X-ray Absorption Spectroscopy : Application to Biological Systems

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U.S. DEPARTMENT OF ENERGY
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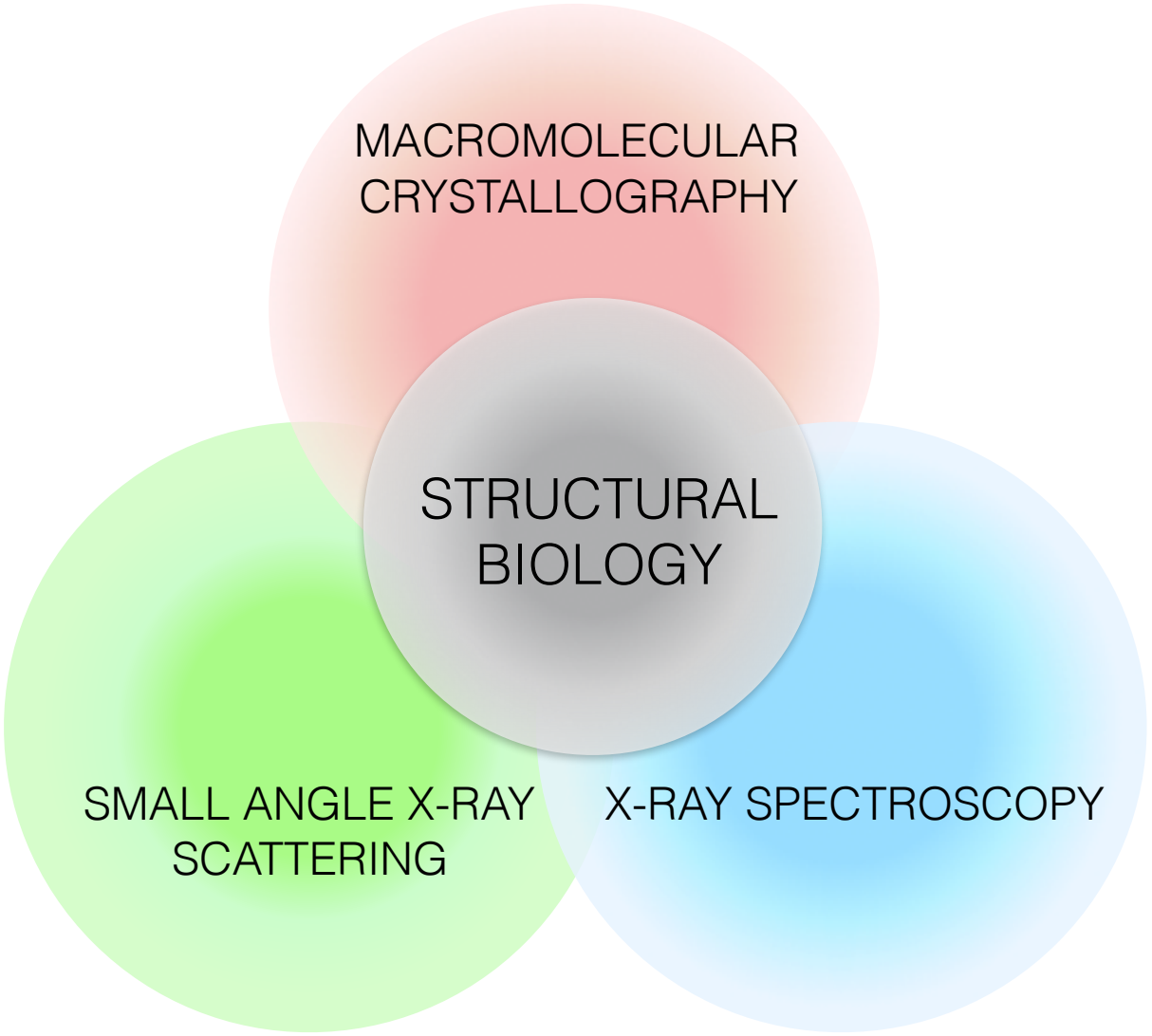
Synchrotron Science



Synchrotron Science

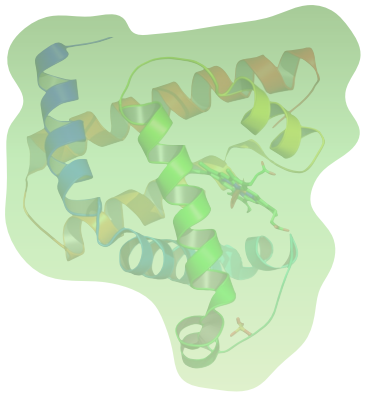
SMALL ANGLE X-RAY SCATTERING

MACROMOLECULAR CRYSTALLOGRAPHY

X-RAY SPECTROSCOPY

Synchrotron Science

Conformation



~50 –7 Å

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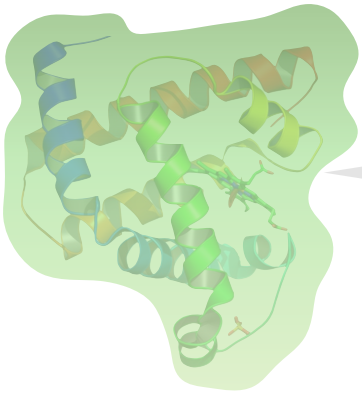

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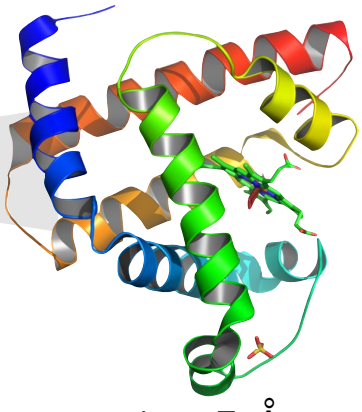
Atomic Position



~1 – 5 Å

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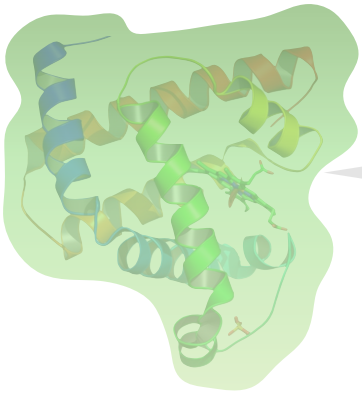

