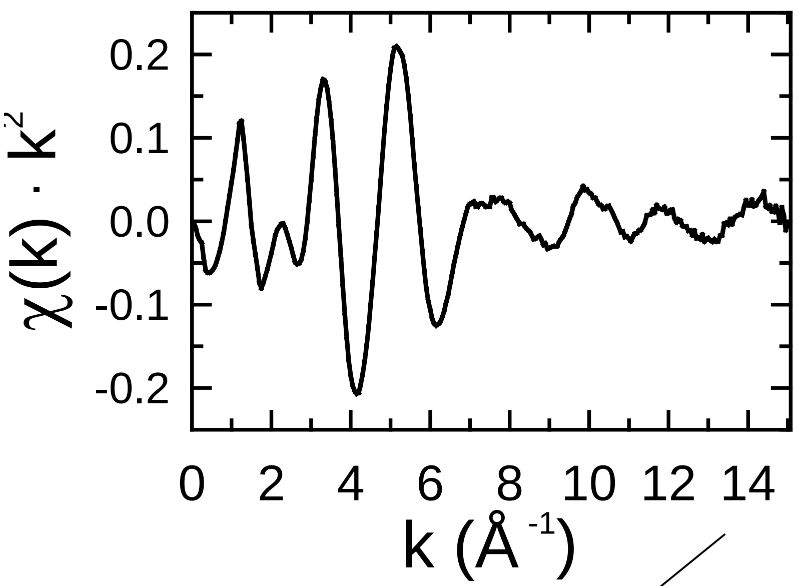
X-ray Absorption Near Edge Structure-(XANES)



- **Position of edge depends on valence state of absorbing atoms**
- U(VI) for All U-biomass samples

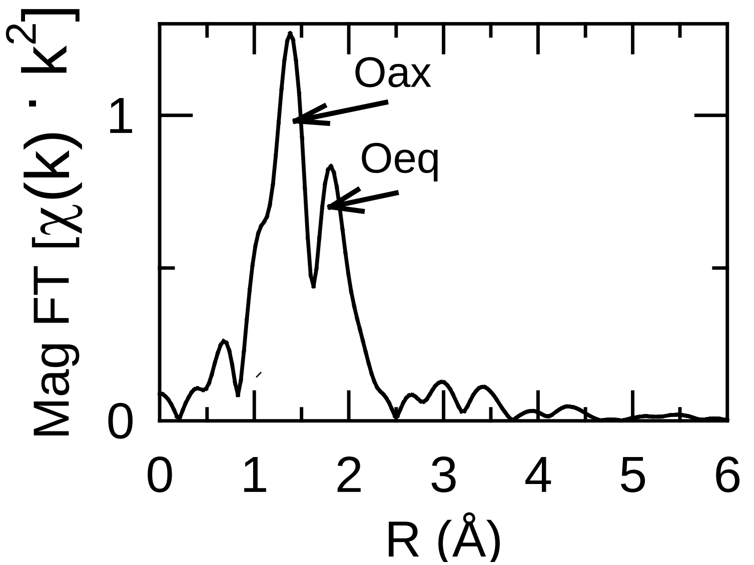
Extended X-ray Absorption Fine Structure-(EXAFS)

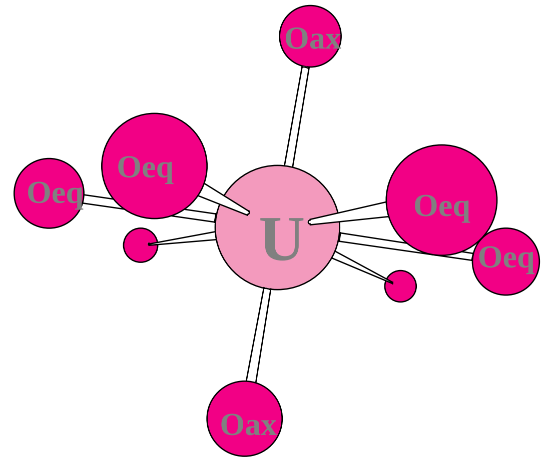




Fourier Analysis

Fourier Transform of $\chi(k)$





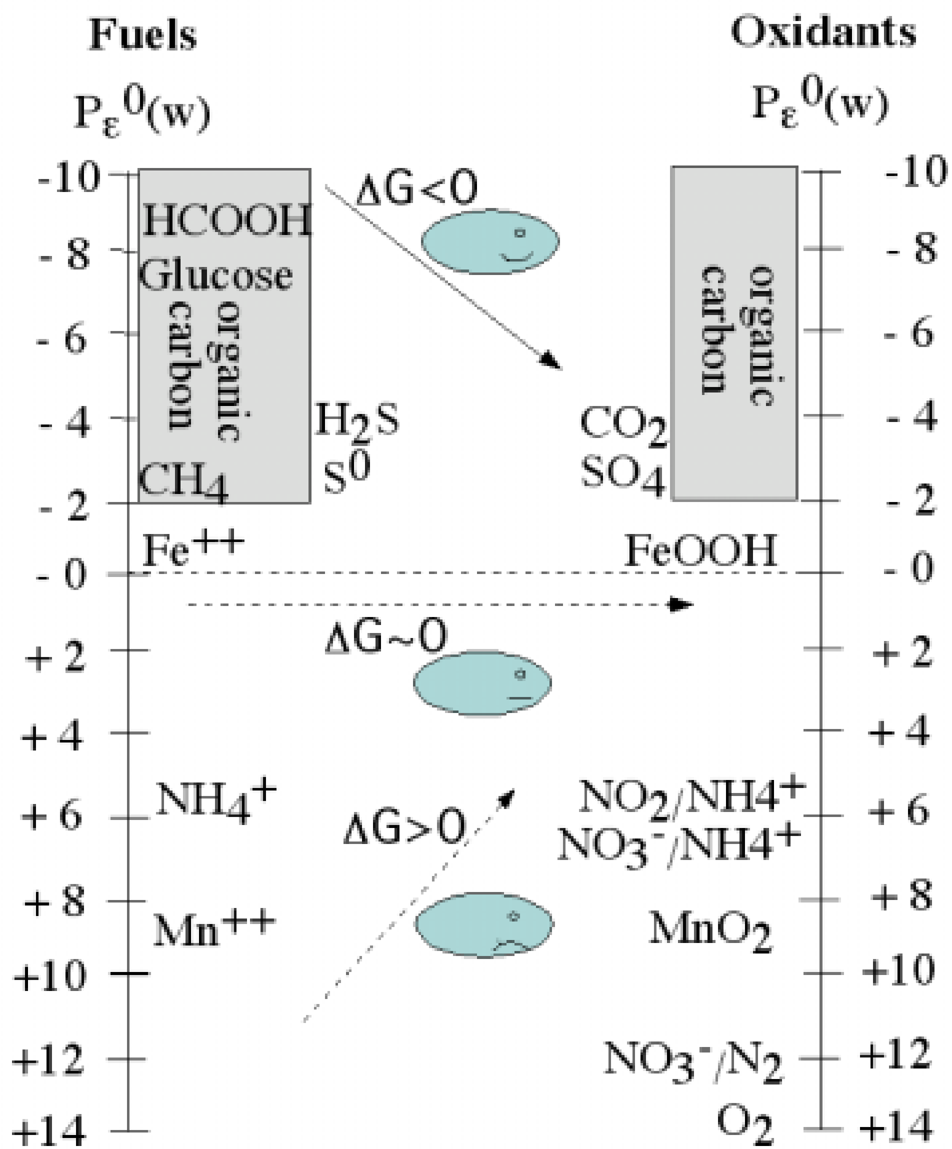
- Like an atomic radial distribution function
  - Distance
  - Number
  - Type
  - Structural disorder

Why are microbes/bacteria important in Environmental Science?

- **Microbes make up ~1% of human biomass but are responsible for ~90% of digestion.**
- **Microbes can transform poisons (heavy metals) into harmless compounds, or repackage them so they are physiologically unavailable (bioremediation).**
- **Microbes degrade organic pollutants, restore key nutrients to depleted soil, or act as a sink for greenhouse gases (CO2), from the atmosphere.**
- **Microbial processes can have a profound effect on major societal issues such as groundwater quality, environmental contamination, the loss of productive agricultural lands, and global warming.**

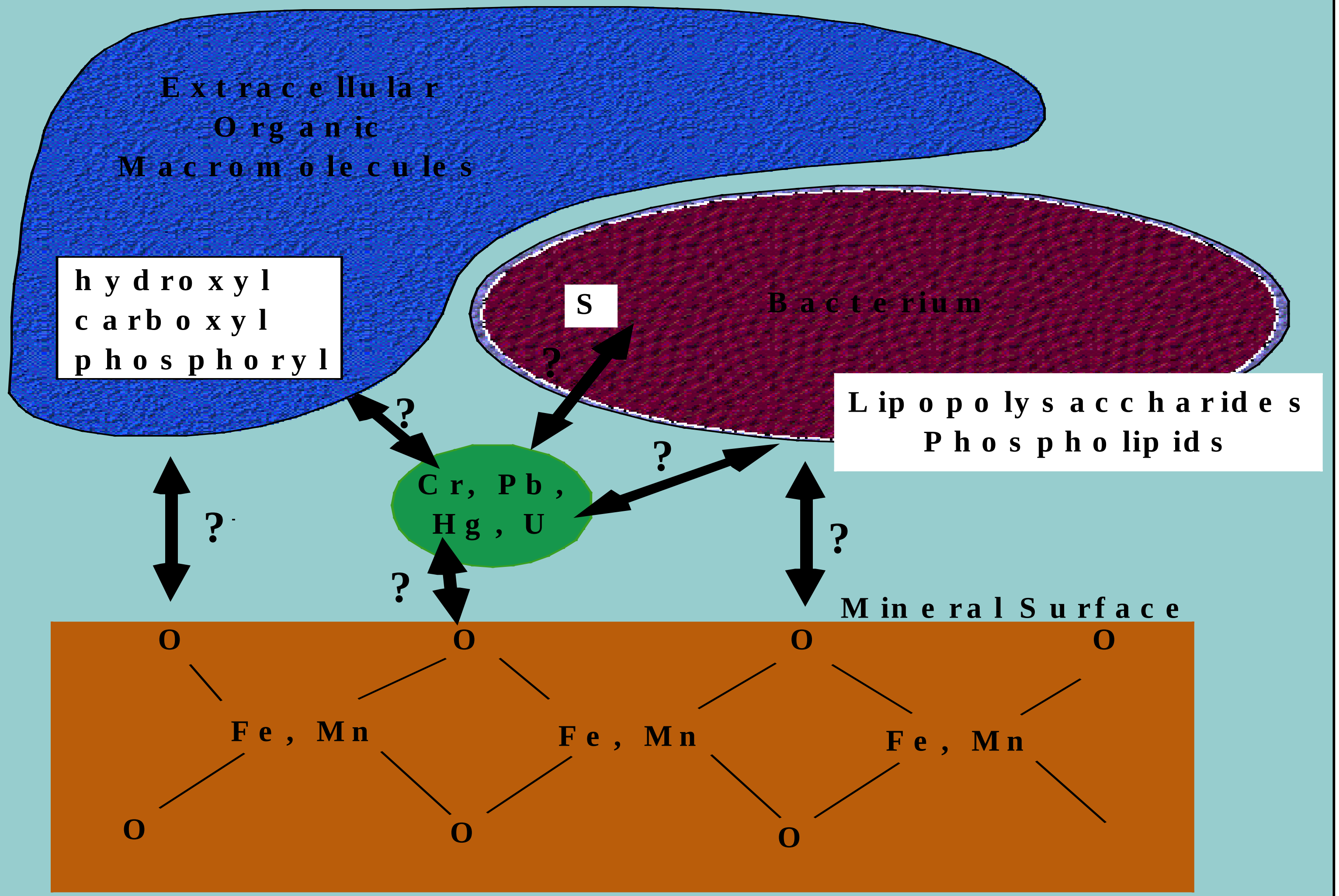
- *** "*Geobiology: Exploring the interface between the biosphere and the geosphere*," American Academy of Microbiology**

Thermodynamics:
The Chemical Fuels and Oxidants of Life



Nealson and Stahl in *Geomicrobiology*, Rev. Min. **35**, 1997.

Investigation of the Spatial Distributions and Transformations of Contaminant Ions at the Bacteria-Geosurface Interface



An oversimplified view of uranium in the subsurface

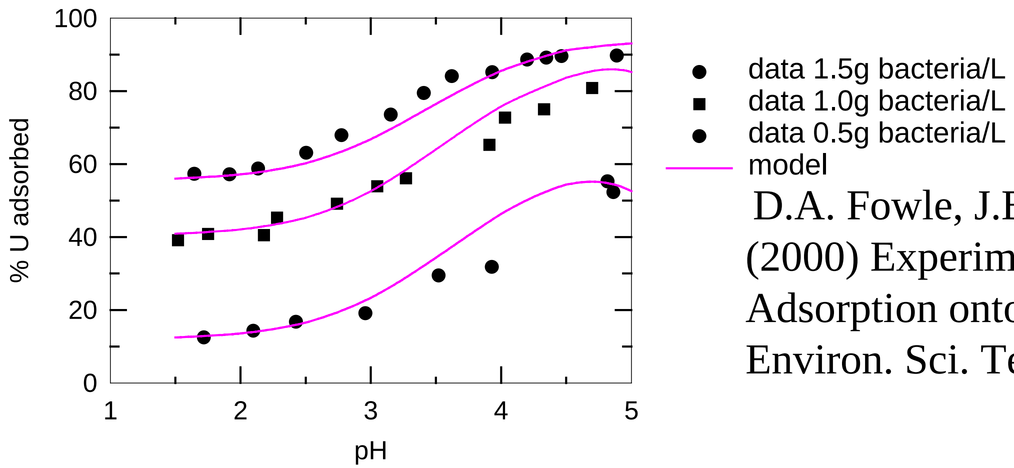
- Laboratory and field studies have demonstrated that bacterial cell walls and mineral surfaces efficiently adsorb a variety of aqueous metal cations like uranium.

