

# *EXAFS of Complex Environmental and Biological Samples*

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EXAFS provides an elegant probe for *element speciation* in biological and environmental samples

Periodic Table of Absorption Data																	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	S	Cl	Ar
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Kr
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Rf	Ha	Sg	Bh	Hs	Mt									
Lanthanides			Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
Actinides			Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	

Periodic Table courtesy of "Hephaestus"

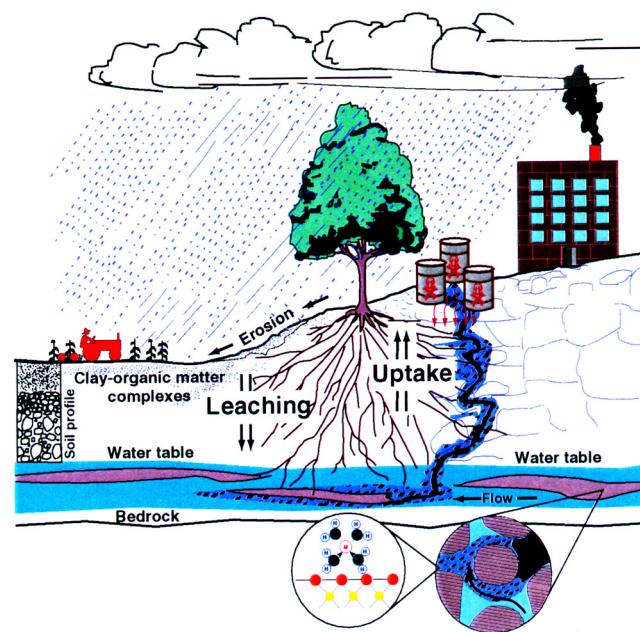
- “Hard X-rays” are sufficiently penetrating to permit studies:
  - under ambient conditions
    - ie, open to air, water
    - vacuum conditions required for “soft X-ray” analysis could alter samples
  - under controlled conditions
    - ie, oxidizing/reducing environments
  - of hazardous samples that need to be encapsulated for safe analysis

- Broad range of applications since **most** of the periodic table is accessible to the technique.
- Will restrict comments to “hard X-rays” which have seen the most widespread applications

## Obligatory Safety Comment

- The study of environmental/biological samples poses the most difficult details for Synchrotron Safety Staff.
- They are **dedicated** to helping you accomplish your experiment whether it involve “Bubonic plague” or “radioactive samples laced with plutonium”.
  - Begin talks with the safety staff early. They can put you in touch with others who may already have solutions to “difficult” samples.
  - Be honest
  - Leave extra time to address experiment details
  - With patience and good communication the safety staff can ensure most any experiment will be accommodated and conducted **safely**.

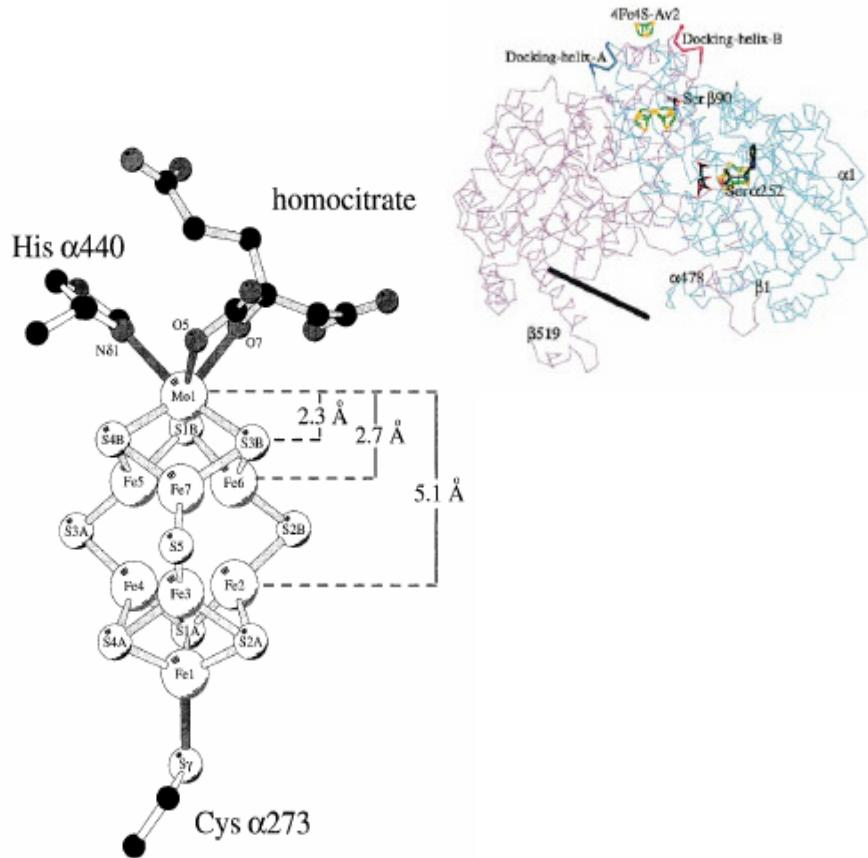
## Environmental and Molecular Sciences



**Multidisciplinary:**

- Biology
- Geology
- Soil Science
- Chemistry
- Physics

## Health Sciences



**Metallo-protein structure  
function relationships**

## Common Themes for EXAFS Sample Preparation

“Need to think like a chemist and physicist at the same time”

- Samples are often dilute (<5% by weight element of interest)

Examples:

1. Reasonably trace levels of contaminants
2. Single metal site in large biomolecules
3. Keep activity of radioactive samples as low as possible
4. Complex matrices with overlapping absorption/fluorescence elements

Solutions

1. Ensure the sample is “infinitely thick” for fluorescence measurements
  1. Dependant on absorption energy and matrix density
  2. Software such as Hephaestus or NRLXRF can estimate this parameter
2. For thick samples ensure sample is large enough for 45 degree sample mount geometry
  1. Beam size on sample increases by  $\sqrt{2}$  in x-plane
3. If matrix is low enough z, then it may be possible to conduct transmission with a long pathlength sample.

## Sample Preparation

- Lambert-Beer's Law

Absorption to EXAFS Physicists:

$$\frac{I}{I_0} = e^{-\mu(E)x} \quad \mu(E)x = \ln\left(\frac{I_0}{I}\right)$$

Absorbance to Chemists:

$$Absorbance = \log\left(\frac{I_0}{I}\right) = \frac{\mu(E)}{2.303}x$$

Rule of thumb for chemists:

Keep **Absorbance** less than 1 to maintain linear relationships

Rule of thumb for physicists:

Keep **Absorption** less than 2

Instructions for Hephaestus discuss how to calculate path lengths for your sample prep

One absorbance length is  $x=1/\mu(E)$   
 $I/I_0=.37$

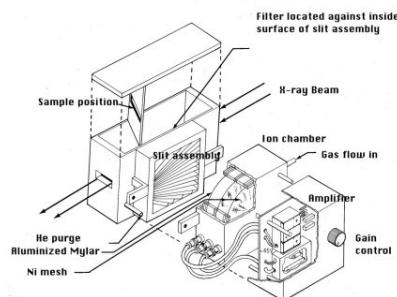
Although this approach can maximize you signal; you need to always remain cognizant whether your detectors maintain linearity

## Acquisition

- Samples are often dilute

## Solutions

1. Maximize absorption/fluorescence path-length
2. Minimize background
  1. Lytle filters for fluorescence ion chambers



**Be prepared to spend significant time setting-up the beamline and knowing its performance before data acquisition on “tough” samples**

## Acquisition

2. Minimize background
  2. Energy dispersive detectors
    1. Selecting a “region of interest” (ROI) allow collection of data from only the fluorescence energy of interest
    2. When using a multi element detector, be sure that alignment is such that all detector elements give a similar signal
      1. I run a small routine that auto-normalizes the above edge region to the edge step and calculates the variance of the last ~20 data points for the spectrum from each element
      2. The spectra for each element are then ranked in quality, and then averaged consecutively (ie spectra 1+2, 1+2+3, 1+2+3+4...)
      3. The set of averaged data is compared and if the “noisiest” elements degrade the averaged spectrum (as judged by the variance in the last few normalized data points), they can be discarded.
  3. Maximize signal-to-background
    1. Sometimes aperturing about the central portion of the beam will lower the background scatter and improve stability of the beam