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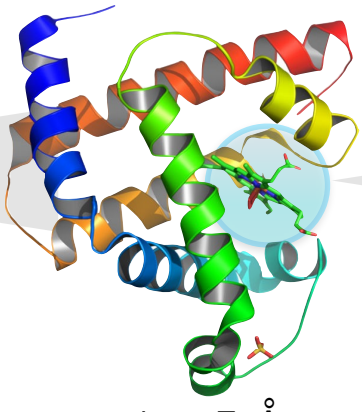
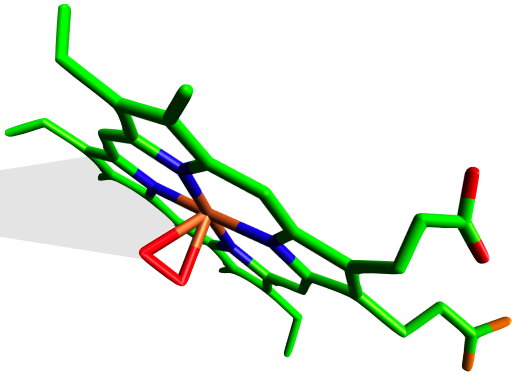


~1 – 5 Å

MACROMOLECULAR CRYSTALLOGRAPHY

Electronic Structure



>>1 Å

X-RAY SPECTROSCOPY

Outline

- Introduction of X-ray Absorption Spectroscopy (XAS)
- Importance of XAS
- Factors affecting the XAS Spectra : Information Content
- Some XAS Examples
- EXAFS and the Biological Complication
- Examples from Homogeneous Biological (and otherwise) Catalysis
- Single Crystal XAS : Combination of XAS & EXAFS

Basics of X-ray Absorption Spectroscopy (XAS)

An edge results when a core electron absorbs energy equal to or greater than its binding energy.

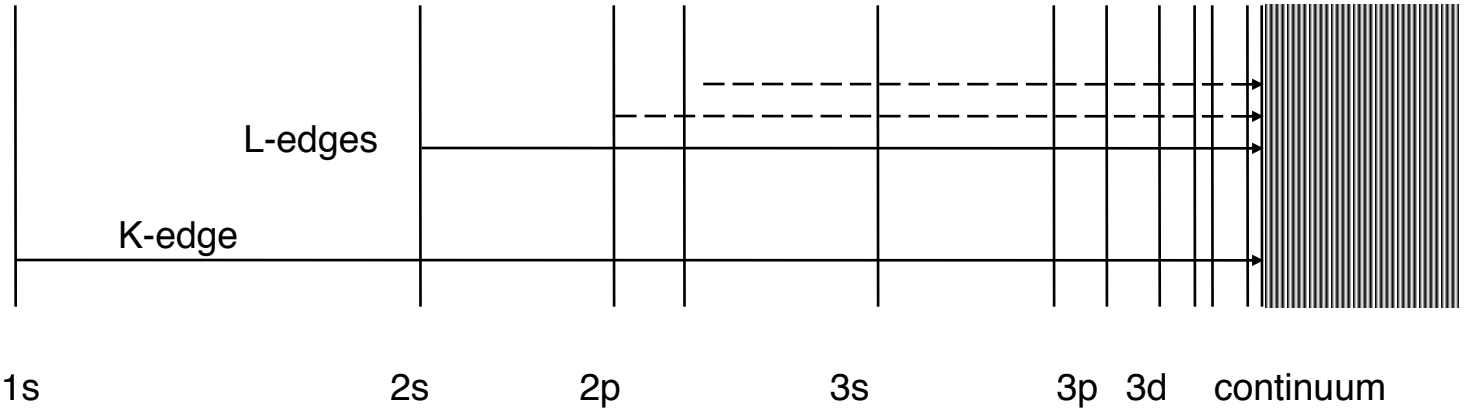
Edges are labeled according to the shell the core electron originates from.

XAS is an element specific technique.

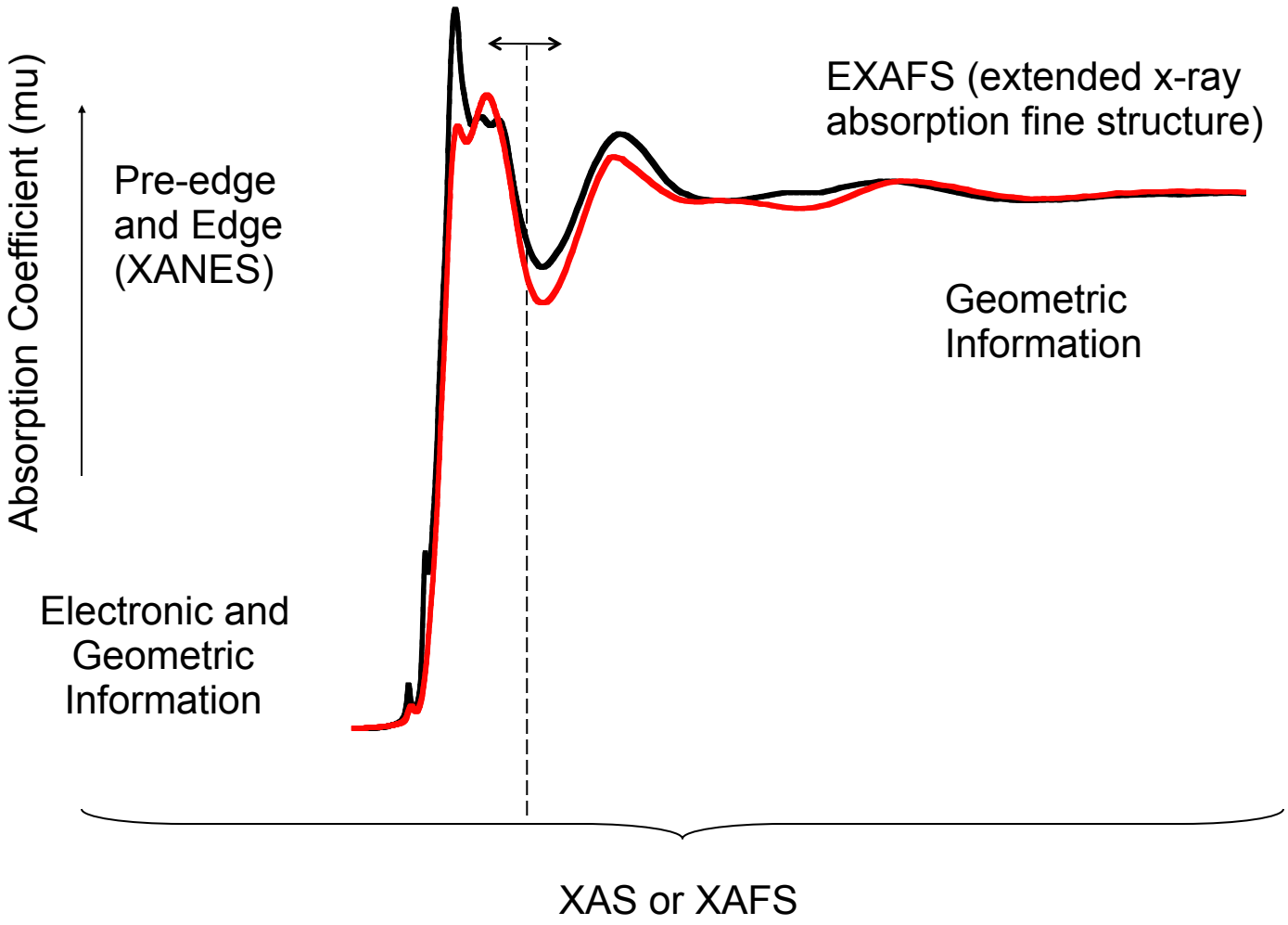
Cu K-edge ~9000 eV
Cu L-edges ~930 eV
Cu M-edges ~70-120 eV