- Because bacteria and minerals are abundant in near-surface geologic systems, adsorption reactions to these constituents may significantly affect the mobility of metals in aqueous systems.

- The extent of adsorption of aqueous metals onto bacterial and mineral surfaces can vary markedly with changing conditions such as pH, ionic strength, and fluid composition.

- Changes to the oxidation state of uranium [i.e. from U(VI) to U(IV)] can drastically reduce its solubility and hence its mobility.

Uranium Adsorption to *B. subtilis*



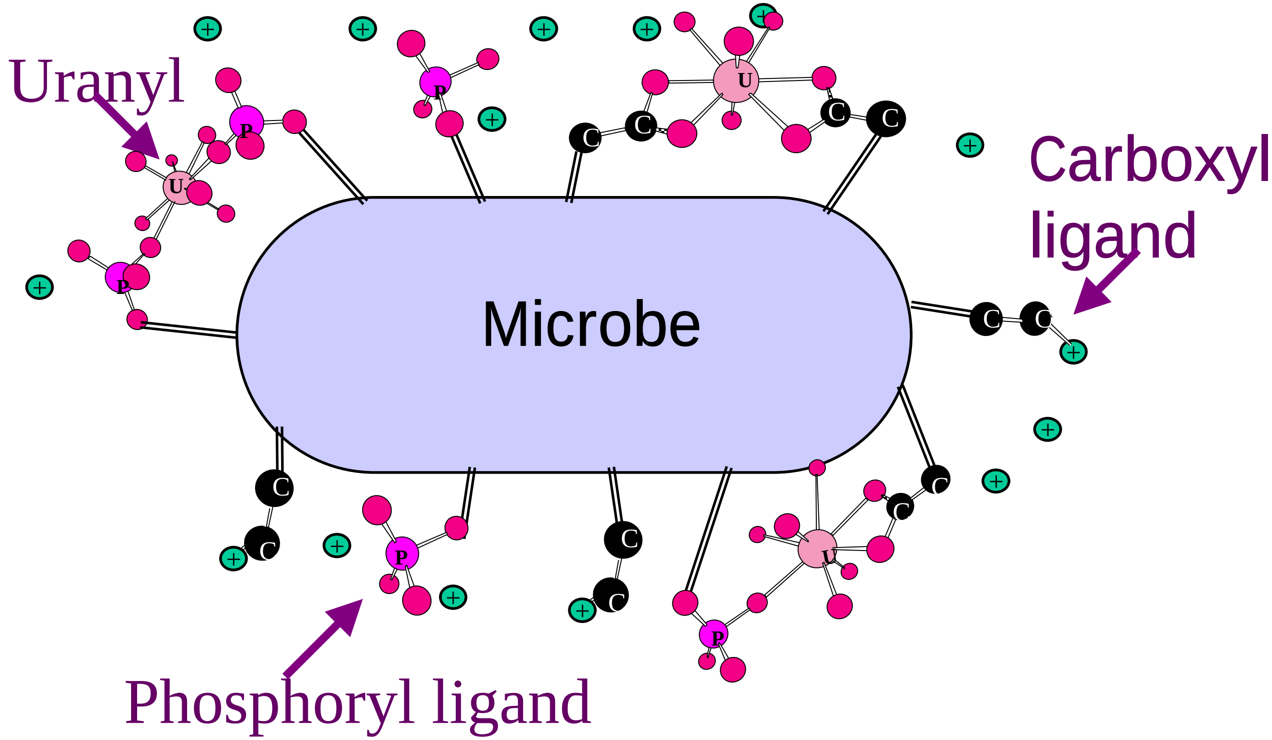
D.A. Fowle, J.B. Fein, and A.M. Martin (2000) Experimental Study of Uranyl Adsorption onto *Bacillus subtilis*. Environ. Sci. Technol. **34**(17), 3737.

- The surface complexation model is used to quantify U adsorption
  - acid/base titrations determine acidity constants of functional groups
  - metal adsorption experiments yield site-specific stability constants
- These batch adsorption measurements, provide only circumstantial evidence regarding the mechanism of adsorption and the stoichiometry of the adsorption reaction.
- Successful application of a surface complexation model requires detailed understanding of the binding mechanism.

provided directly by XAFS spectroscopy

Bacterial Cell Wall

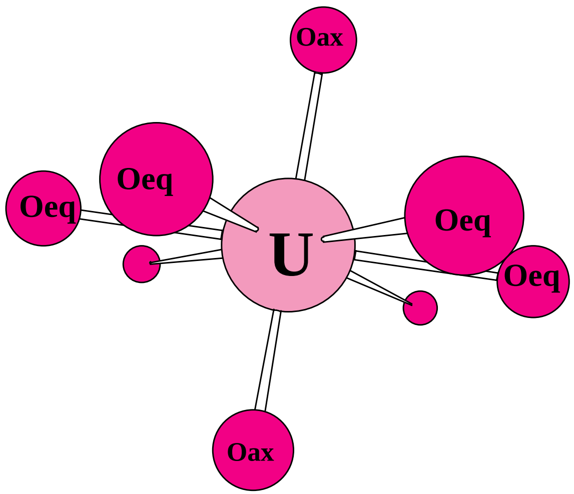
Bacterial cell walls display pH dependent charging and acid-base characteristics.



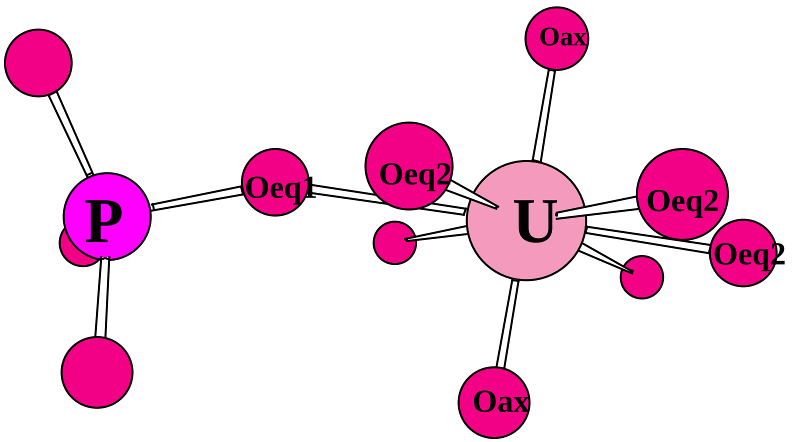
Cd, Cu, Pb, Co Ni. Zn and Sr have negligible adsorption by *Bacillus subtilis* Under low-pH conditions, however above pH 3.0 adsorption increases with increasing pH as the surface functional groups successively deprotonate.