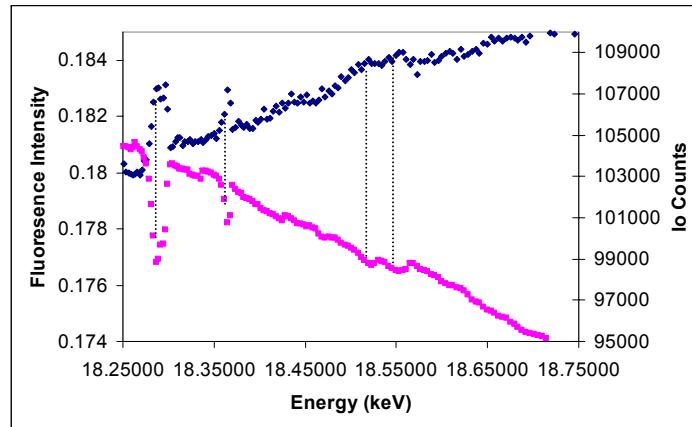
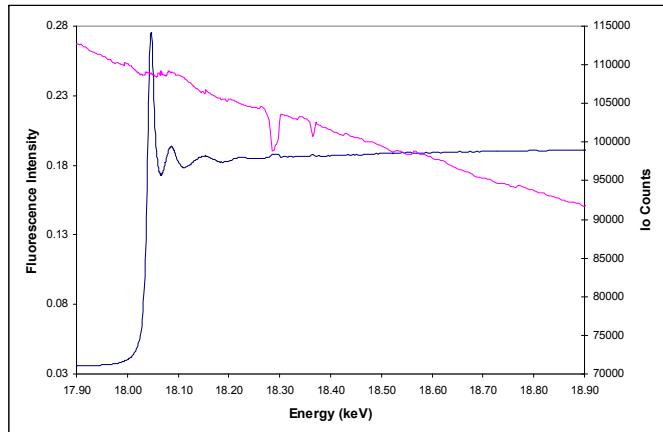
**Be prepared to spend significant time setting-up the beamline and knowing its performance before data acquisition on “tough” samples**

## Acquisition

### Solutions

#### 4. Maximize linearity between detectors

1. Remember that EXAFS is already a challenge of measuring signal to background that only becomes more challenging for weak signals
2. Monitor Io signal as a reference for the quality of beam reaching the sample



5. Maximize beamline stability for long acquisition times
6. Find the appropriate compromise between length of scan and number of scans

**Be prepared to spend significant time setting-up the beamline and knowing its performance before data acquisition on “tough” samples**

## Acquisition

- Samples are concentrated (>5% by weight element of interest)

Issues:

1. Minerals; metals; alloys

Solution: Transmission

- Rarely lucky enough to have foils
- Grind to a fine powder and evenly distribute on scotch tape
- Grind to a fine powder and homogeneously dilute in a low-z matrix (NaCl, sucrose etc...)
- Pathlength for solutions can be calculated

## Acquisition

- Samples contain pure phase particles which are dispersed but dilute

### Issues:

1. Fluorescence self-absorption can occur at 10 um particle size

### Solutions

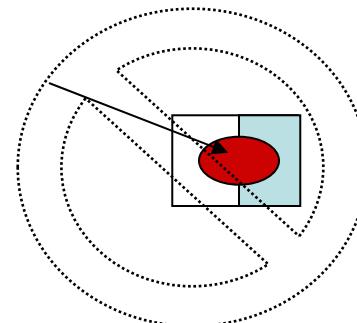
1. Transmission measurements if possible
2. Fluorescence corrections may be necessary if fluorescence is measured

- Natural samples are spatially heterogeneous

Issues:

1. Homogenize the sample appropriately for the scale of the x-ray beam size

For accurate determination of coordination numbers, the magnitude of the oscillations must be accurately normalized to  $\mu(E)$



2. Beware of crystallites that may yield diffraction peaks or multi-electron transitions of L lines
3. Remember that it is unlikely that the element of interest is a single chemical environment and that the EXAFS data represents an average of every absorbing element in the sample.

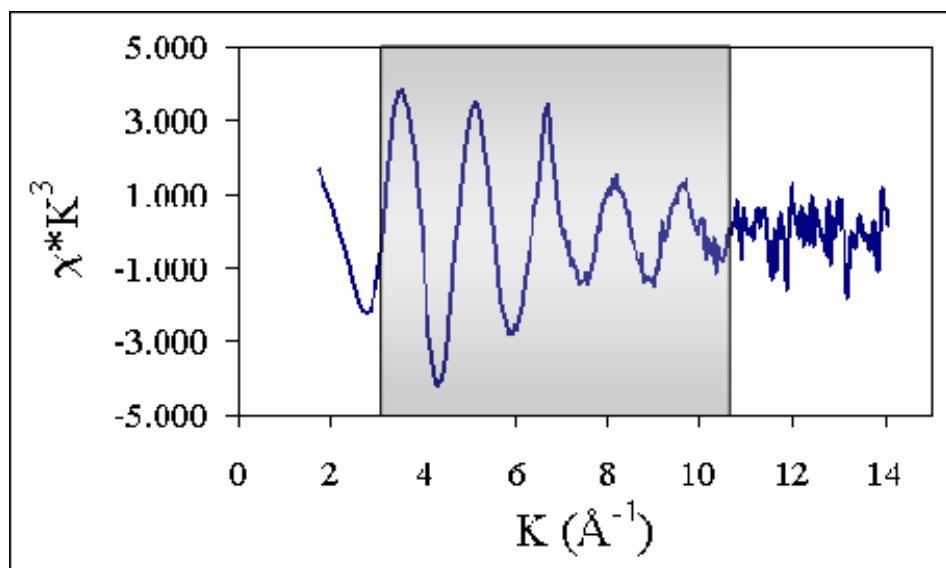
## Common Themes for EXAFS

- First shell analysis is most often oxygen (perhaps sulfur) in the first shell

Elemental form       $\longleftrightarrow$       Oxide (sulfide) form

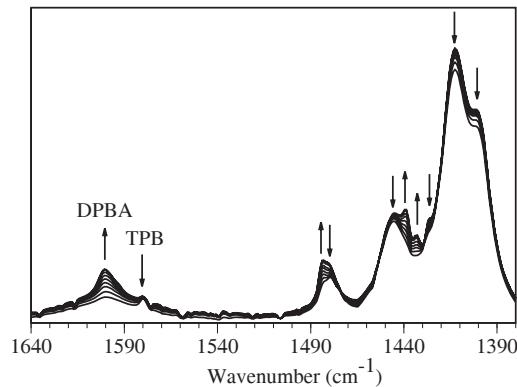
### Issues:

1. Oxygen is a moderately weak backscatterer
2. Focus on quality data and scan no further above the edge than you need

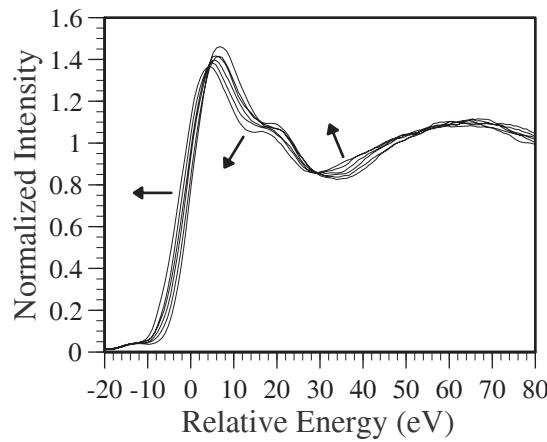


10 mM SrCl dissolved in water  
The hydration sphere of  $\text{Sr}^{2+}$  consists of 9-10 water molecules; ie 9-10 first shell water molecules