Fe K-edge ~7000 eV
Fe L-edges ~720 eV
Fe M-edges ~50-100 eV

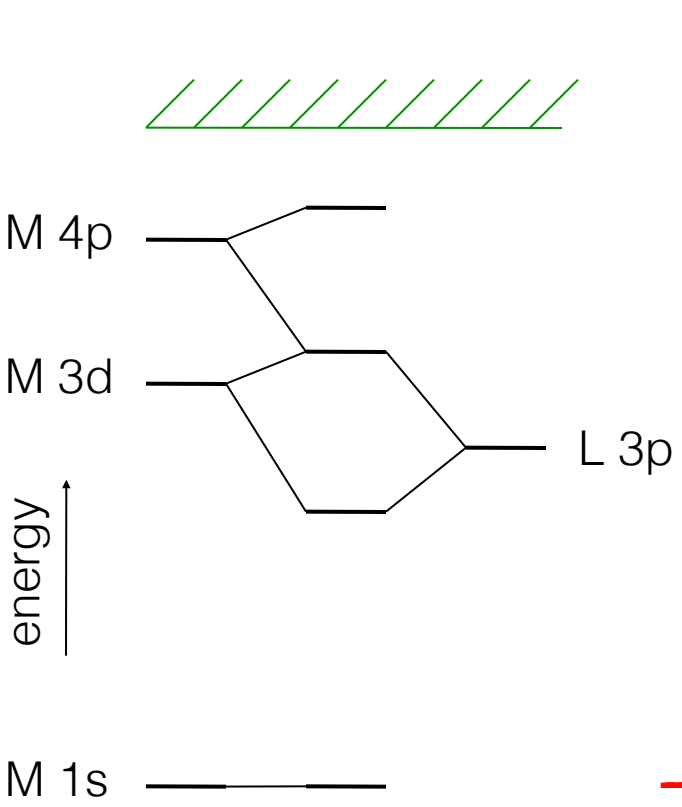
S K-edge ~2472 eV
S L-edges ~200 eV

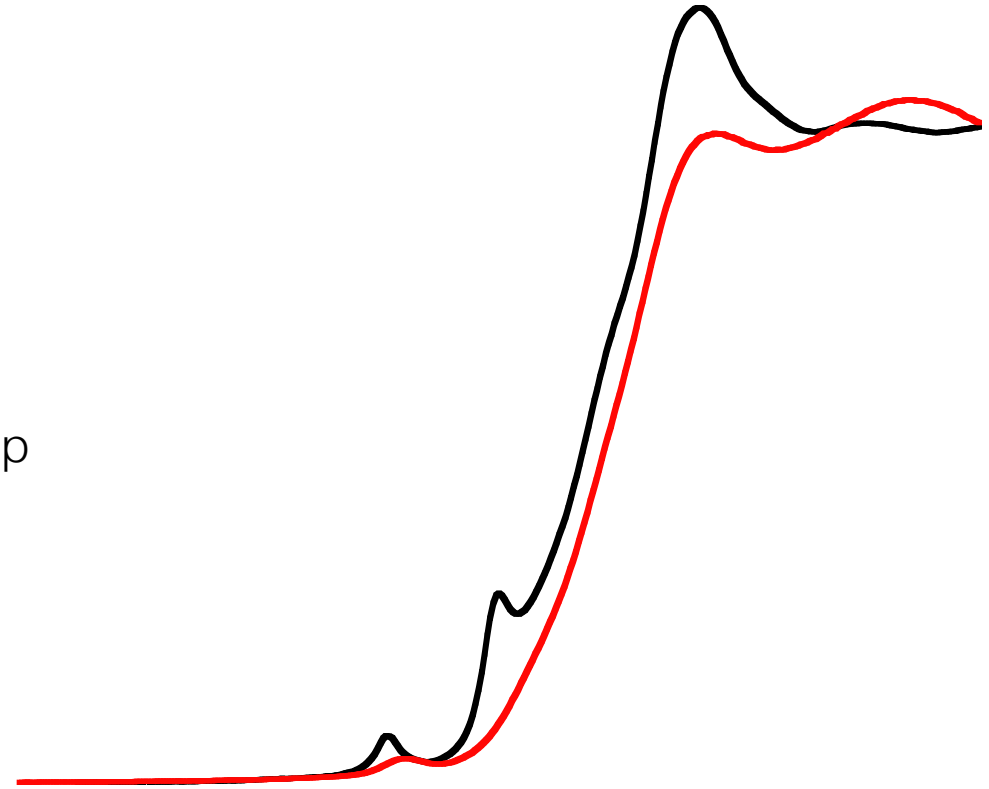


X-ray Absorption Spectrum (XANES + EXAFS Region)