Models for U-biomass data

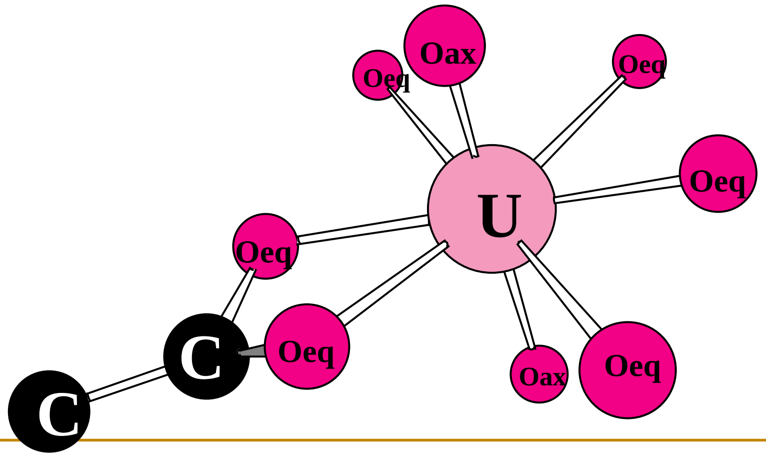
Hydrated Uranyl → hydroxyl



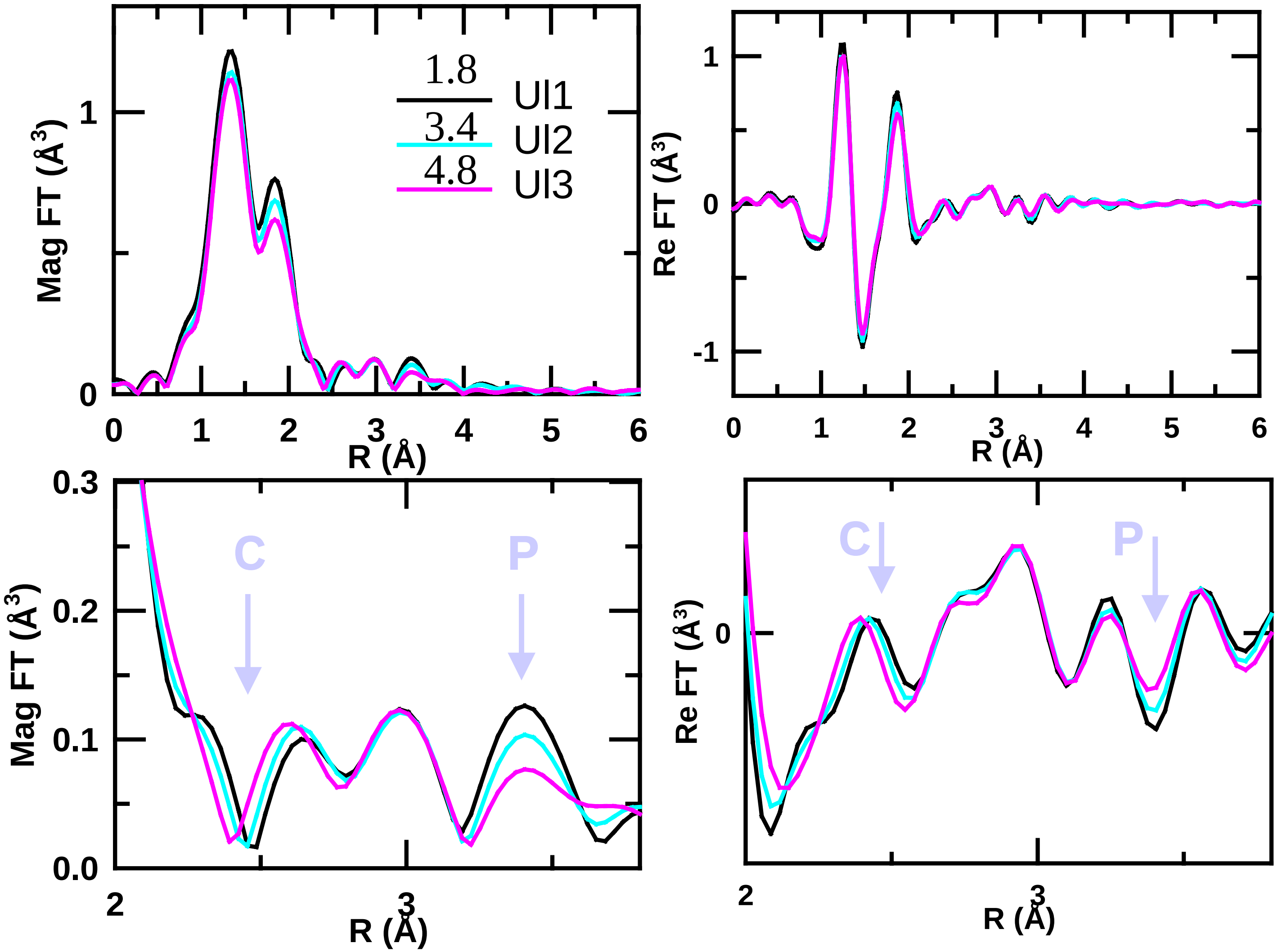
Uranyl Phosphate → phosphoryl



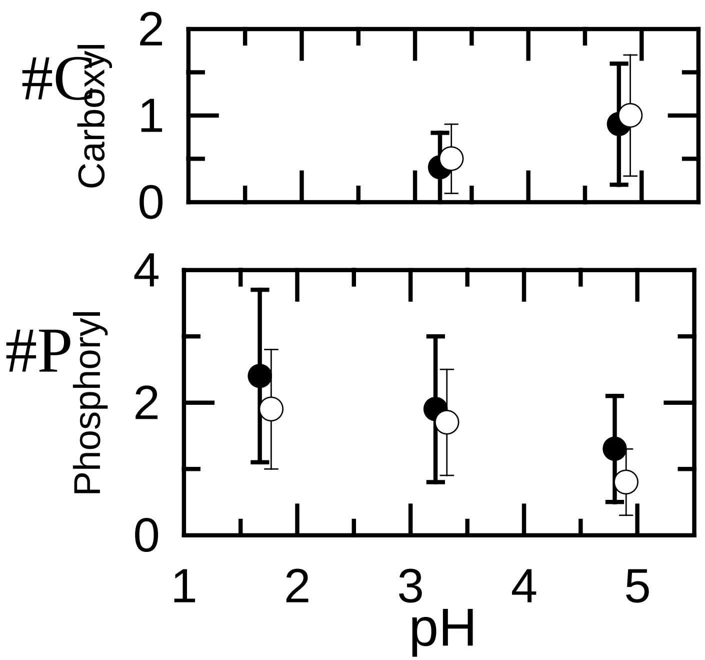
Uranyl Acetate → carboxyl



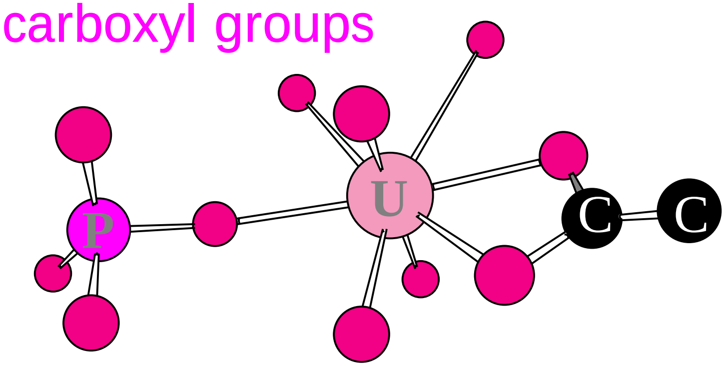
Comparison of U-Biomass Data



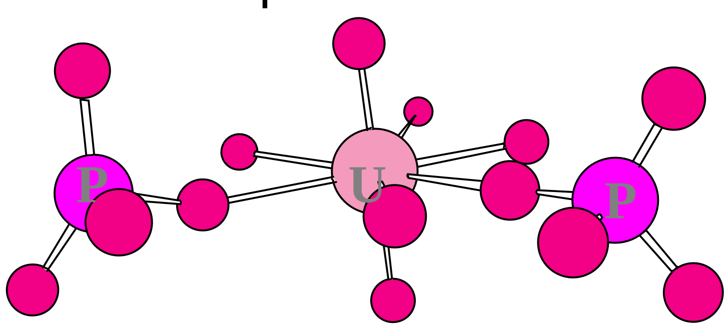
Adsorption Sites for U(VI)

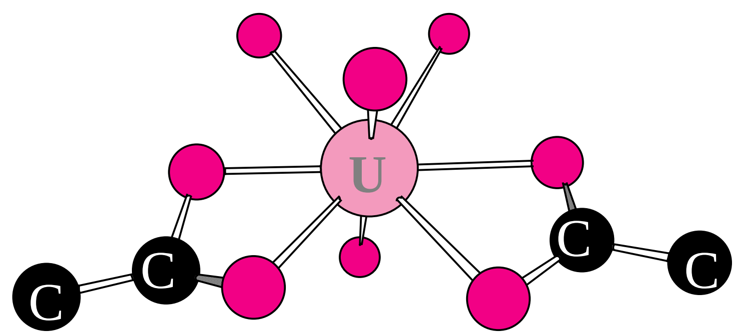


Some mixing of phosphoryl and carboxyl groups



Or 50% of Uranyl with 2 phosphoryl groups and 50% of Uranyl with 2 carboxyl groups



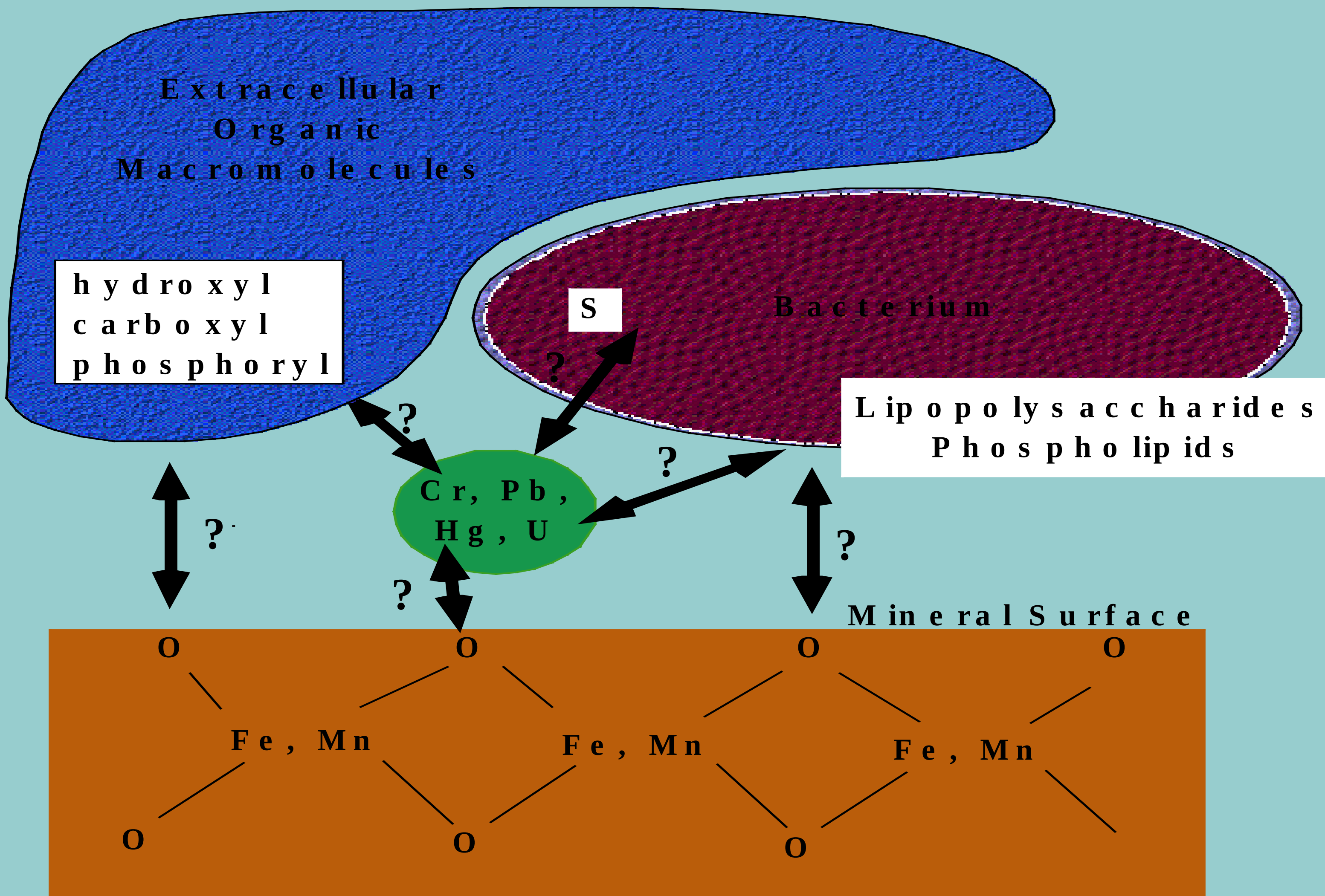


Conclusions

- XAFS Results
  - U-biomass data clearly indicates U(VI) added to the biomass samples was not reduced.
  - Uranyl adsorbs primarily to phosphoryl functional groups at the lowest pH value (1.67).
  - An increase in uranyl adsorption to carboxyl functional groups with increasing pH (3.20 and 4.80).
- The XAFS results are consistent with the surface complexation models proposed by Fein *et al.* and Fowle *et al.*
- These results demonstrate the complementary roles of XAFS spectroscopy and bulk adsorption measurements in determining metal distribution behaviors in the environment.

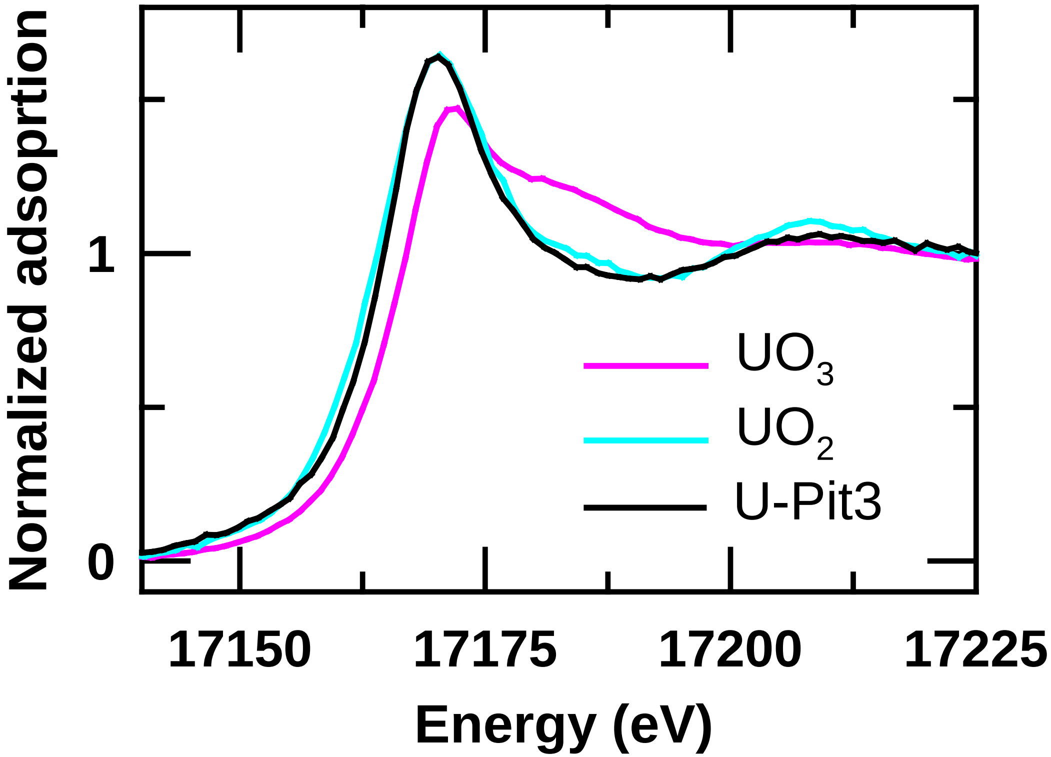
Fein, *et. al*., Geochim. Cosmochim. Acta, 1997, **61** 33.19
Fowle, *et. al*., Environ. Sci. Technol., 2000, **34**, 3737.
Kelly *et al.,* Geochim. Cosmochim. Acta, 2002, **66** 3855.

Investigation of the Spatial Distributions and Transformations of Contaminant Ions at the Bacteria-Geosurface Interface