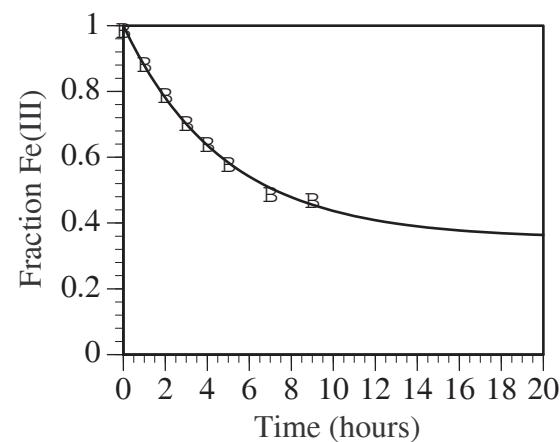
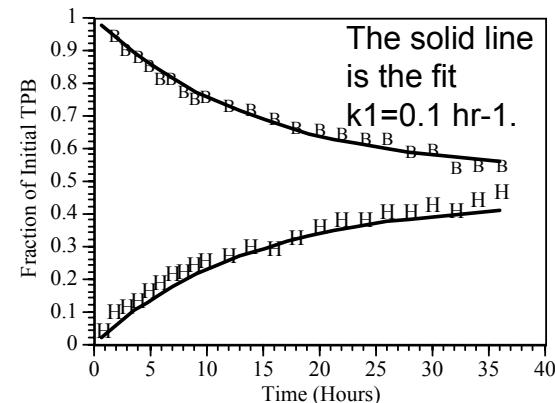
# Catalyzed Organic breakdown on Clay Surfaces



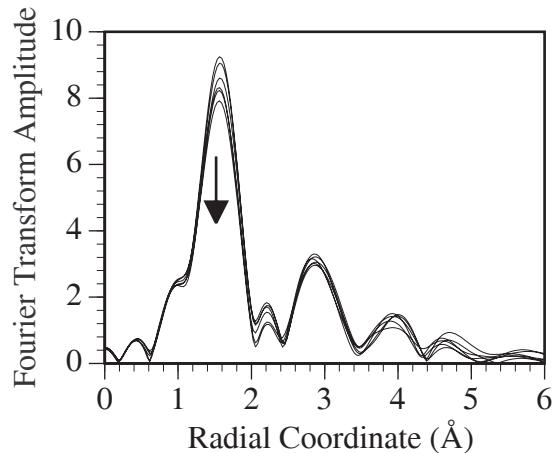
- We knew tetraphenylboron (TPB) could be catalytically degraded with clays containing Fe using FT-IR to track TPB and breakdown products.
- The slow reaction rate was ideal for spectroscopic analyses



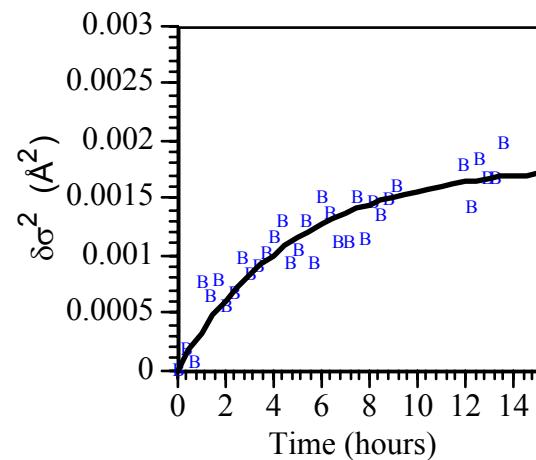
- Fe-XANES confirmed that structural Fe(III) in the clay structure was being reduced to Fe(II).
- The rate of Fe reduction was 2x that of TPB oxidation
- TPB liberates 2 electrons during oxidation



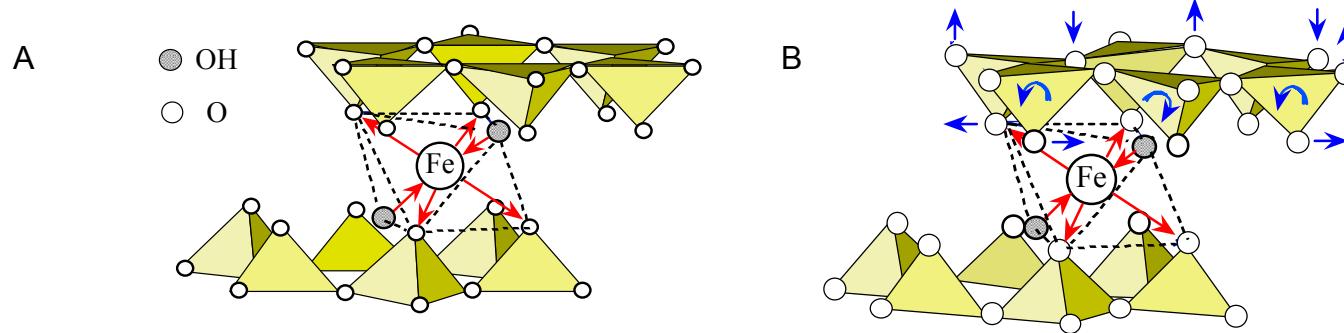
EXAFS interpretation is trickier: there are always 2 populations of sites: Fe(II) and Fe(III)



- Fe-EXAFS exhibited a slight (?) change of 0.01Å during the course of the reaction
- However the Debye-Waller Factor increased significantly
- The reaction rate equaled that measured from the XANES measurements

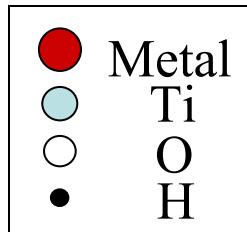


One explanation for this increase in the EXAFS Debye-Waller factor could be that there is an increase in the spread of Fe-O bondlengths about their initial average value, i.e. the shorter Fe-O bonds become shorter and the longer Fe-O bonds become longer.

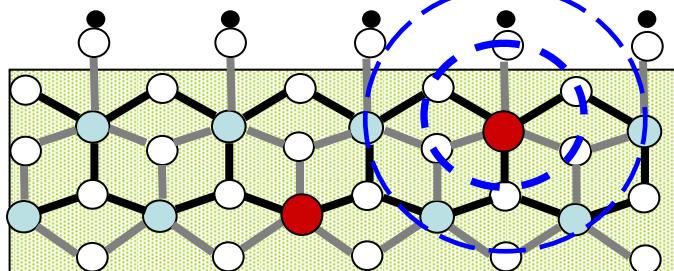


- Polyhedral representation of montmorillonite showing the linkage of the tetrahedral sheet with the octahedral sheet.
- Effective change in the average Fe-O bondlength upon reduction of Fe(III) to Fe(II) results in the distortion of the local clay crystal structure.

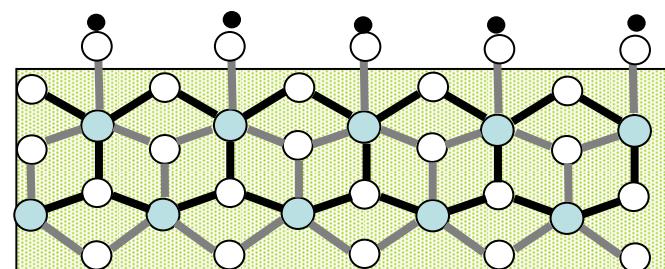
# Example of Metal Uptake by Surfaces-- What Can XAFS Tell Us?



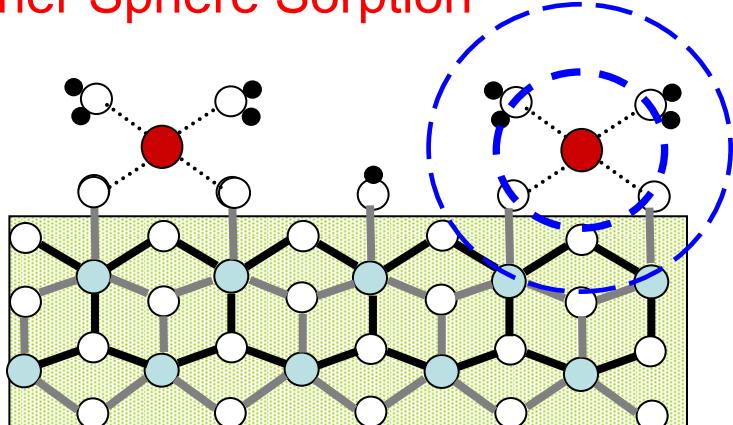
Structural  
Incorporation



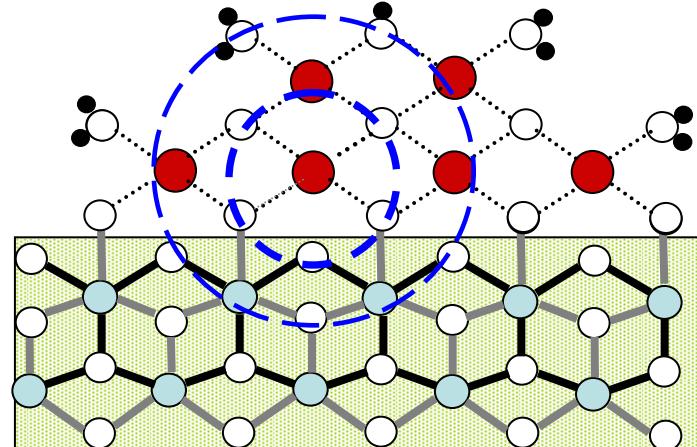
Outer Sphere/  
Ion Exchange/  
Electrostatic  
Sorption