Metal K-edge XAS





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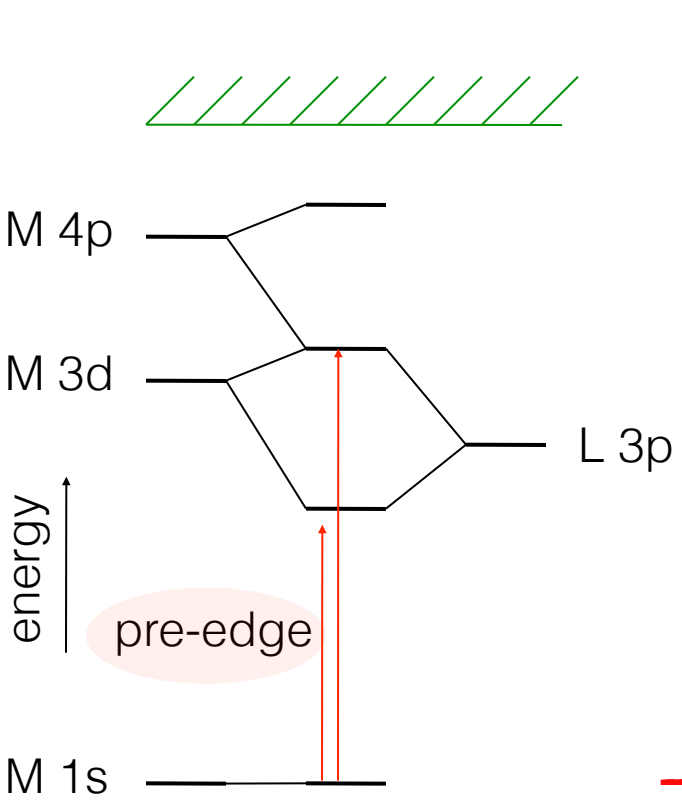

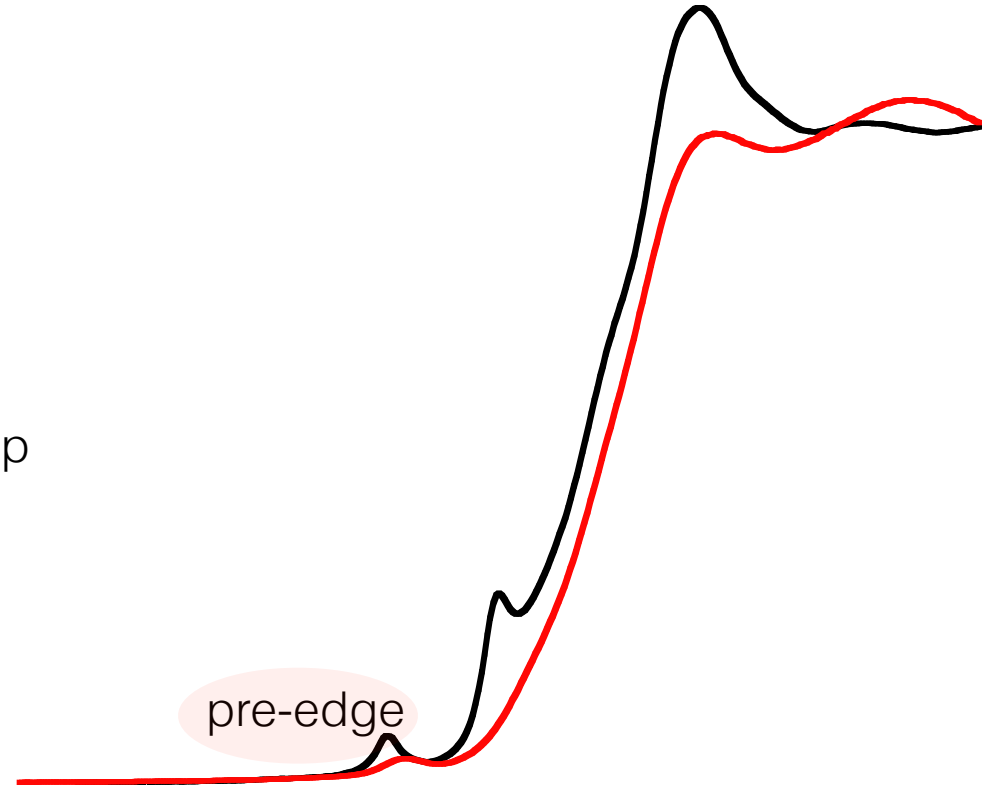

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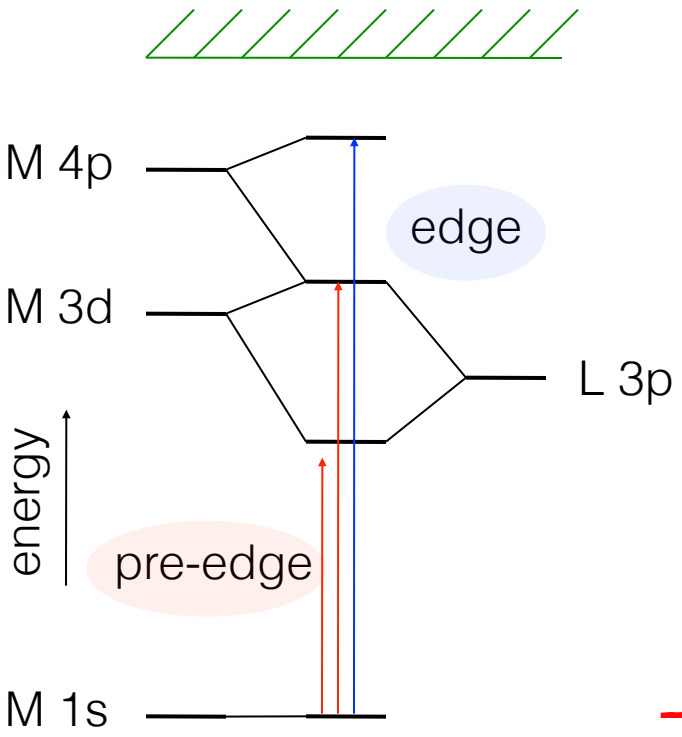