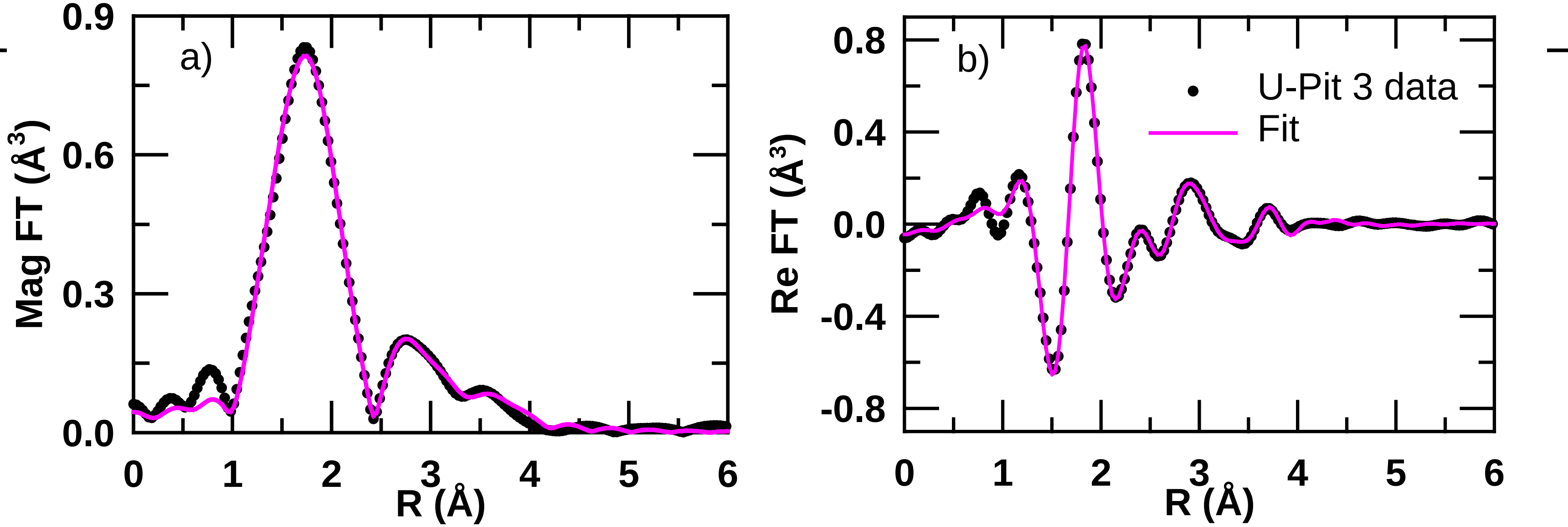
Midnight Mine

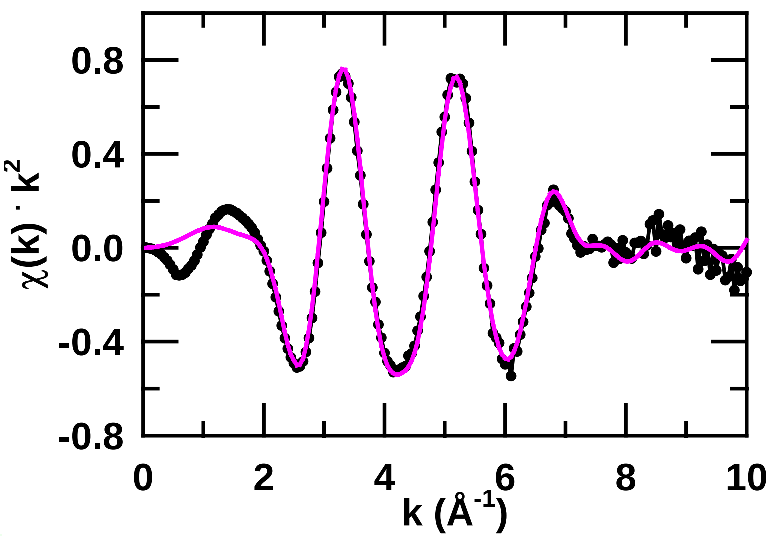
U XANES for U-Pit 3 sediment



- Mostly U(IV) for U-Pit 3 sediment

U EXAFS for U-Pit 3 sediment

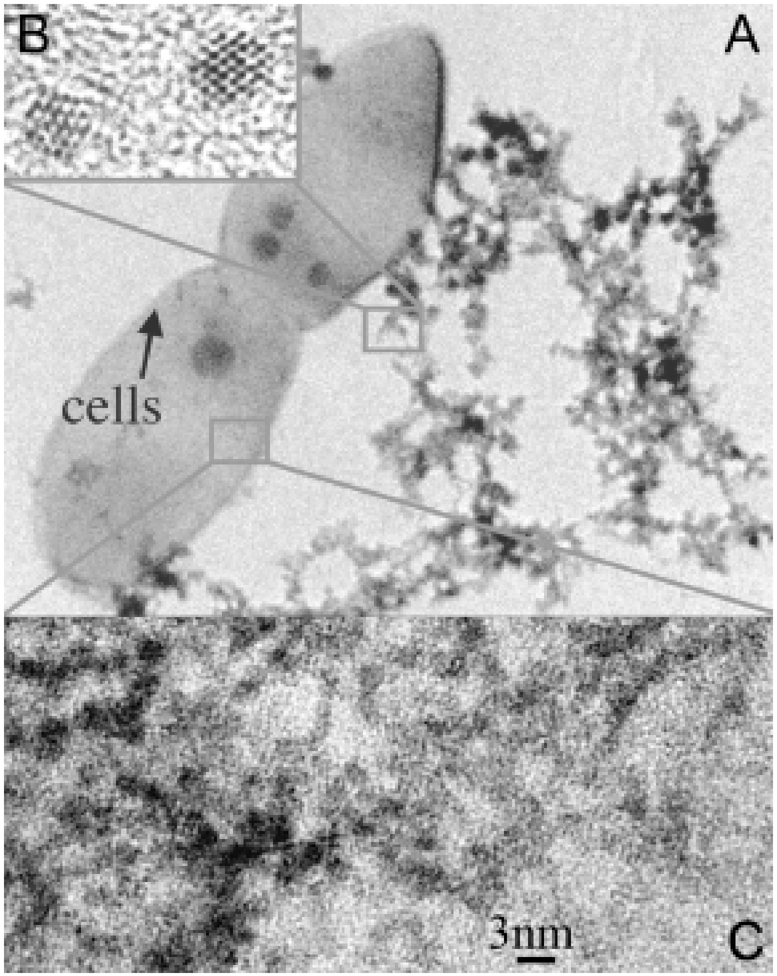




Path	N_{degen}	Distance (Å)	σ^2 ($\times 10^{-3}$ Å^{-2})
U→Oax	0.5 ± 0.1	1.77 ± 0.01	2 ± 1
U→O1	7.4 ± 1.0	2.34 ± 0.01	12 ± 1
U→C	2.6 ± 0.8	2.92 ± 0.01	1 ± 4
U→O2	7.0 ± 5.7	3.85 ± 0.05	19 ± 15
U→U	5.6 ± 4.0	3.80 ± 0.02	19 ± 10

- 5.6 U neighbors → ~ 2.2-1.3 nanometer particles

TEM Image of UO_2 Nanoparticles

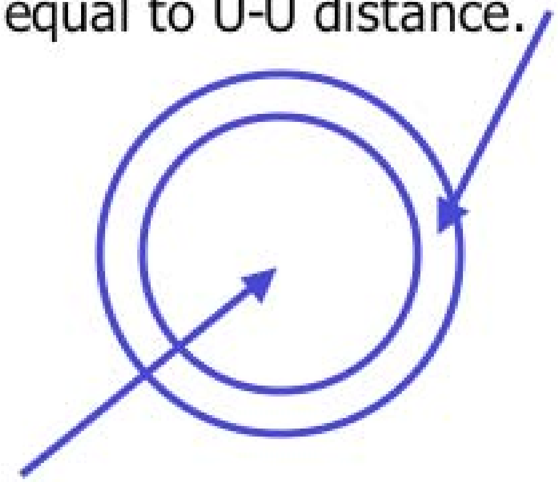


A. *Desulfosporosinus sp.* isolate and associated flocculated UO_2 nanoparticles
B. High-resolutions lattice fringe images of individual particles
C. Cell surface coated with ~ 1.5-2.5 nm diameter UO_2 nanoparticles

J.F. Banfield and Y. Suzuki
Department of Geology and Geophysics
University of Wisconsin-Madison

Particle size from number of near neighbors

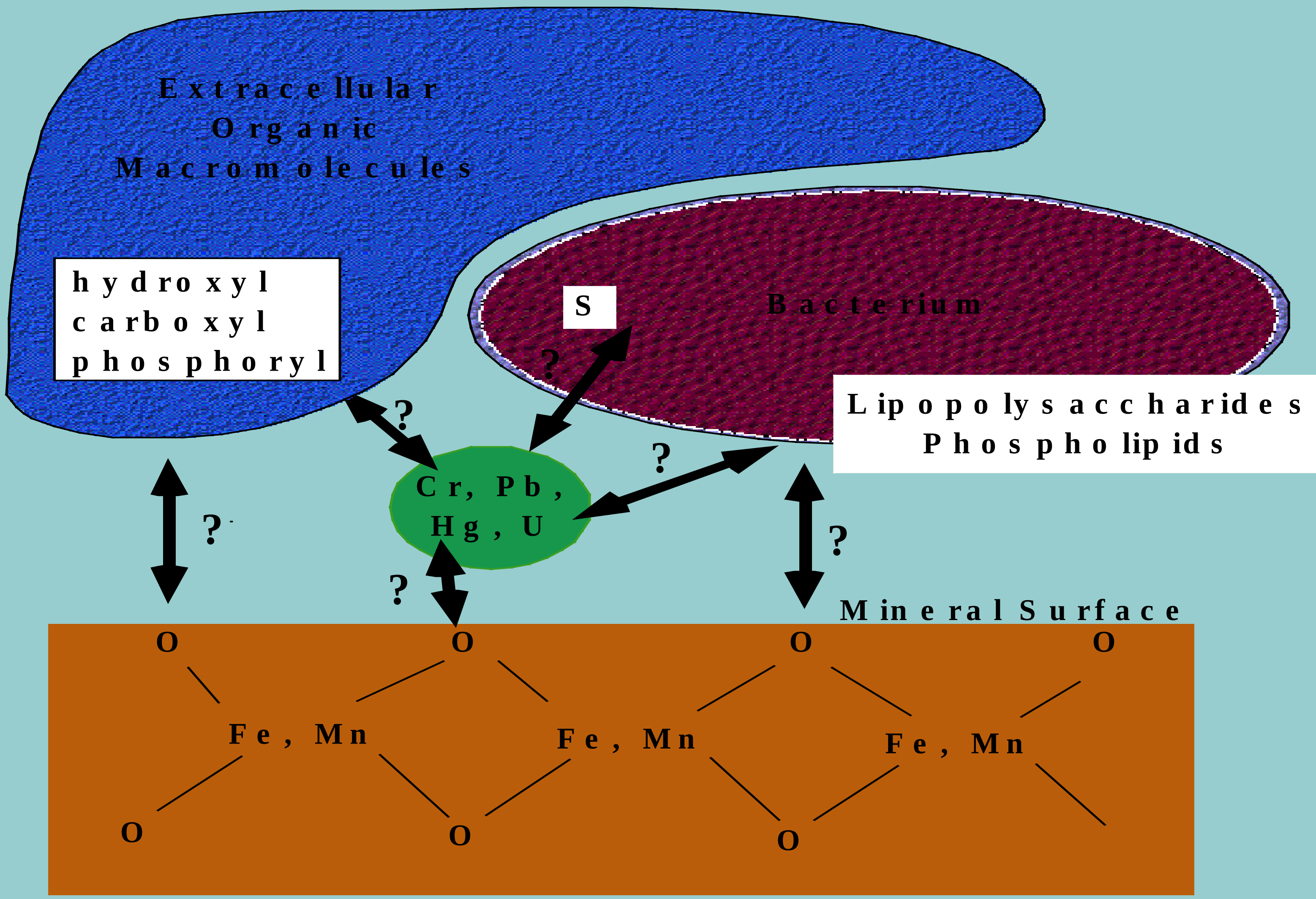
Surface volume depth is equal to U-U distance.



In the interior the of particle, each U has 12 neighboring U atoms.

Assume that the particle is a sphere of uniform U density given by the XAFS result for the U-U distance, with one layer of surface U atoms with 4-8 neighbors. Then the average number of neighboring U atoms is equal to the percent of interior volume multiplied by 12 plus the percent of surface volume multiplied by 4-8.

Investigation of the Spatial Distributions and Transformations of Contaminant Ions at the Bacteria-Geosurface Interface



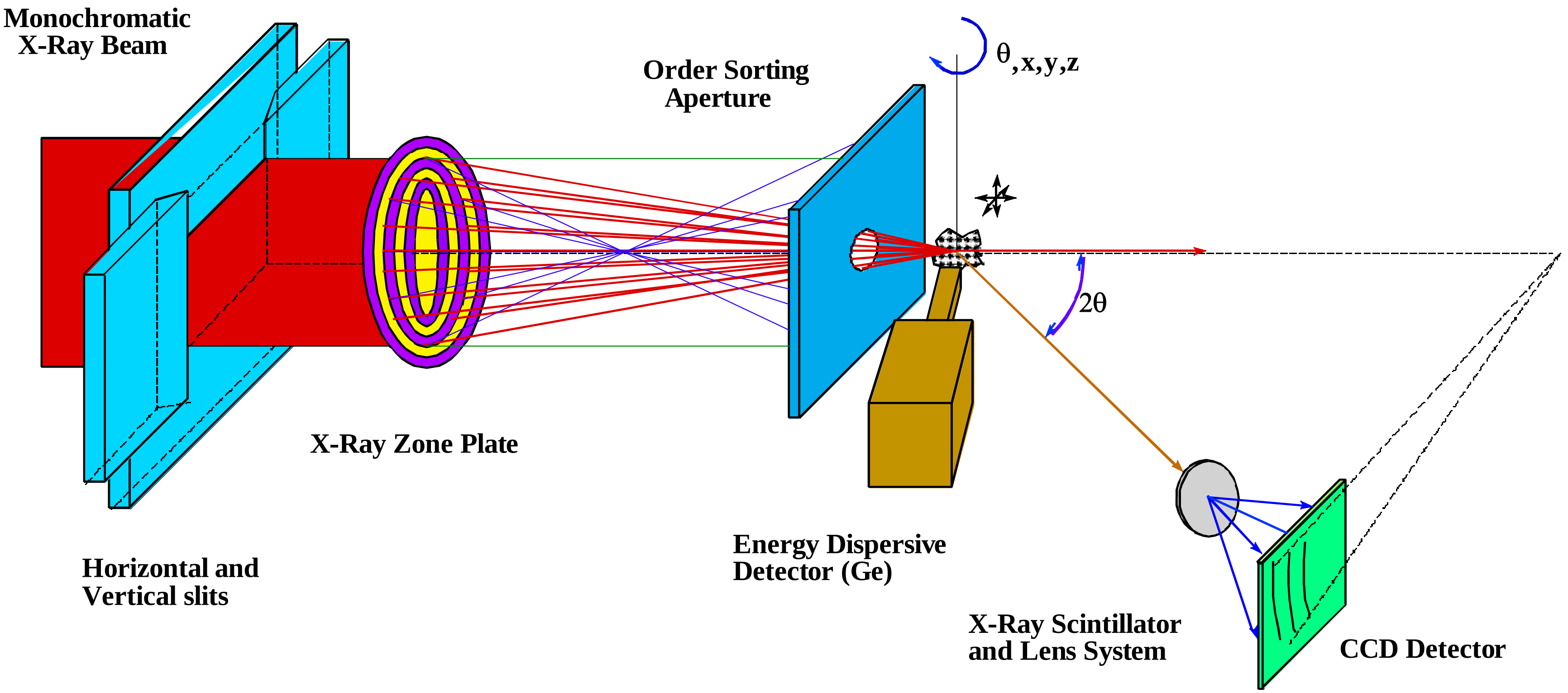
Differences between planktonic and surface-adhered bacteria to heavy metal exposure