Specific Adsorption/ Chemisorption  
/ Inner Sphere Sorption



Surface Precipitation/Polymerization



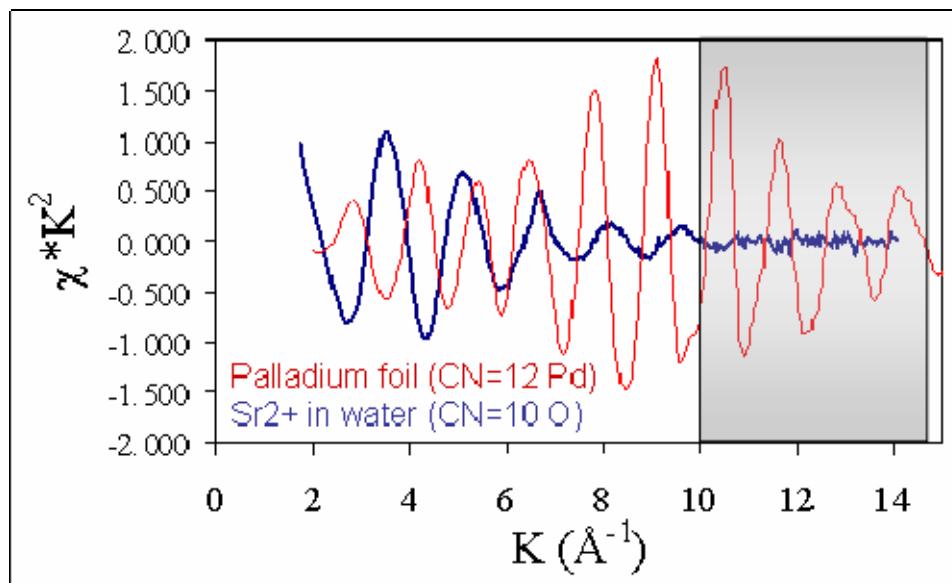
Blue circles denote coordination shells.

- Second shell information to determine self-precipitate from adsorption

Inner sphere adsorption       $\longleftrightarrow$       Surface Precipitation

### Issues:

1. Is the second shell a moderately weak/strong backscatterer
  - If it is high-z, the oscillations will be identifiable beyond  $10\text{\AA}^{-1}$  where oxygen oscillations are very weak. Focus on getting high quality data as far out in k-space as possible.
  - Analyze different windows to convince yourself that structures exist across the scan



- The second shell high-z backscatterer will be the dominant contributor to the EXAFS signal for  $k > 10\text{\AA}^{-1}$
- The increased resolution from the expanded data set may help distinguish overlapping peaks in R-space

Structural incorporation  $\leftrightarrow$  Inner sphere adsorption  $\leftrightarrow$  Surface Precipitation

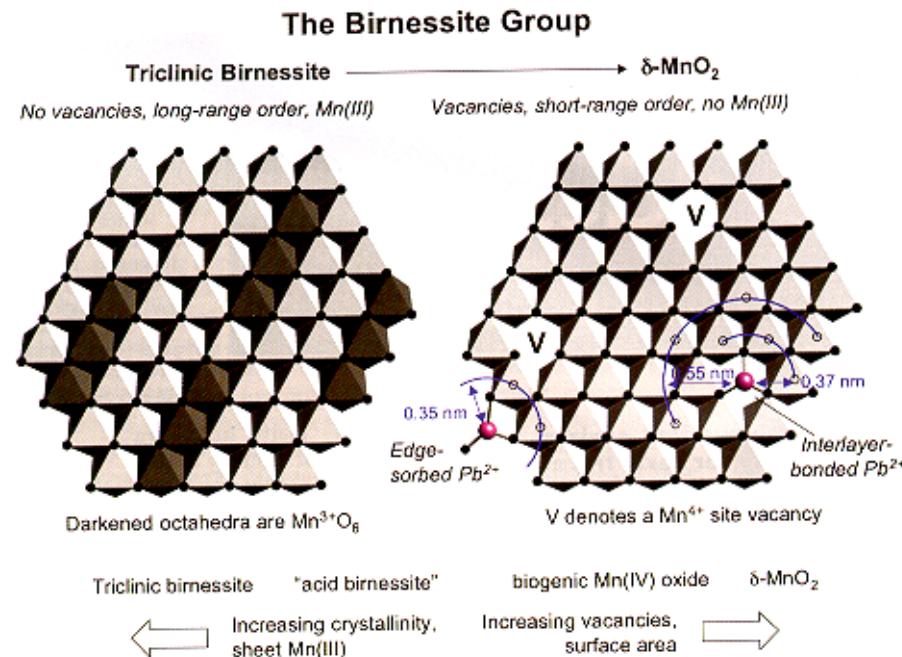
Issues:

1. Multiple scattering paths from first shell becomes increasingly important
2. Multiple environments escalate the uncertainty of second shell N and r determinations
  1. Given the uncertainty in higher shell analysis, I try to restrict interpretations to the most basic conclusions:
    1. Determination of  $r = \pm 0.2\text{\AA}$  is close enough to answer most questions (examples given later)
    2. Determine what the 2<sup>nd</sup> shell element is
    3. Class coordination number determinations
      - If  $CN \leq 1$  and element belongs to surface then inner sphere adsorption
      - If  $CN > 1$  and element belongs to surface then structural incorporation
      - If 2<sup>nd</sup> shell is the same element as absorbing element, then surface precipitation

# X-Ray Absorption Measurements on Hydrous Manganese(IV) Oxides Prepared from Permanganate $[KMn(VII)O_4]$

## RELEVANCE TO METAL SORPTION

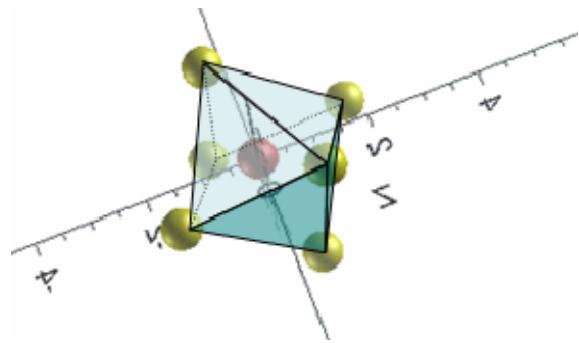
- Oxidation of permanganate yields an **amorphous** precipitate of hydrous manganese(IV) oxide (loosely termed  $MnO_2$ ) that is a useful adsorbent for heavy metals
- Since long range order is lacking, XRD provides little useful information



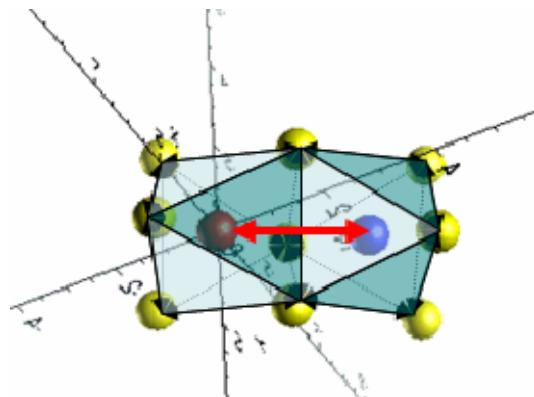
Metal adsorption to crystalline  $MnO_2$  has been extensively studied