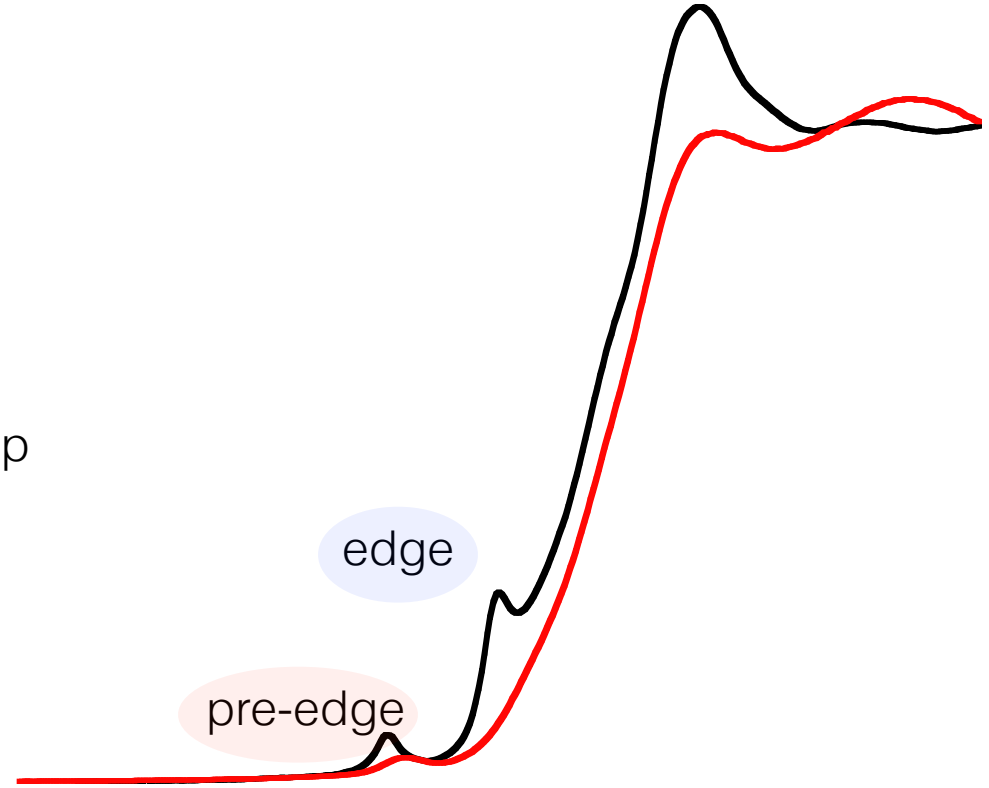

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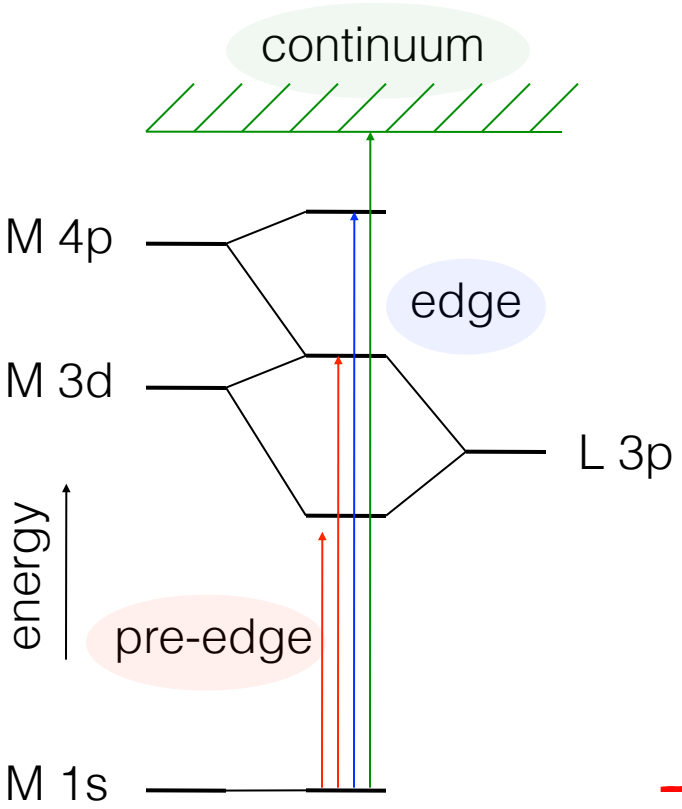