- **Attachment of cells to surfaces during biofilm formation leads to major changes in metabolism, resistance, and survivability.**
- **Although microbes appear to be able to catalyze almost any reaction from which energy can be obtained, it is difficult to determine the mechanisms whereby catalysis occurs at the microbe-substrate interface.**
- **It is difficult to quantify the concentrations of metals, their cellular locations, and their redox states.**

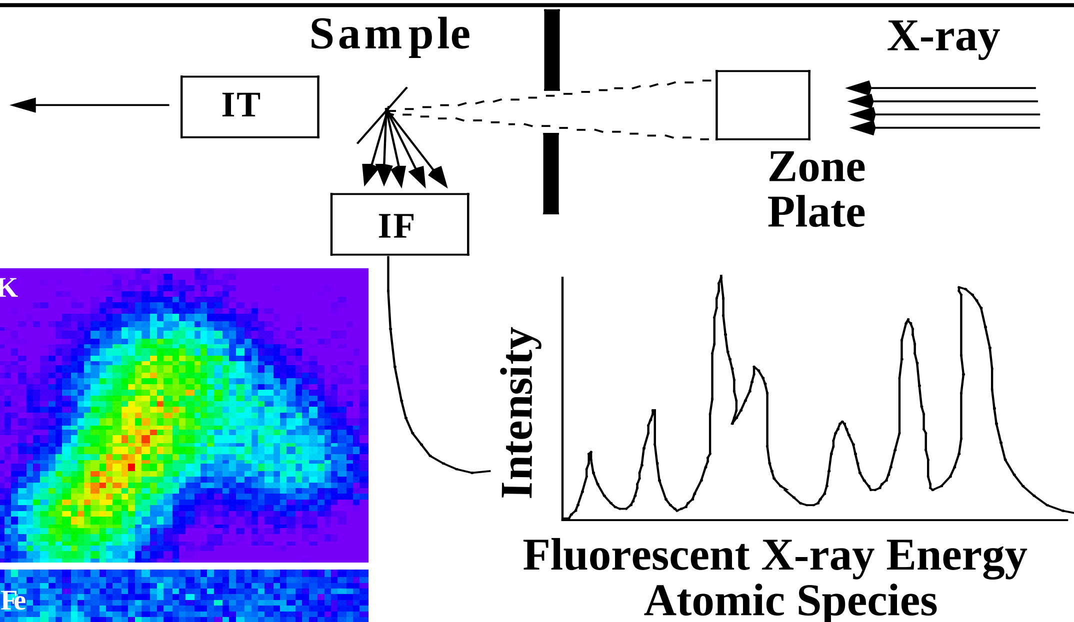
- ***Can XRF microscopy identify differences in planktonic and surface-adhered bacteria upon exposure to heavy metals?***

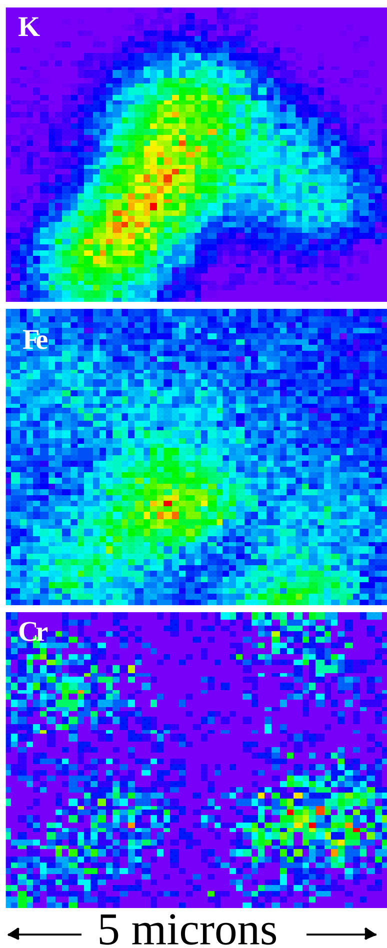
XRM with Fresnel zone plates:

X-RAY MICROPROBE BEAMLINE AT APS (2-ID-D/E)



2-D X-ray Fluorescence Imaging of Individual hydrated Bacterium with Zone Plates at the APS





What can it do for me?

Spatially resolve (150 nm):

- ***Distribution***
- ***Valence state***
- ***Chemical speciation***

of elements

K. M. Kemner, K. H. Nealson, B. Lai*
J. Maser, Z. Cai, D. Legnini, P. Ilinski
*M. A. Schneegurt**, C. F. Kulpa, Jr.***
Argonne National Laboratory
*Caltech/Jet Propulsion Laboratory
**University of Notre Dame

Biological Abundance

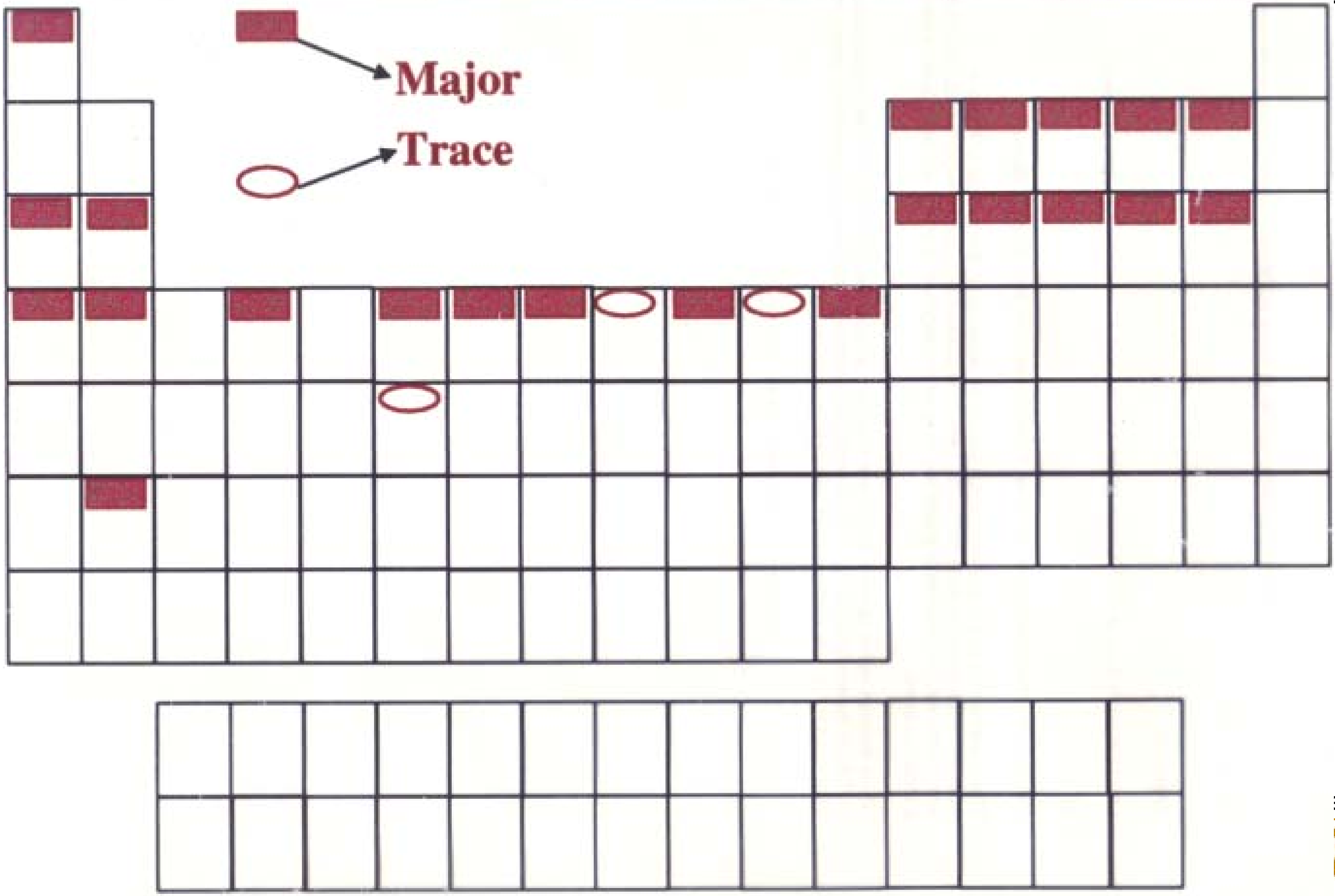
Major

Trace

1 H 1.01																	2 He 4.00
3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31											13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.06	17 Cl 35.45	18 Ar 39.95
19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.88	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.39	31 Ga 69.72	32 Ge 72.59	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.94	43 Tc (98)	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.75	52 Te 127.60	53 I 126.91	54 Xe 131.29
55 Cs 132.91	56 Ba 137.33	57 La 138.91	72 Hf 178.49	73 Ta 180.95	74 W 183.85	75 Re 186.21	76 Os 190.2	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po (209)	85 At (210)	86 Rn (222)
87 Fr (223)	88 Ra 226.03	89 Ac 227.03	104 Rf (261)	105 Ha (262)	106 Sg (263)	107 Ns (262)	108 Hs (265)	109 Mt (266)	110 Uun (269)	111 Uuu (272)	112 Uub (277)						

58 Ce 140.12	59 Pr 140.91	60 Nd 144.24	61 Pm (145)	62 Sm 150.36	63 Eu 151.96	64 Gd 157.25	65 Tb 158.93	66 Dy 162.50	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.97
90 Th 232.03	91 Pa 231.04	92 U 238.03	93 Np 237.05	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (260)

Crustal Abundance



Biological Abundance

1 H 1.01																	2 He 4.00
3 Li 6.94	4 Be 9.01											5 B 10.81	6 C 12.01	7 N 14.01	8 O 16.00	9 F 19.00	10 Ne 20.18
11 Na 22.99	12 Mg 24.31											13 Al 26.98	14 Si 28.09	15 P 30.97	16 S 32.06	17 Cl 35.45	18 Ar 39.95
19 K 39.10	20 Ca 40.08	21 Sc 44.96	22 Ti 47.88	23 V 50.94	24 Cr 52.00	25 Mn 54.94	26 Fe 55.85	27 Co 58.93	28 Ni 58.69	29 Cu 63.55	30 Zn 65.39	31 Ga 69.72	32 Ge 72.59	33 As 74.92	34 Se 78.96	35 Br 79.90	36 Kr 83.80
37 Rb 85.47	38 Sr 87.62	39 Y 88.91	40 Zr 91.22	41 Nb 92.91	42 Mo 95.94	43 Tc (98)	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.75	52 Te 127.60	53 I 126.91	54 Xe 131.29
55 Cs 132.91	56 Ba 137.33	57 La 138.91	72 Hf 178.49	73 Ta 180.95	74 W 183.85	75 Re 186.21	76 Os 190.2	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po (209)	85 At (210)	86 Rn (222)
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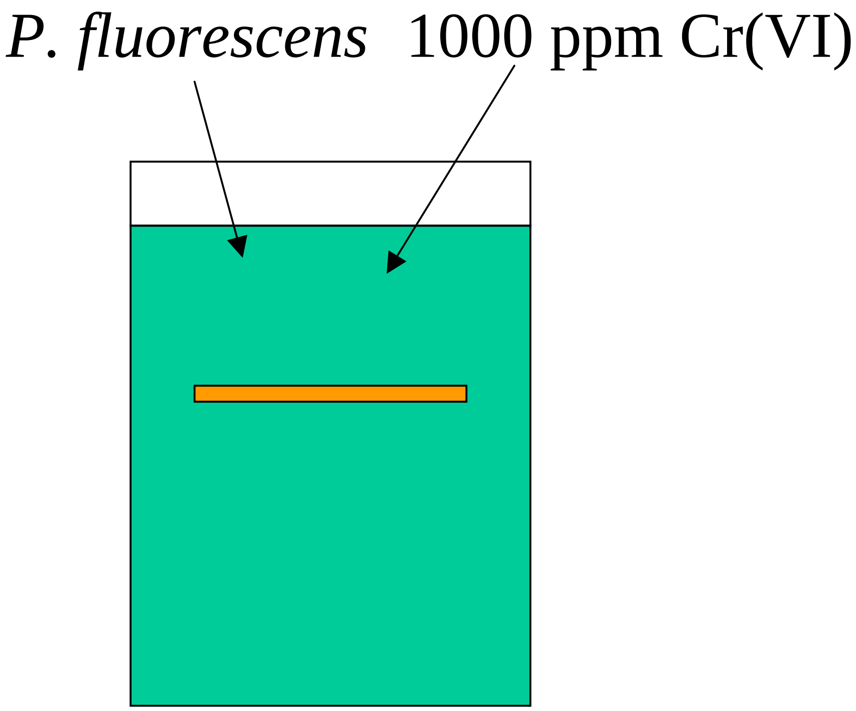
90 Th 232.03	91 Pa 231.04	92 U 238.03	93 Np 237.05	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (260)

What is the role of the physiological state of a microbe (planktonic versus surface-adhered) on its tolerance to heavy metals?