- Note implied multiple sites for Pb adsorption

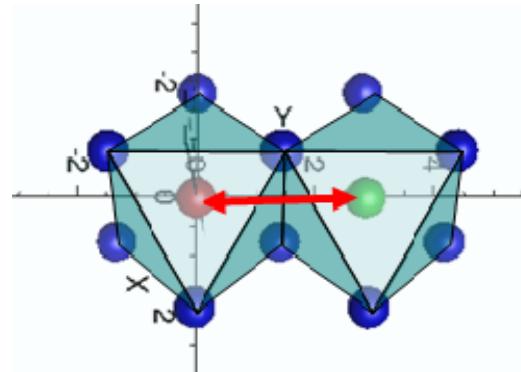
## Basic Building Blocks of Mn(IV) Oxides to demonstrate the interest in second shell structure



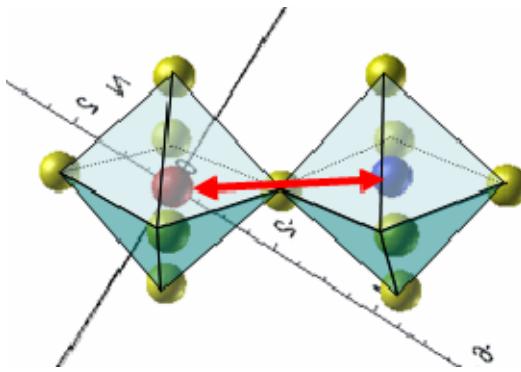
A. Mn(IV) with six first shell oxygens at 1.9 Å and octahedral symmetry



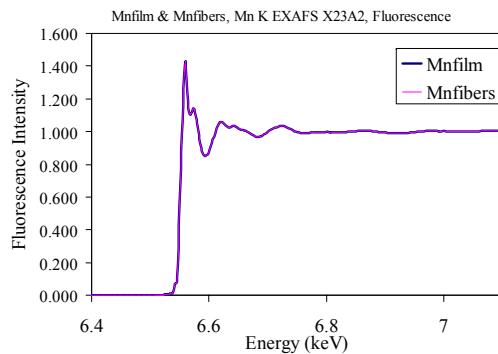
B. Two Mn(IV) octahedra sharing 3 oxygens. Termed “face” sharing, Mn-Mn distance 2.5 Å



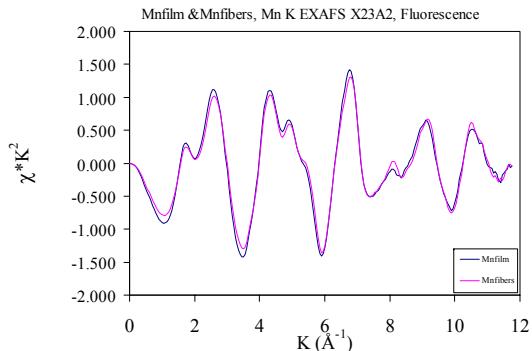
C. Two Mn(IV) octahedra sharing 2 oxygens. Termed “edge” sharing, Mn-Mn distance 2.85 Å



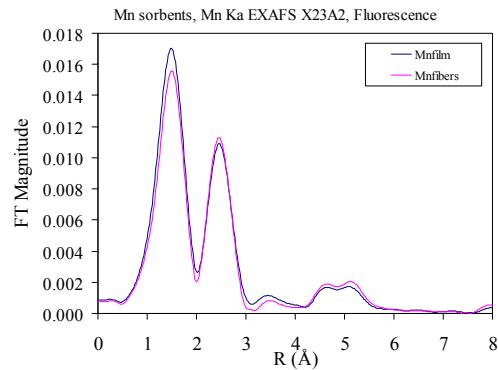
D. Two Mn(IV) octahedra sharing 1 oxygen. Termed “corner” sharing, Mn-Mn distance 3.8 Å



A. Mn EXAFS data on film and cotton fibers



B. Extracted Chi data



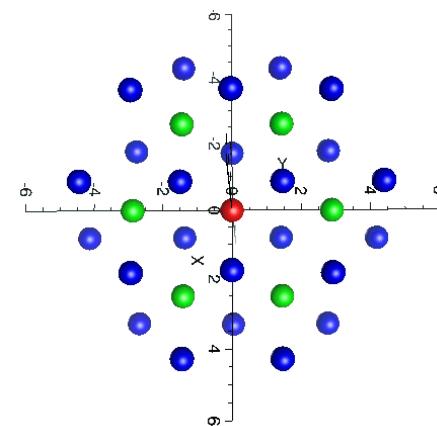
C. Radial distribution function (Fourier transform of Chi)

## RESULTS

- Absorption edge shift consistent with final oxidation state of Mn as Mn(IV)
- First shell analysis indicates 6 oxygen atoms at 1.89  $\text{\AA}$
- Second shell analysis indicates 4 manganese atoms at 2.87  $\text{\AA}$

## INTERPRETATION

- Mn is octahedrally coordinated to oxygen
- Based on Mn-Mn radial distance of 2.87  $\text{\AA}$ , Mn octahedra are primarily “edge” sharing
- Manganese oxides that are primarily edge sharing form sheets with high surface area (ie, birnessite)
- Large sheets would have a Mn-Mn coordination number of 6. The reduced coordination number of 4 indicates that the sheets are limited in size. For example, the sheet below consisting of 6 Mn (green) around a central Mn (red) would have an average Mn-Mn coordination number of 3.43.