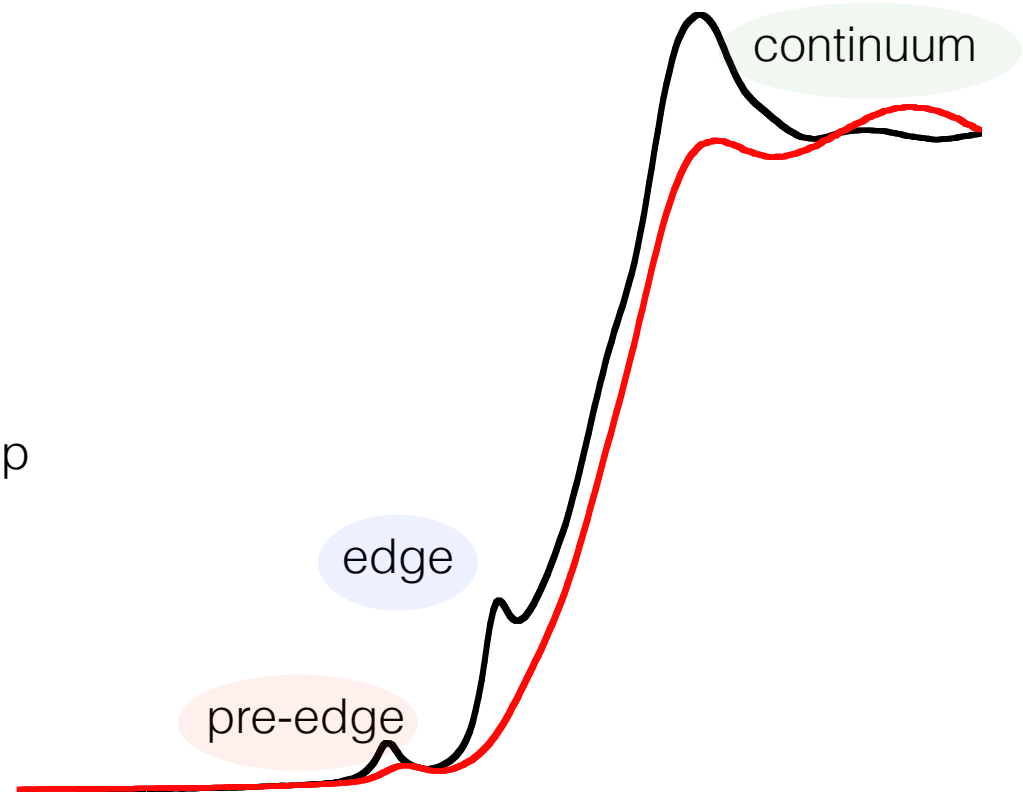

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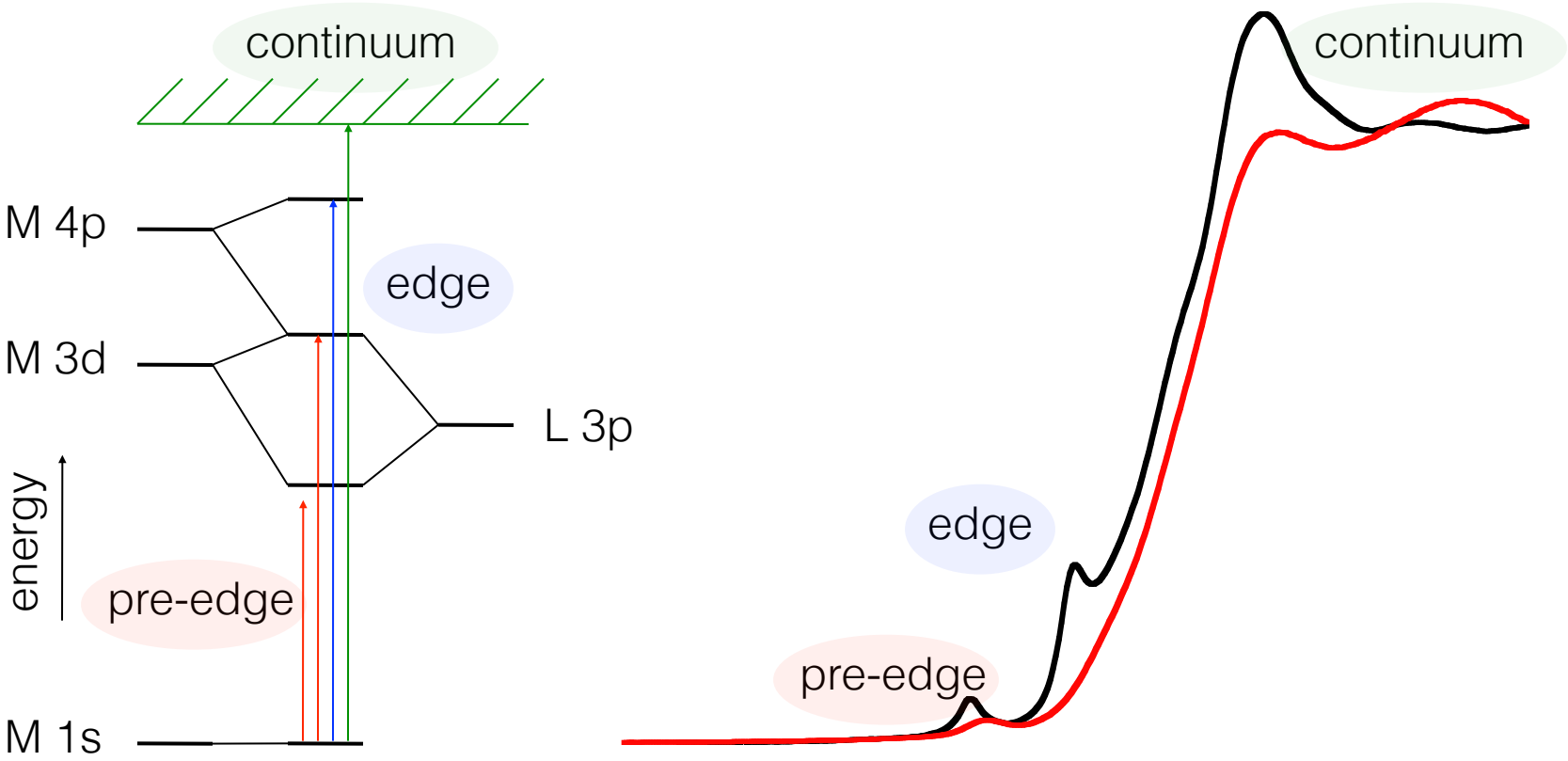

Metal K-pre-edge absorptions arise due to a quadrupole-allowed dipole-forbidden 1s ⟶ 3d excitation ($\Delta l = \pm 2$) - weak

Metal K-rising edge absorptions are electric dipole allowed ($\Delta l = \pm 1$)- Intense

Importance of XAS Edges

Fast data acquisition time and high signal-to-noise ratio. Can be measured at room temperature without depreciation of data quality. Used to estimate:

The pre-edge region can be used to estimate:
Ligand-field
Spin-State
Centrosymmetry

The rising-edge region can be used to estimate:

Geometric Structure
Metal-Ligand overlap via Shakedown transitions
Ligand arrangement in certain cases
Charge on the metal center

Interpretation of XAS Edges

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Qualitatively

Uses edges as a “fingerprint” of the electronic structure.
Compare to known model complexes.
Use in PCA analysis.

Molecular Orbital-Based Approach

Obtain a more quantitative description.
Energy and intensity distributions LF theory.
Works well for bound state transitions.
Fails for rising-edge and beyond.

Multiple Scattering-Based Approach

Required to simulate rising edge.
FEFF, MXAN.
Difficult to relate back to an MO-based picture.

Band Structure Approach

Density of States.

Factors that Affect Metal K-edge Shape and Energy

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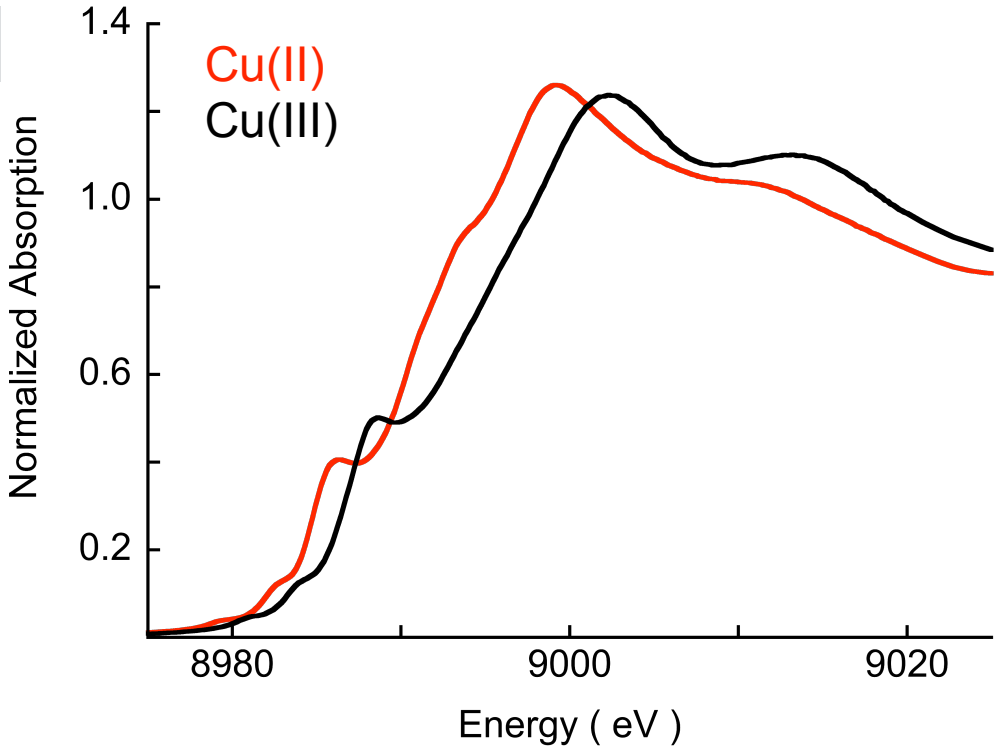
Oxidation State