Elements required for life: H, C, N, O, P, Ca, S, Fe, Ni, Cu….

These elements should be in cells.

Elemental distribution in planktonic *P. fluorescens* w/ and w/out addition of Cr(VI)

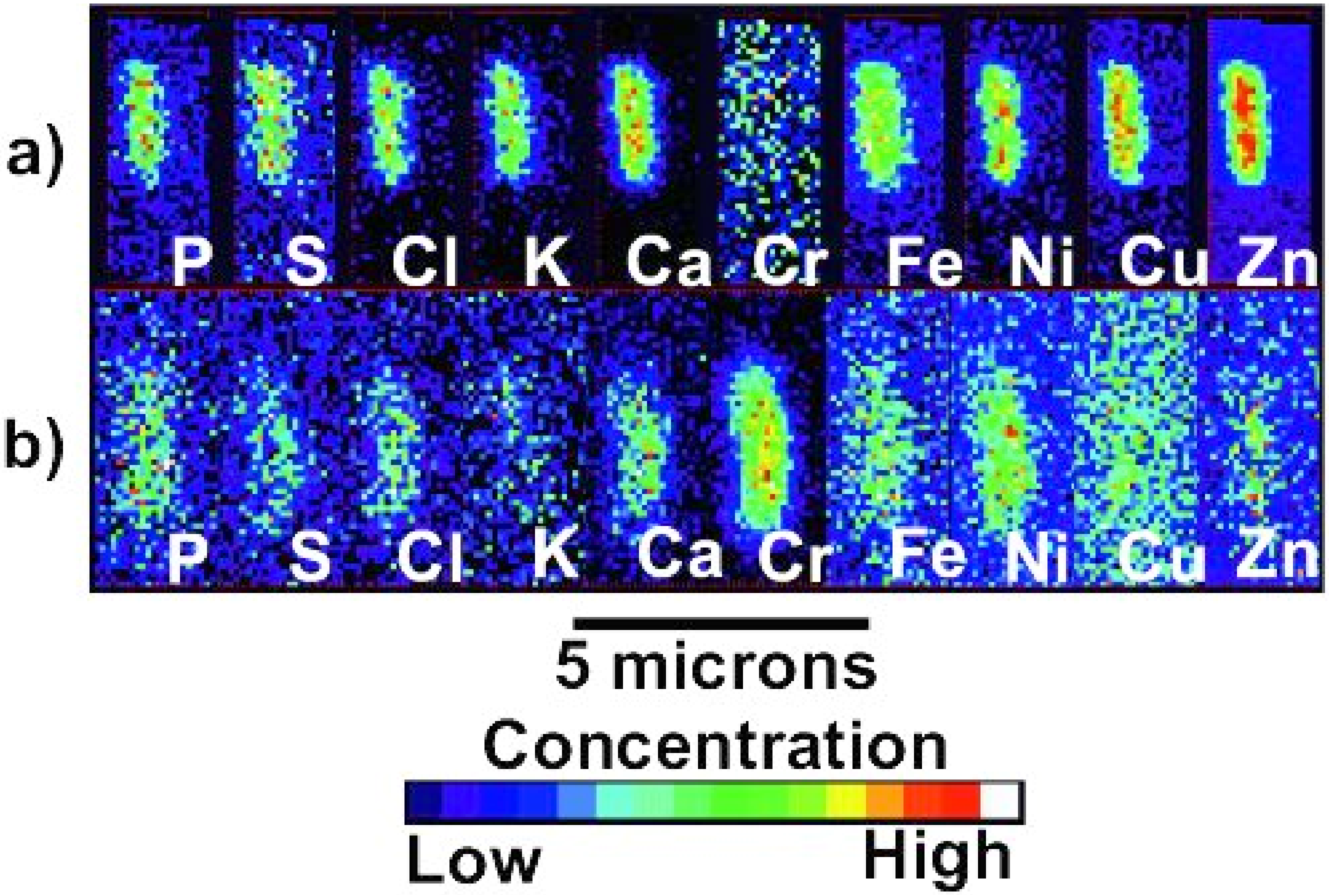


Fig. 1

Elemental distribution in surface-adhered *P. fluorescens* w/ and w/out addition of Cr(VI)

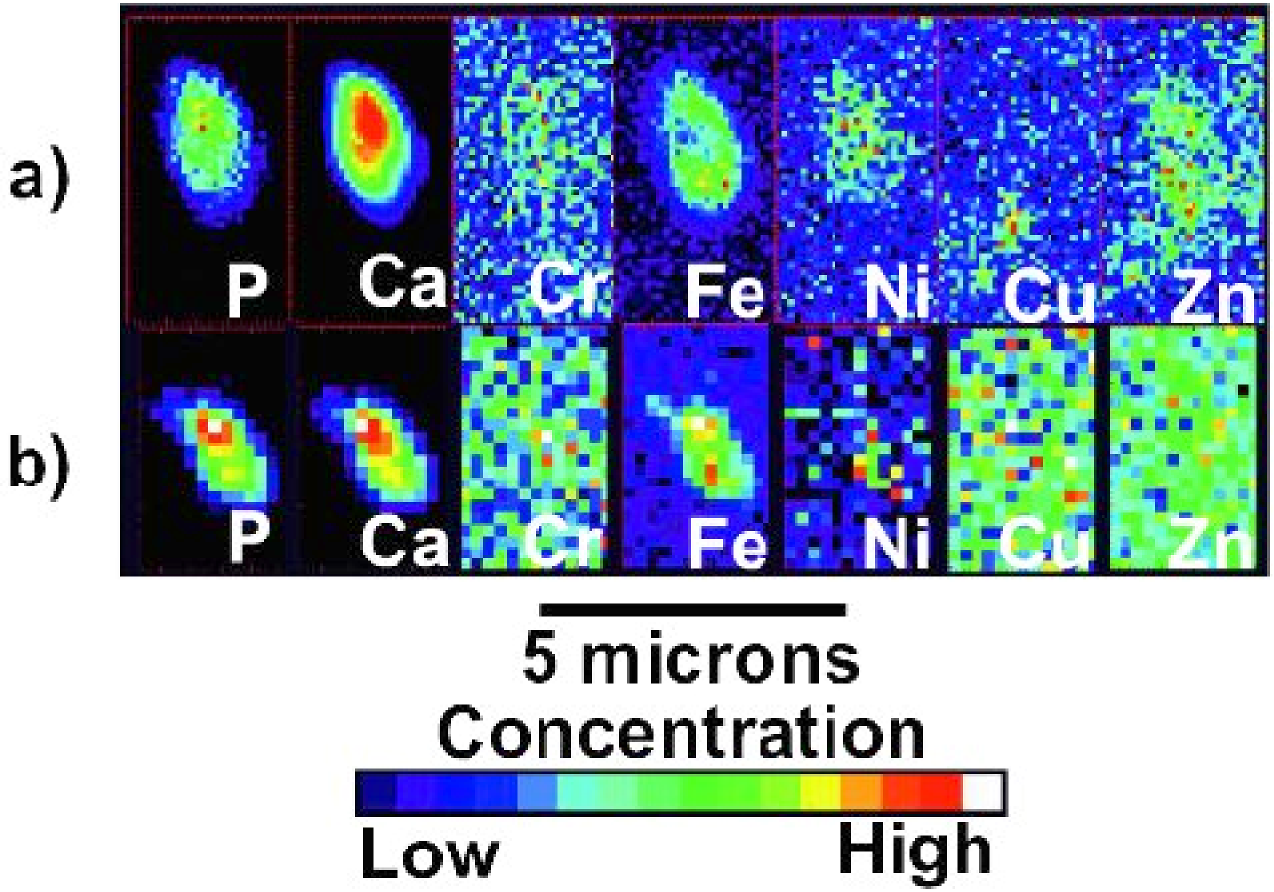
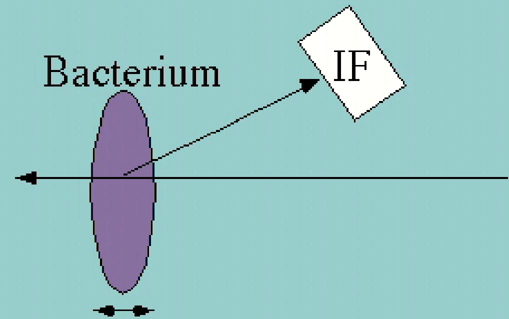


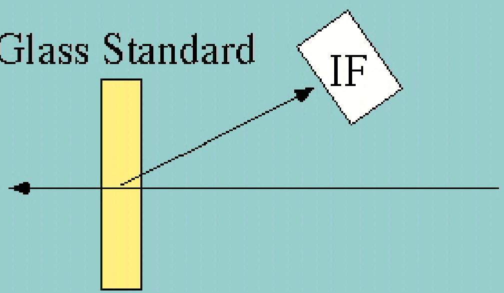
Fig. 2

XRF Elemental Microanalysis of a Bacterium



thickness$_{microbe}$ ~ 1 micron

$\rho_{microbe}$ ~ 1 g/cm^3



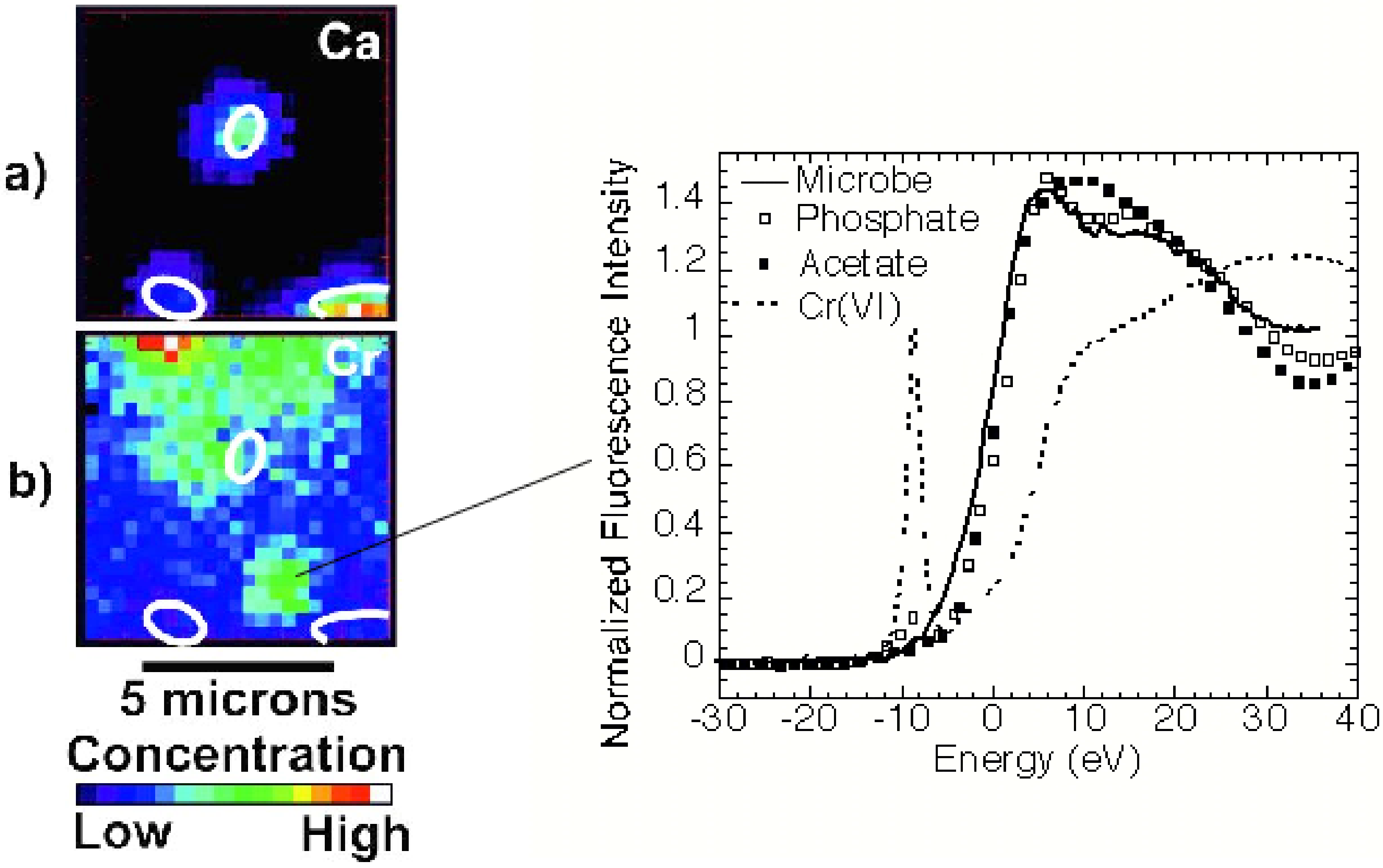
Known elemental area density = σ^2 µg/cm^2

$$[\ \ ] = \frac{(IF_{microbe})}{(\rho_{microbe} * thickness_{microbe})} \frac{(\sigma^2_{glass})}{(IF_{glass})}$$