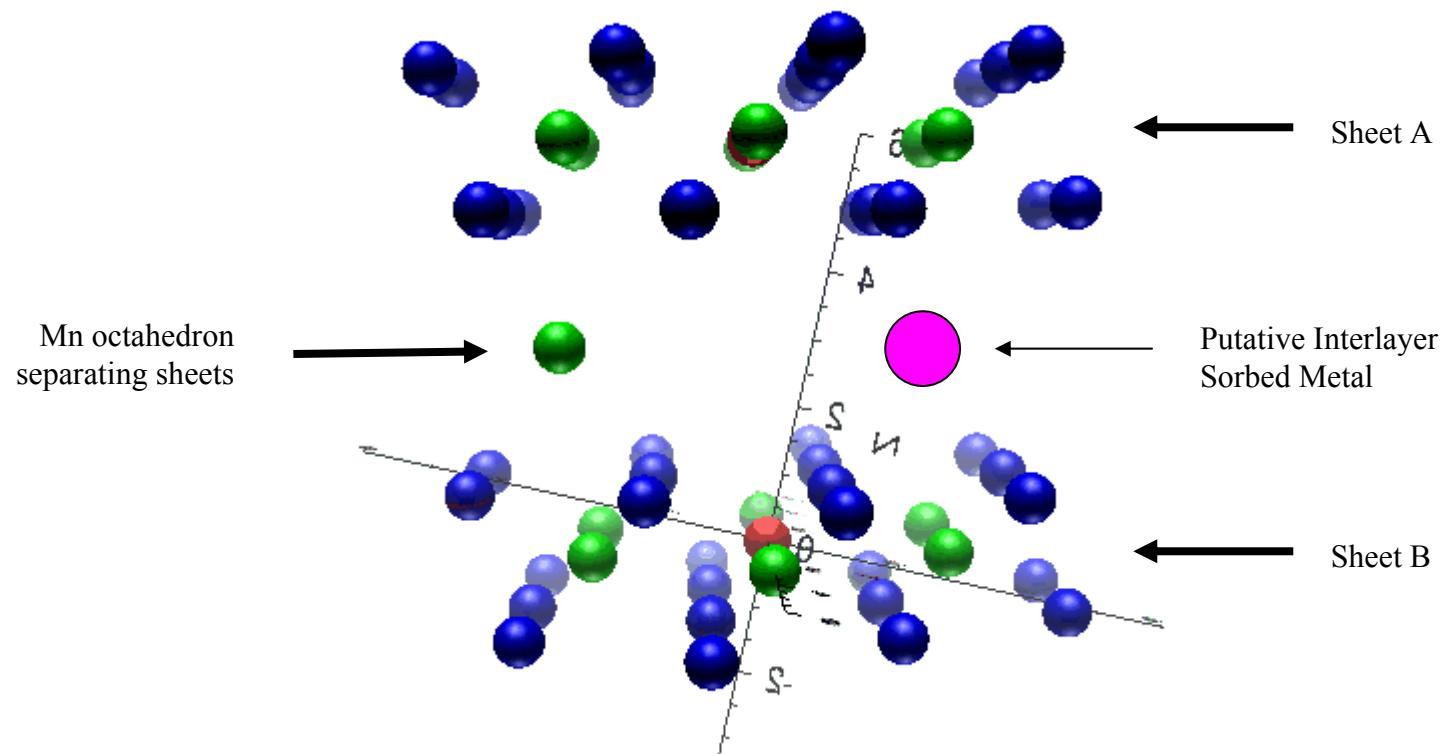


#### Corroboration:

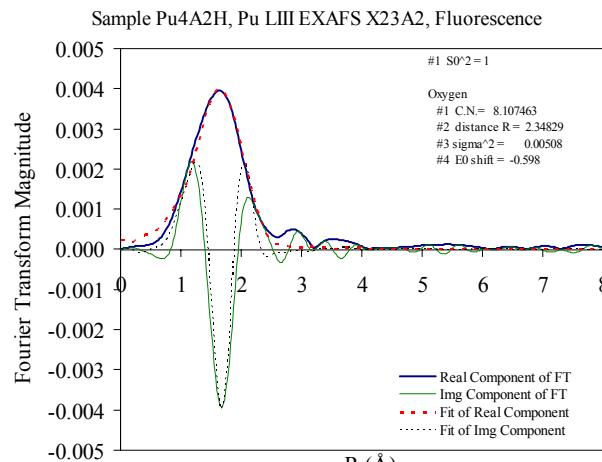
Transmission electron microscopy revealed hexagonal platelets of varying size. A fractal pattern of 10-20 nm was evident when an unstable thin area of the section was pulled apart upon exposure to a high intensity beam. Because the image was moving, it could not be photographed, but large hexagonals pulling apart into what looked like smaller hexagonals.

#### RELEVANCE TO METAL SORPTION

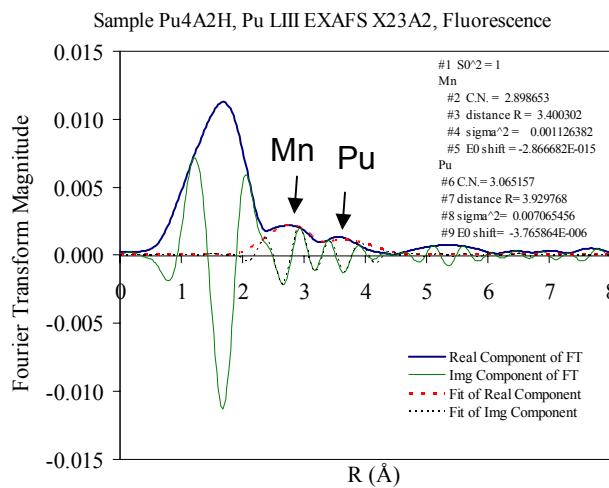
- Sheet structures of Mn(IV) oxides provide high surface areas for metal sorption
- Mn(IV) oxides can also sorb metals through redox reactions (ie, Cr, Pu)
- The EXAFS results indicate a highly structured material at the 10-20 nm size scale.
- Offset sheets can provide further sorptive capabilities by trapping metals between sheets (see below)



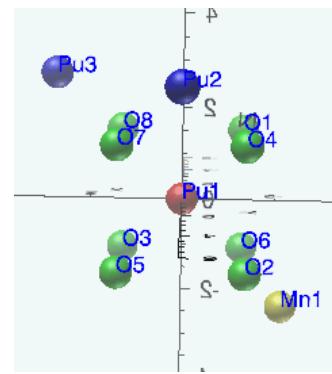
Plutonium EXAFS was measured on Pu co-precipitated during the formation of the hydrous Mn oxide:



First shell analysis consistent with 8 coordinate Pu(IV).



Second shell analysis suggest 2 phases: PuO<sub>2</sub> and Pu sorbed to MnO<sub>2</sub> consistent with 8 coordinate Pu(IV).



- The model started with 8 coordinate PuO<sub>2</sub> and add Mn at Mn-O=1.89 Å from 2 of the Pu-O oxygens.
- 2-3 Mn in the second shell is consistent with diagram on previous page

- XANES for fingerprinting

Many people are trying to take a chemometric approach:

1. Build library of standard spectra
2. Run unknown
3. Perform PCR analyses...because WinXAS software permits this.

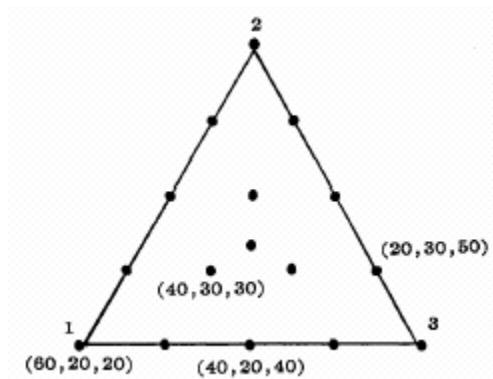
#### 4. PCR vs PLS

Please Read

David M. Haaland and Edward V. Thomas. 1988. *Partial Least-Squares Methods for Spectral Analyses. 1. Relation to Other Quantitative Calibration Methods and the Extraction of Qualitative Information*. Analytical Chemistry, **60**:1193-1202

Issues:

- Self-absorption or any other spectral distortions
- Is your unknown a part of the training set?
  - PLS can handle this, PCR can not
- Are you sure that your fingerprint is unique?
- Is the training set sufficient?
  - Should have at least 16 samples for a three component mixture



- EXAFS fitting/interpretation

Issues:

In a true unknown sample, interpretation can become difficult quickly

1. Supplement data from as many other techniques as possible
2. Constrain analyses with supporting chemical information
3. Avoid over-subscribing the available degrees of freedom for fitting
4. Important to remember entire coordination sphere Molecular modeling software still lags for high-z elements (relies on force-field approximations)
  - Until calculational methods evolve, rely on known properties of elements and crystal structures

## The Use Theoretical Standards (*Matt Newville*)

There are many categories of analysis problems for which empirical standards and Fourier filter techniques are inadequate. With theoretical standards, many such problems become tractable.

- Overlapping shells
- Three-body correlations
- Mixed shells
- Molecules with essential MS

Uranium possesses strong multiple scattering arising from the colinearity of the equatorial oxygens with the central uranium absorber

- Polarized measurements
- Exotic materials

Perhaps the most significant drawback to empirical standards is that one must exist for the compound of interest. For many important problems, no suitable empirical standards exist.

- Uranium, Plutonium, Neptunium

Bonding with these elements involves f electrons.

Multiple valence states exists.

It may be impossible to find a standard with an electronic structure similar to your sample.

# Theory

## Molecular Model

Crystalline → Atoms

Amorphous → Rot\_Atoms, molecular modeling software

Supply a list of atomic coordinates

ab initio multiple  
scattering calculations → FEFF

Analysis

Interpretation

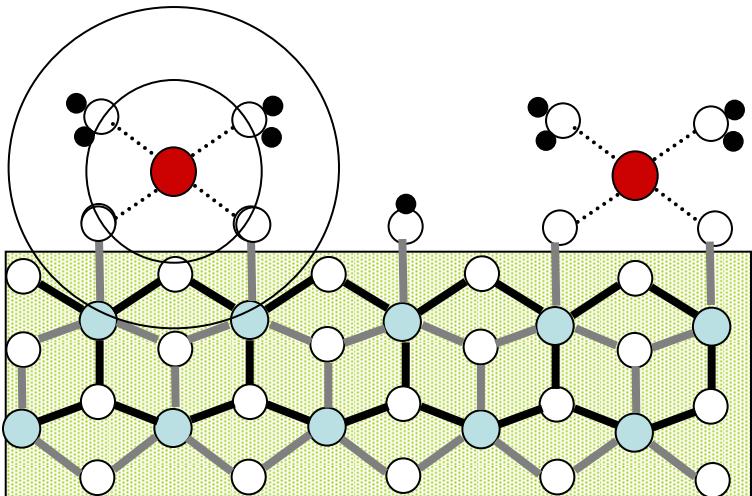
Radial Distribution: RDF(A)