The rising-edge and the edge maxima shift to higher energy as the oxidation state increases.

Important consideration – similar ligand system.

Factors that Affect Metal K-edge Shape and Energy

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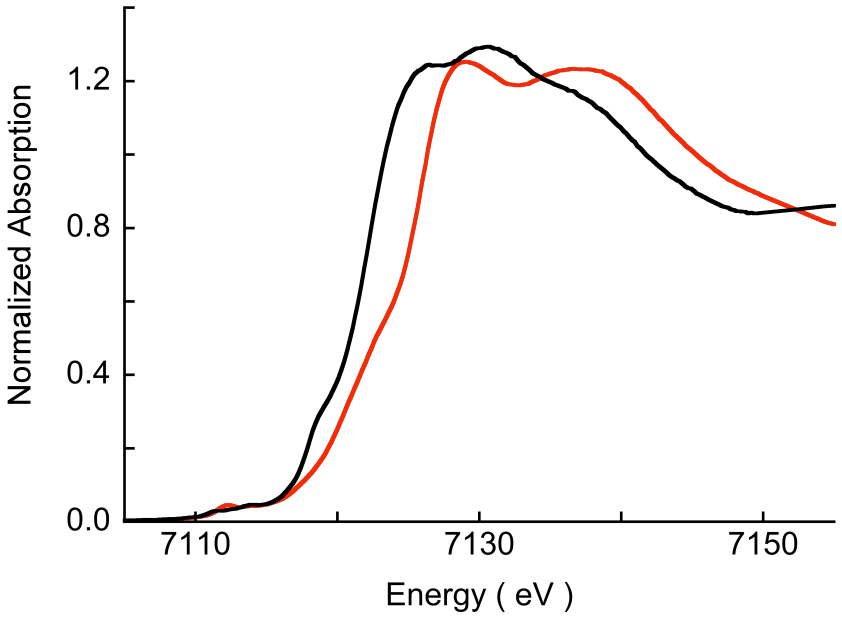
Oxidation State

Factors that Affect Metal K-edge Shape and Energy

Oxidation State



Both Fe samples. What oxidation states do they represent?

Factors that Affect Metal K-edge Shape and Energy

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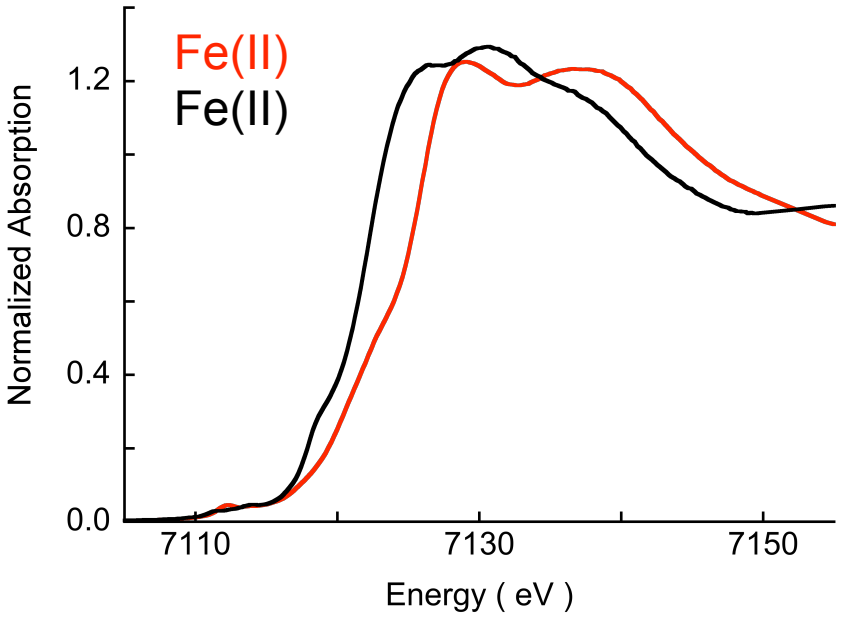
Oxidation State