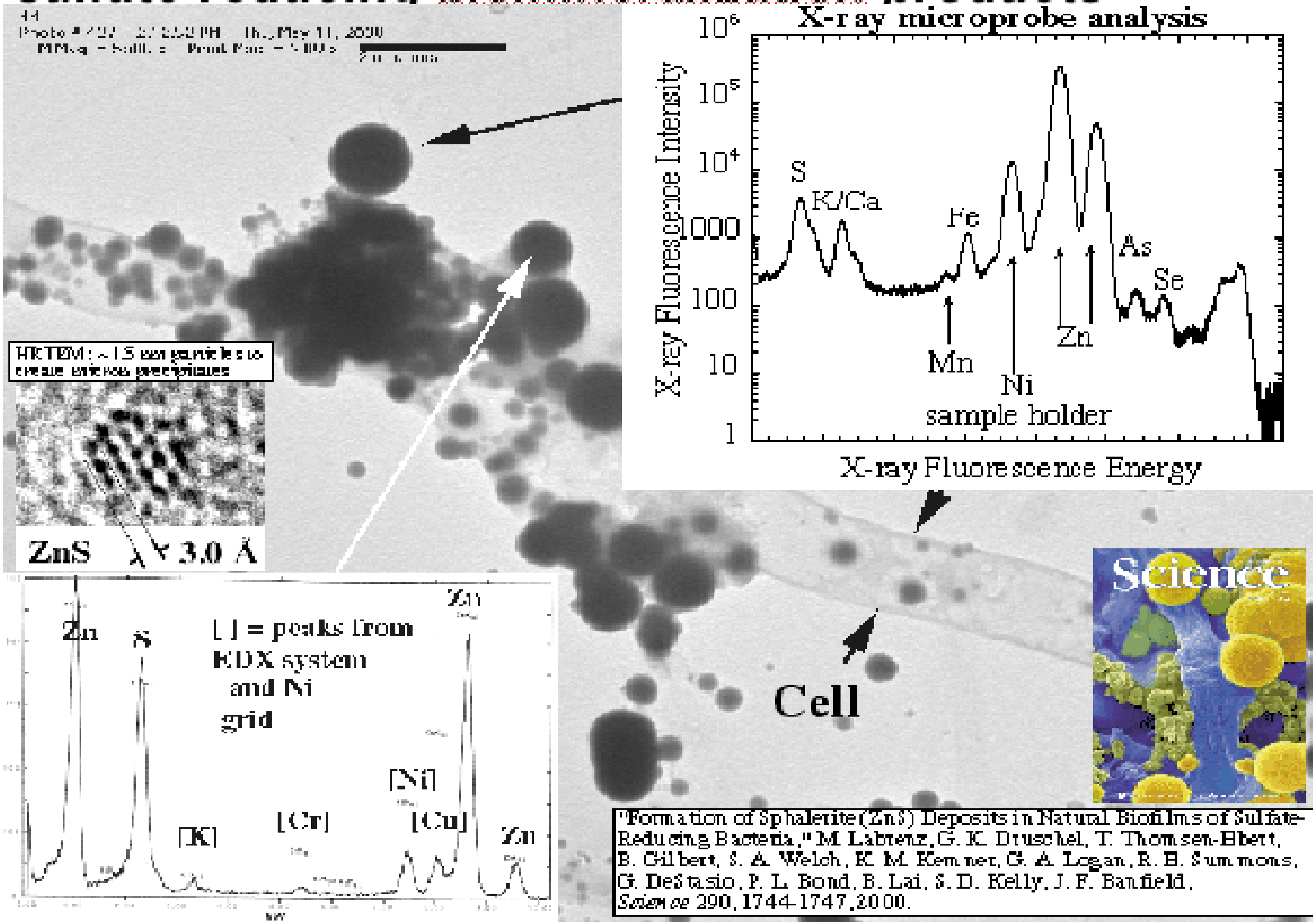
Results of quantitative XRF elemental analysis of single cells

	[P]	[S]	[Cl]	[K]	[Ca]	[Cr]	[Mn]	[Fe]	[Co]	[Ni]	[Cu]	[Zn]
Planktonic (5)	16,048 (2,446)	6,625 (1,117)	8,421 (2,628)	3,604 (1,173)	3,815 (392)	9 (2)	22 (4)	156 (23)	190 (37)	120 (33)	201 (46)	1,175 (176)
Planktonic + Cr(VI) (6)	6,156 (1,034)	3,719 (1,516)	3,908 (1,814)	2,201 (1668)	673 (230)	949 (323)	22 (4)	58 (29)	13 (12)	26 (18)	105 (76)	94 (30)
Surface-Adhered (8)	661,032 (139,416)	*	*	*	570,855 (92,831)	32 (10)	40 (7)	360 (216)	14 (7)	26 (10)	0 (14)	25 (13)
Surface-Adhered +Cr(VI) (10)	419,034 (362,728)	*	*	*	427,987 (147,983)	24 (15)	23 (8)	326 (177)	12 (7)	18 (9)	2 (5)	15 (7)

Spatial distribution and valence state of Cr relative to Surface-adhered cells



X-ray & electron microscopy investigations of sulfate-reducing biomineralization products



"Formation of Sphalerite (ZnS) Deposits in Natural Biofilms of Sulfate-Reducing Bacteria," M. Labrenz, G. K. Druschel, T. Thomsen-Ebert, B. Gilbert, S. A. Welch, K. M. Kemner, G. A. Logan, R. H. Summons, G. De Stasio, P. L. Bond, B. Lai, S. D. Kelly, J. F. Banfield, *Science* 290, 1744-1747, 2000.

X-ray and electron micro(spectro)scopy investigations of internal biomineralization products

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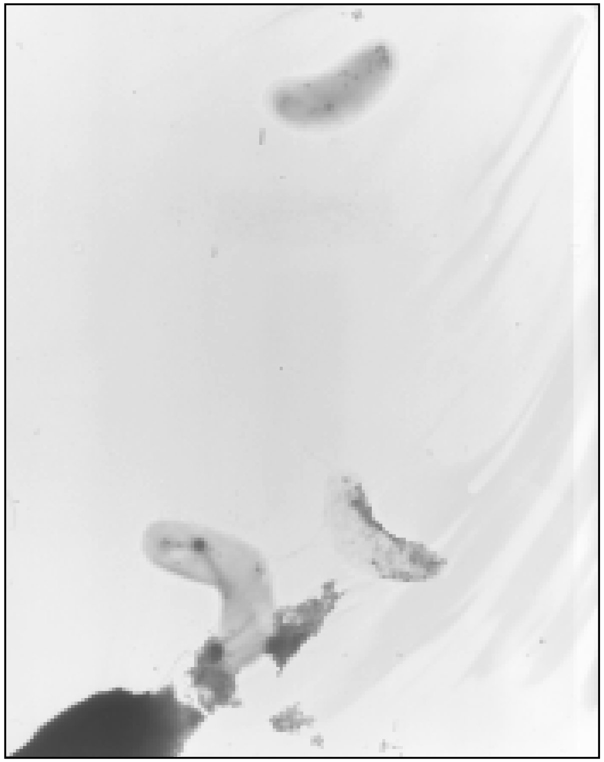
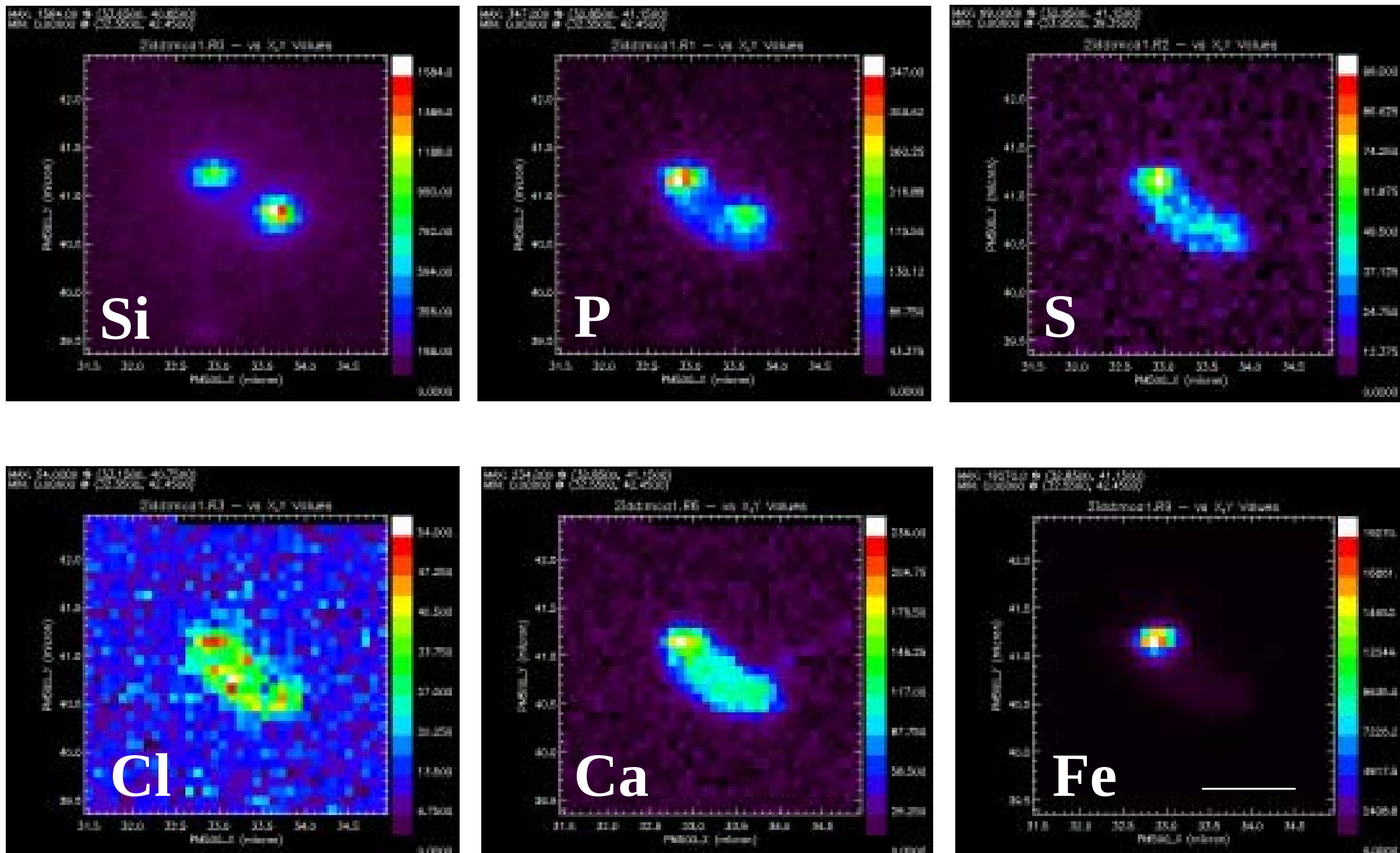
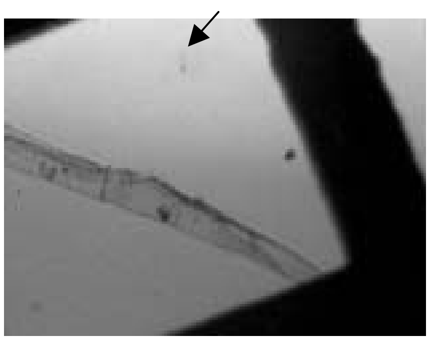


Electron acceptor: HFO (*S. oneidensis*, 16 and 24 days)