Chi data:  $\chi(k)$  → WinXAS, Athena

Raw data:  $\mu(E)$  → Autobk

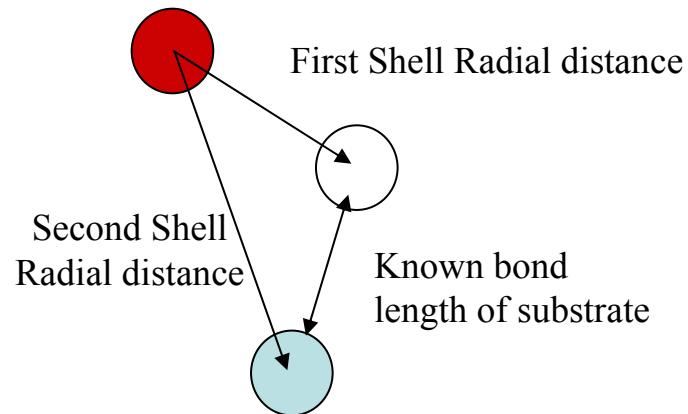
Other Techniques

# Experiment



- EXAFS gives radial information
- Crystallography gives atom position

By utilizing geometry, some  
Relative positional information can be  
gained from  
the EXAFS data



# Rotate\_Atoms: A Program for Generating Cartesian Coordinates in Atom Clusters

Coordinate Table:  
X,Y,Z positions for each atom  
can be edited

Pull Down File Menu:  
Reading and writing  
data sets

Pull Down Animation Menu:  
Spin the image in X,Y or Z  
planes

Pull Down Make Movies Menu:  
Spin the image in X,Y or Z planes  
and saves as an mpeg movie

Pull Down Selects for any  
three combinations of atoms:  
angles and distances can be  
edited and resultant changes  
in cartesian coordinates are  
updated in Coordinate Table

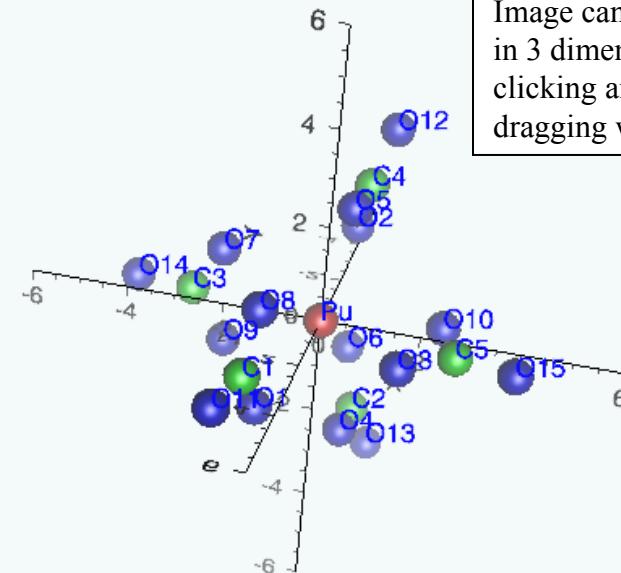
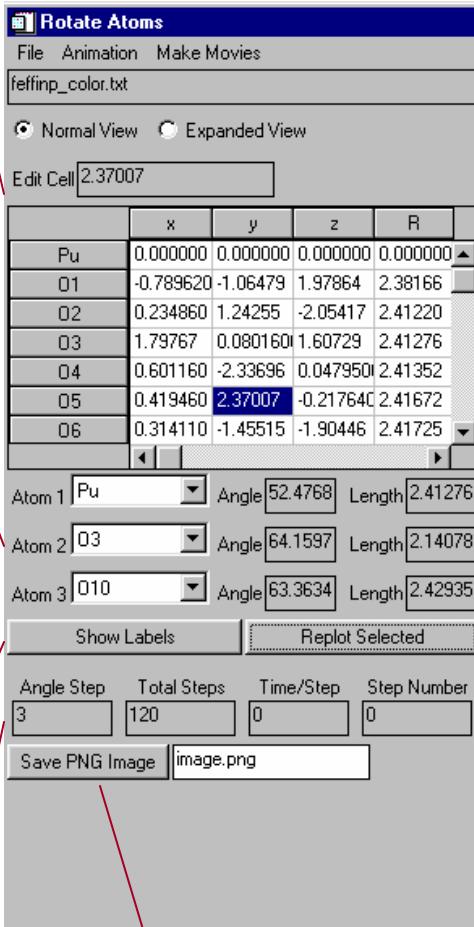
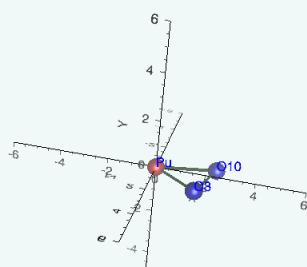


Image Window:  
Image can be rotated  
in 3 dimensions by  
clicking and  
dragging with mouse

Control Buttons to show/hide  
atom labels;plot all atoms/plot  
3 selected atoms

Animation parameters for  
spinning the image

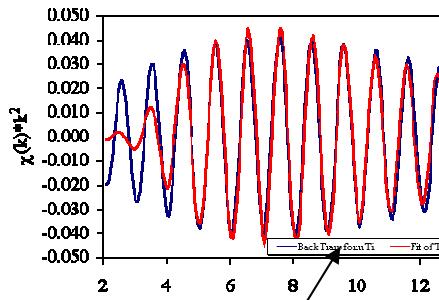
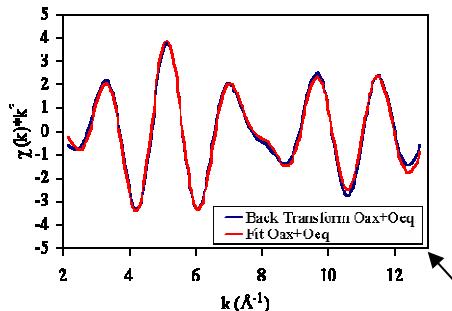
Save current image to portable  
network graphics file format

Current X,Y,Z angles of rotation

X Rotation -26.253383 Y Rotation 8.4214119 Z Rotation 5.1266959

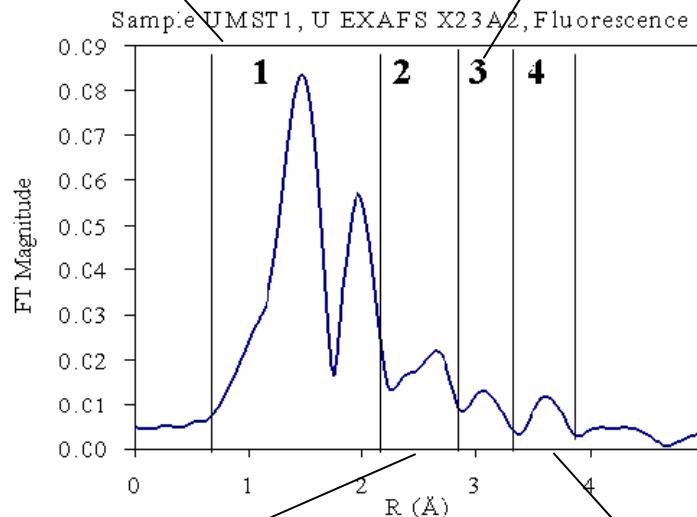
### Example: Uranium Adsorbed to Mono-Sodium Titanate

Region 1:  
High quality fit over  
entire back-transform  
for 2 sub-shells of  
oxygen



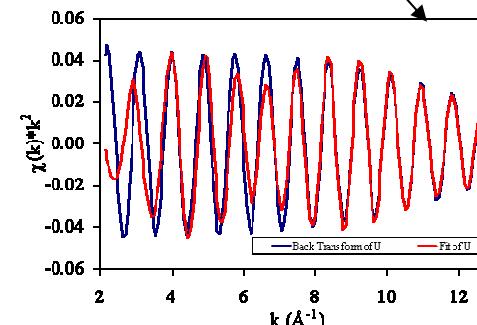
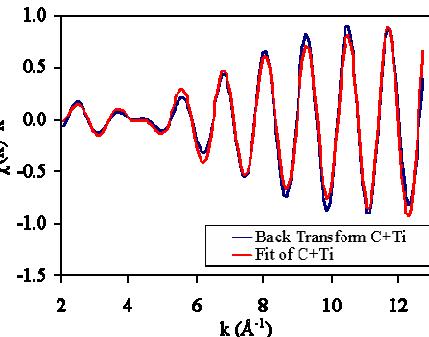
Region 3:  
Greater discrepancies  
in low  $k$  fit-probably  
due to other  
backscatters not  
included in fit