Optical, Electron, and X-ray Fluorescence imaging of DMRB

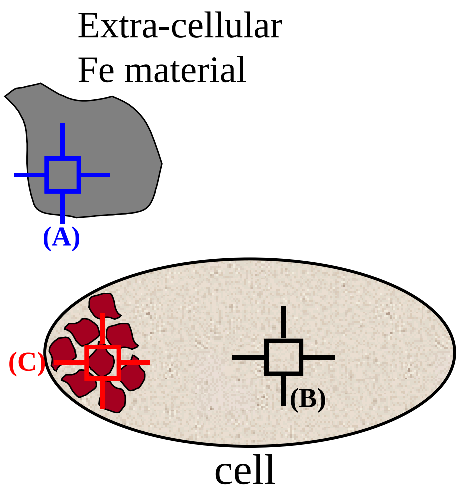
TEM X12,000

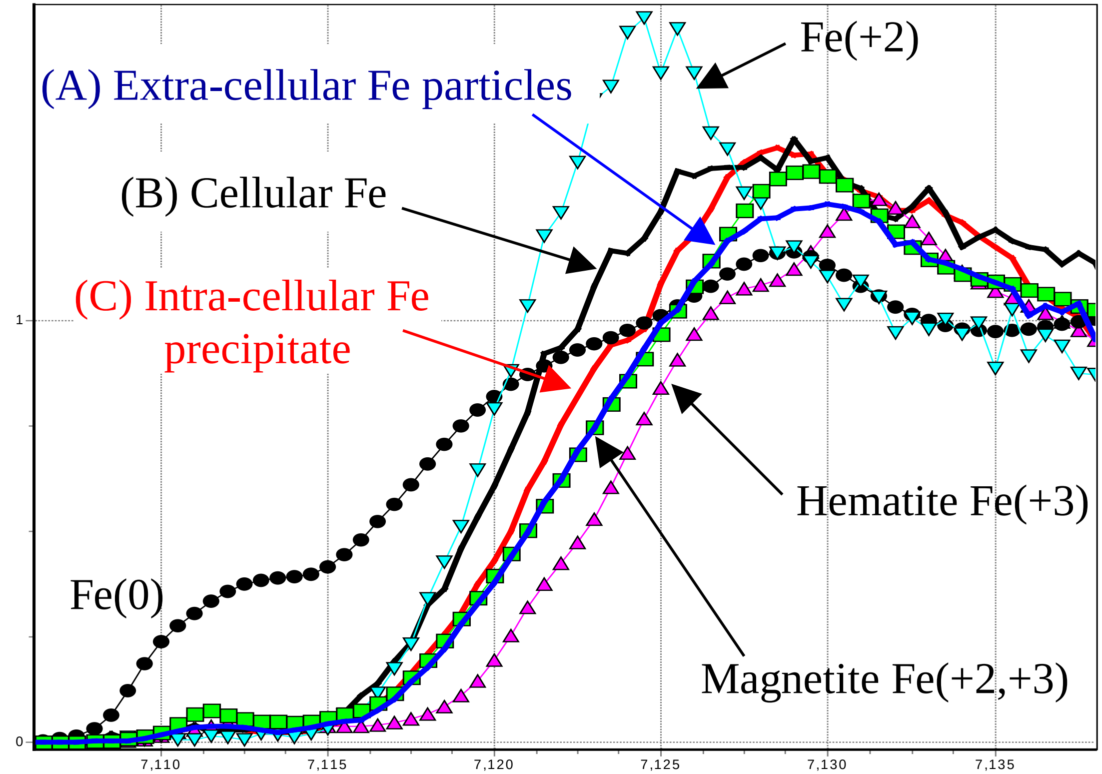




Optical X50

Fe X-ray Absorption Microspectroscopy Analysis of Biomineralization Products Produced by DMRB

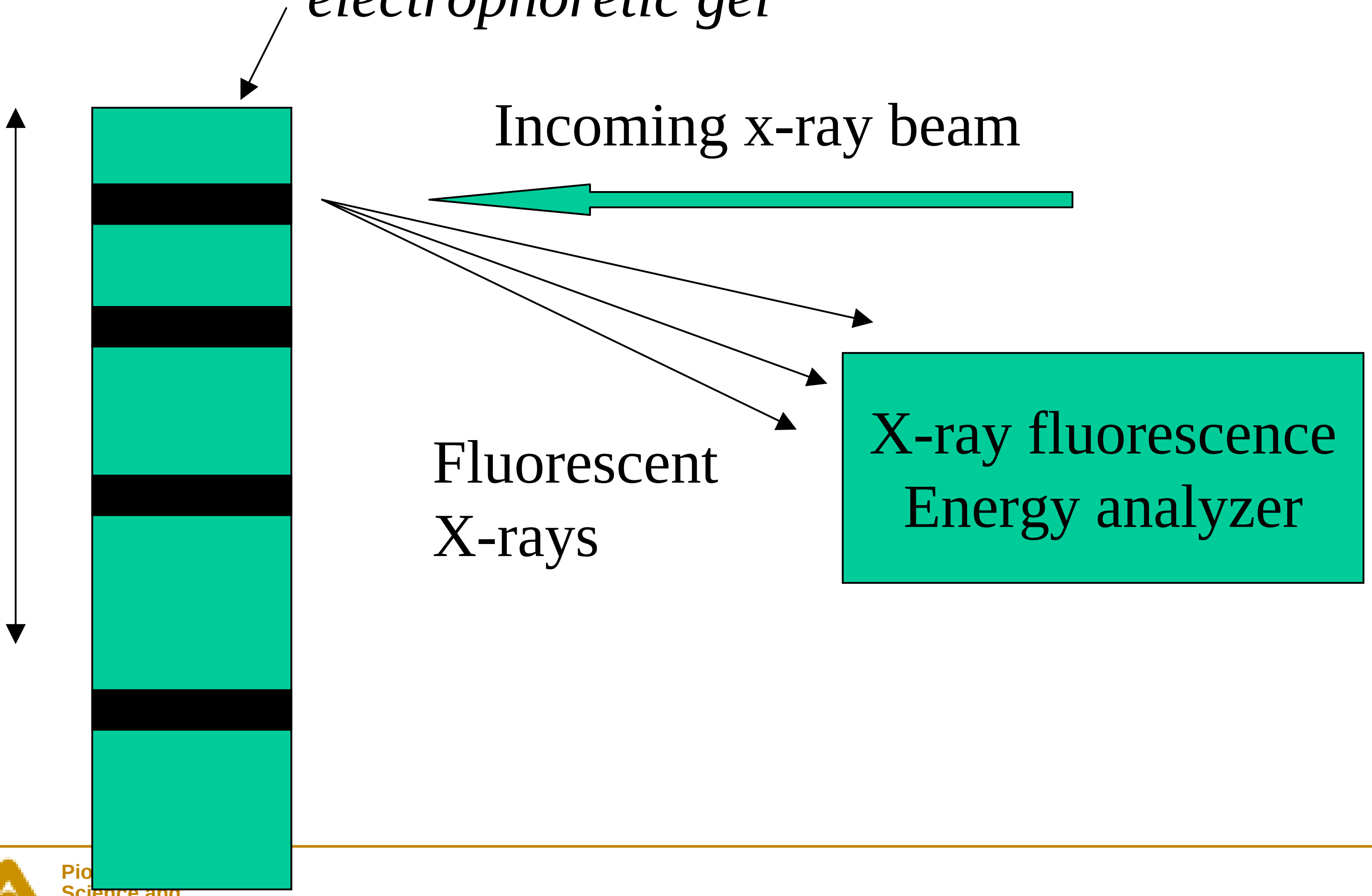




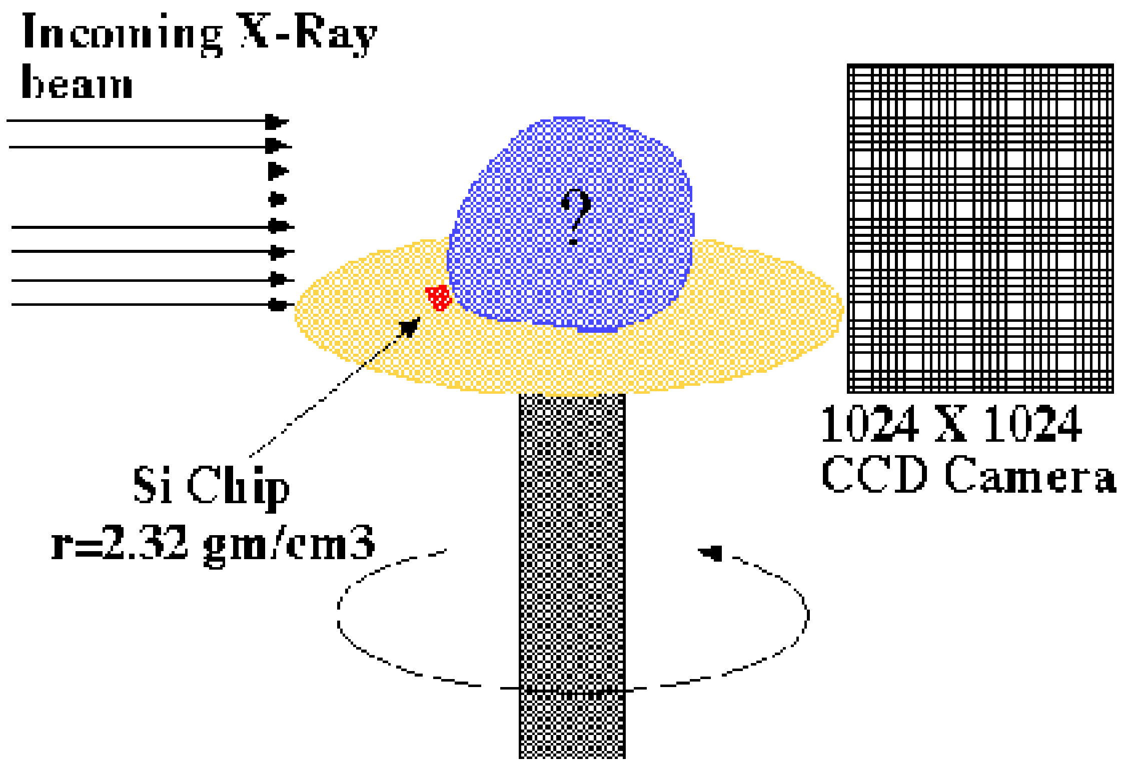
- (A) Fe valence state of extracellular precipitate near cell consistent with magnetite
- (B) Fe valence state associated with cell consistent with highly reduced Fe
- (C) Fe valence state of internal biomineral consistent with Green rust (more reduced than magnetite)

Other uses for spatially resolved x-ray fluorescence elemental analysis

Plant root, Banded Fe formations/life on Mars?, Beethoven's hair, Metalloproteins on 1 dimensional electrophoretic gel



3-D Imaging
X-Ray Microtomography



What can it do for me?

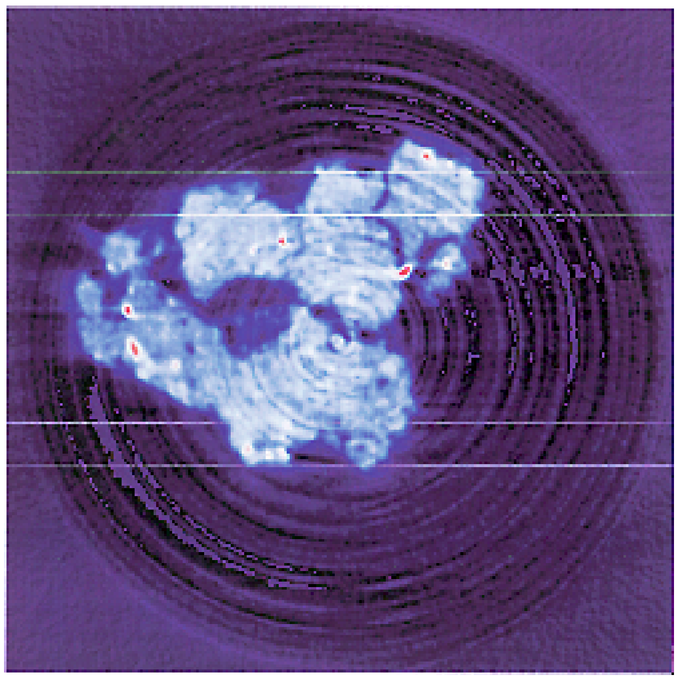
3-D information of material's electron density
(pore space)

Future:
Combine XAS to get 3-D
elemental and chemical speciation information

X-ray Microtomographic Investigations of Soil Porosity

Tomographic Reconstruction of Corn Field Soil

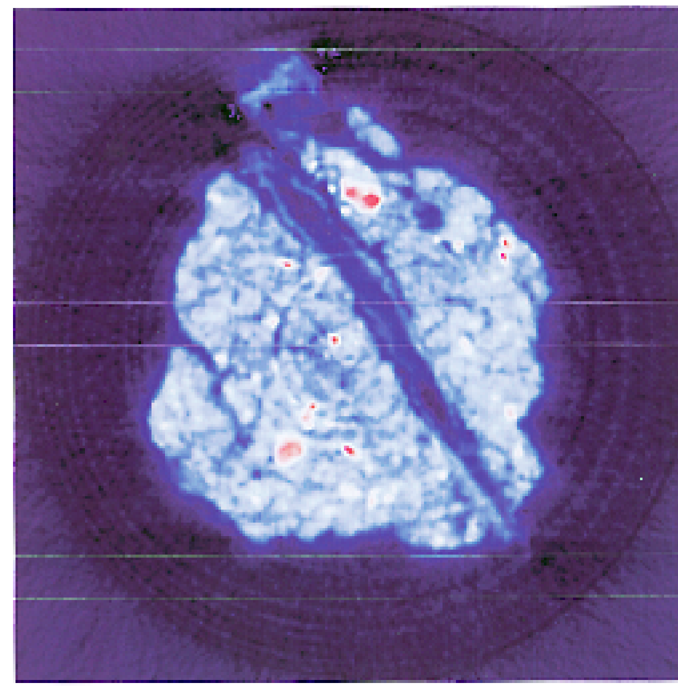
Size: 400 x 400
Resolution: 5 μm



Electron Density
grams/cm^3

Tomographic Reconstruction of Virgin Soil

Size: 400 x 400
Resolution: 5 μm



Electron Density
grams/cm^3

Summary

- **The integration of new techniques/tools such as the Advanced Photon Source with multiple scientific disciplines provides new and exciting opportunities for addressing a variety of highly relevant Environmental Science issues.**
- **Hard x-ray (micro)(spectro)(scopy)(tomography)offers many exciting possibilities for future environmental/biogeochemical investigations.**
- **The integration of the strengths of both x-ray and electron microscopies to investigate geomicrobiological systems is especially promising.**