		CN	$R[\text{\AA}]$	$\sigma^2[\text{\AA}]^2$
Region 1	$U-O_{\text{ax}}$	2.11	1.86	0.002
Region 1	$U-O_{\text{eq}}$	4.98	2.41	0.007
Region 2	$U-C$	1.48	3.09	0.003
Region 2	$U-Ti$	0.53	2.97	0.001
Region 3	$U-Ti$	0.64	3.70	0.003
Region 4	$U-U$	1.20	3.84	0.007

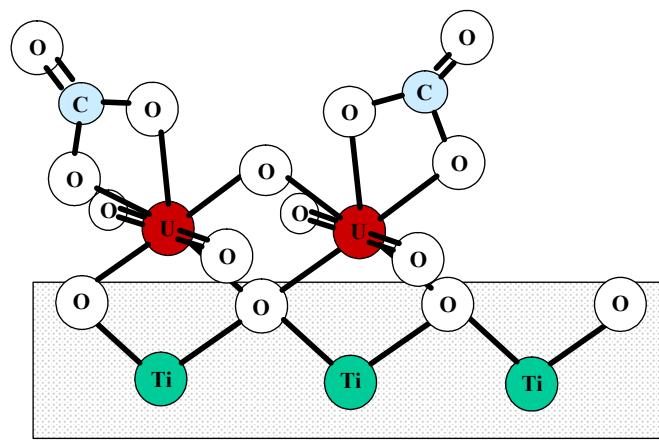
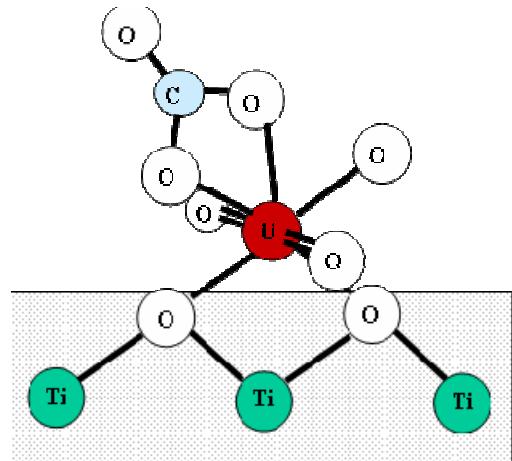


An extremely  
complicated spectrum  
  
Do you believe the fit  
results?

Region 2:  
The beat pattern exists  
in low  $k$ -suggested the  
possibility of a second  
subshell attributable  
to a low  $z$ -  
backscatterer



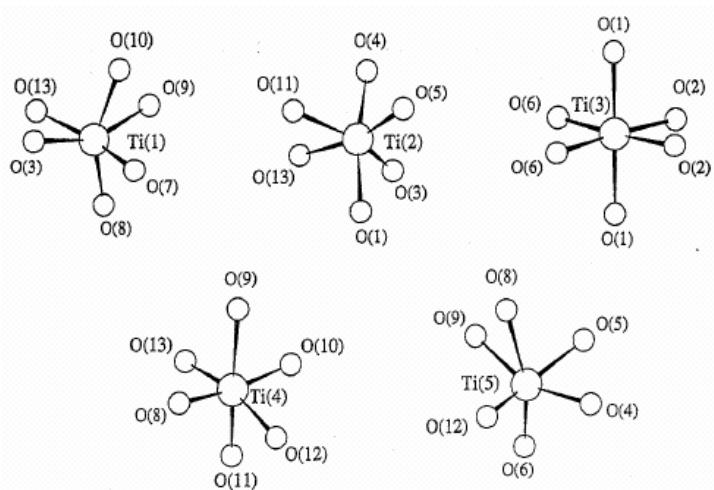
Region 4:  
Fit is even worse-but  
sufficient to identify  
primary component  
arising from a heavy  $z$   
backscatterer



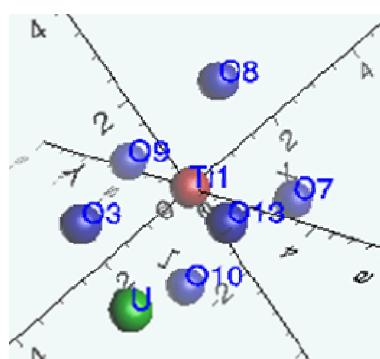
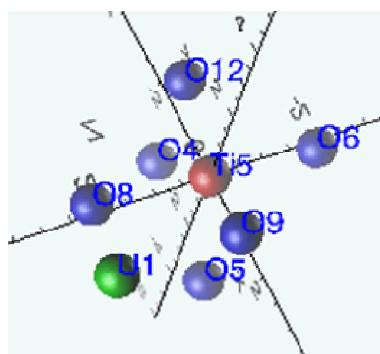
Do you believe the fits or at least the element identifications?

- The oxygen fits are typical for uranyl in CN, R and  $\sigma^2$
- The distance for carbon is typical for uranyl carbonate complexes ( $\text{CO}_2$  liberation was later measured on the samples). Not much can be said for CN and  $\sigma^2$ .
- The U-U distance is typical of oxyhydroxide precipitates of uranium(VI).

		CN	R[Å]	$\sigma^2[\text{\AA}]^2$
Region 1	U-O <sub>ax</sub>	2.11	1.86	0.002
Region 1	U-O <sub>eq</sub>	4.98	2.41	0.007
Region 2	U-C	1.48	3.09	0.003
Region 2	U-Ti	0.53	2.97	0.001
Region 3	U-Ti	0.64	3.70	0.003
Region 4	U-U	1.20	3.84	0.007



Long U-Ti Distance

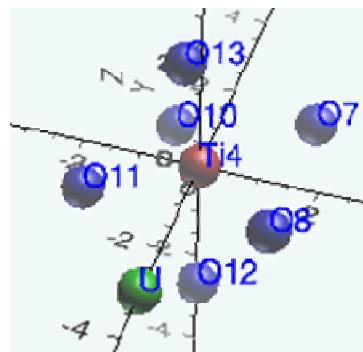


## What about the U-Ti fits?

- Monosodium titanate is amorphous but is considered to be very similar to nonatitanate for which the crystal structure was determined at NSLS
- There are 5 distorted Ti octahedra in the structure
- U-Ti distances are consistent with potential binding sites on specific distorted Ti octahedron

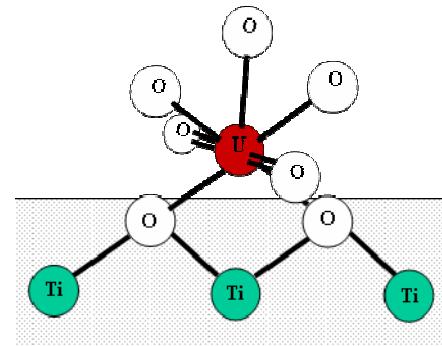
		CN	R[Å]	$\sigma^2[\text{Å}]^2$
Region 1	U-O <sub>ax</sub>	2.11	1.86	0.002
Region 1	U-O <sub>eq</sub>	4.98	2.41	0.007
Region 2	U-C	1.48	3.09	0.003
Region 2	U-Ti	0.53	2.97	0.001
Region 3	U-Ti	0.64	3.70	0.003
Region 4	U-U	1.20	3.84	0.007

Short U-Ti Distance



## What Can I conclude?

- The presence of carbon raised the issue of carbonate complexation that was not initially considered. There are many examples in the literature where researchers are so fixated on the complexation being studied that other ternary or alternate complexes are missed or mis-identified. In this case, the uranyl carbonate complexes may not have been associated with the surface...ie, *multiple phases present*.
- The uranium-titanium distances are consistent with distorted titanium octahedron. Such distortions may be more highly prevalent in amorphous systems than in highly crystalline systems. Uranium does not appreciably adsorb to anatase ( $\text{TiO}_2$ ).
- The uranium-titanium distances are all consistent with **bidentate adsorption** which is typically more stable than unidentate adsorption



I have not really touched on metallo-protein work

- In general these samples are already reasonably pure and consist of a low-z matrix
- Changes in conformation may be more subtle and require care that quality data is collected
- Interest in catching conformational changes during catalytic reactions-see personnel at X9 for these issues

Questions?