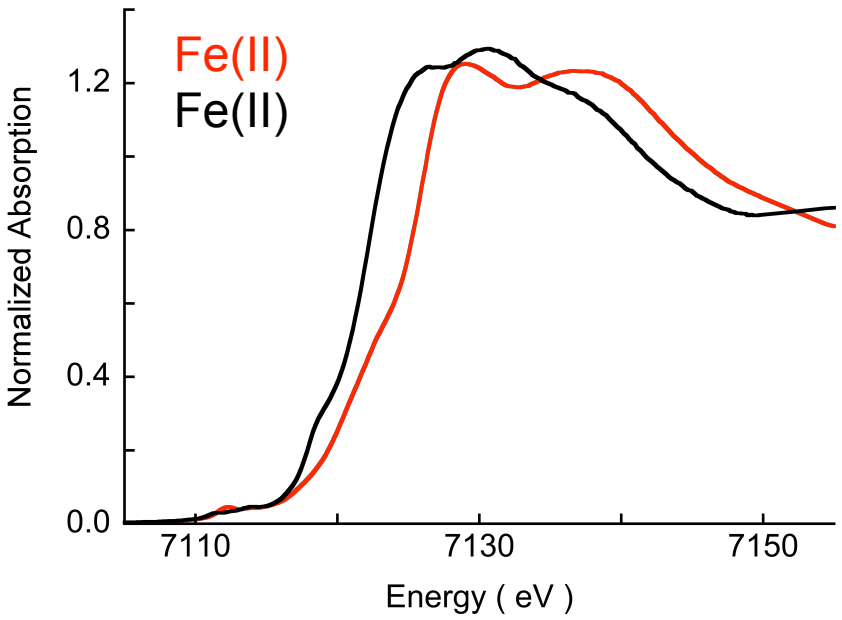


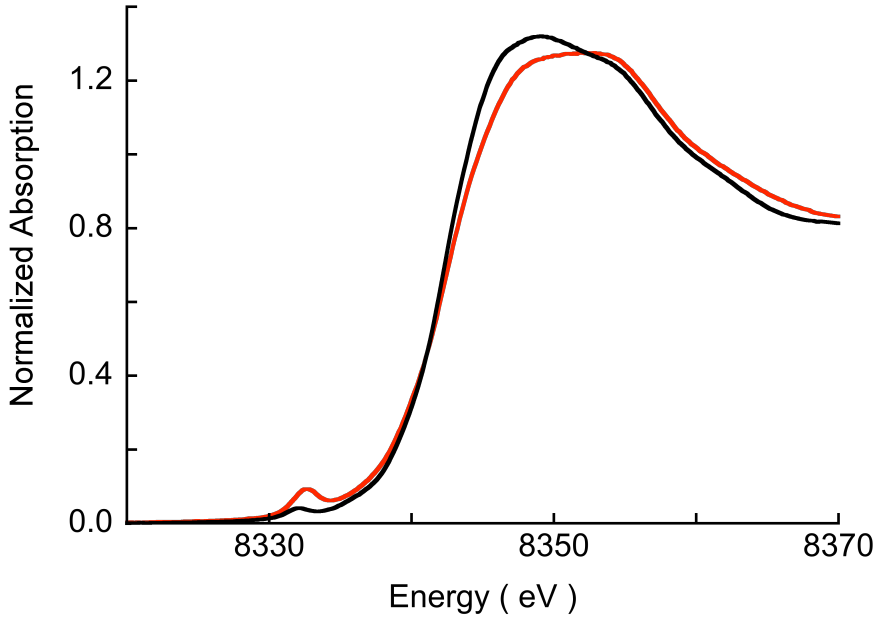
Both Fe samples. What oxidation states do they represent?

Spin states are different!
High-Spin (S=2) and Low-Spin (S=0)

Factors that Affect Metal K-edge Shape and Energy

Oxidation State





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Both Ni samples. What oxidation states do they represent?

Factors that Affect Metal K-edge Shape and Energy

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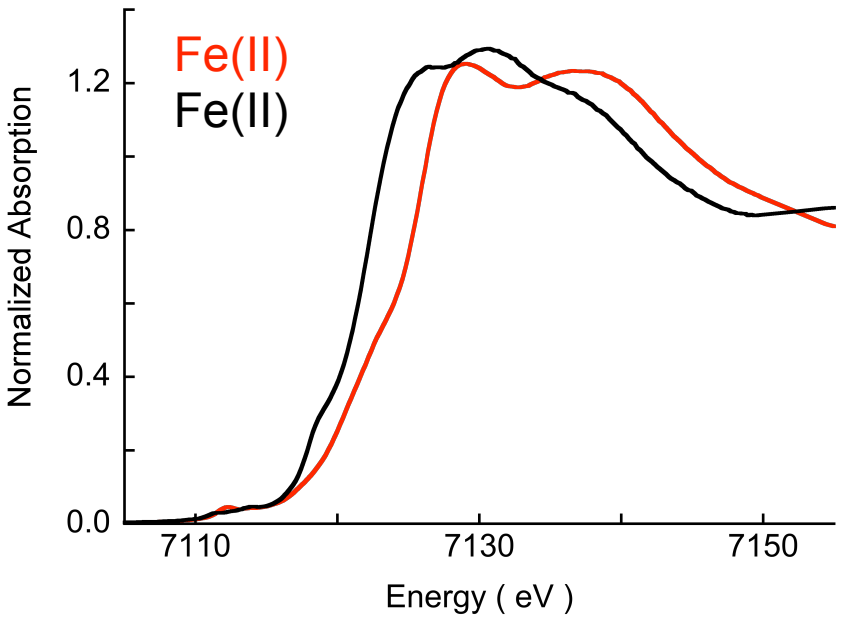
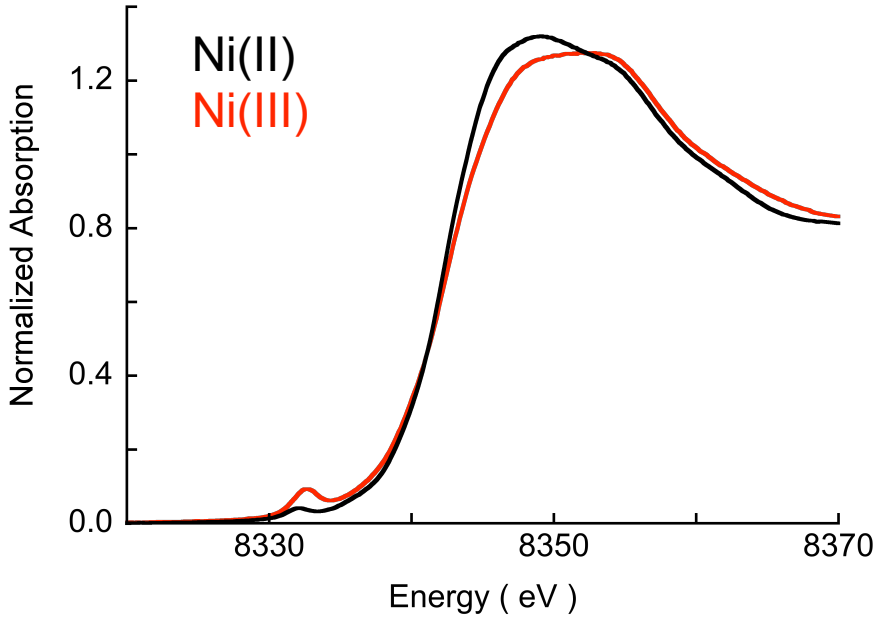
Oxidation State





Both Fe samples. What oxidation states do they represent?

Spin states are different!
High-Spin (S=2) and Low-Spin (S=0)

Both Ni samples. What oxidation states do they represent?

Ni is special case with little change upon oxidation.

Factors that Affect Metal K-edge Shape and Energy

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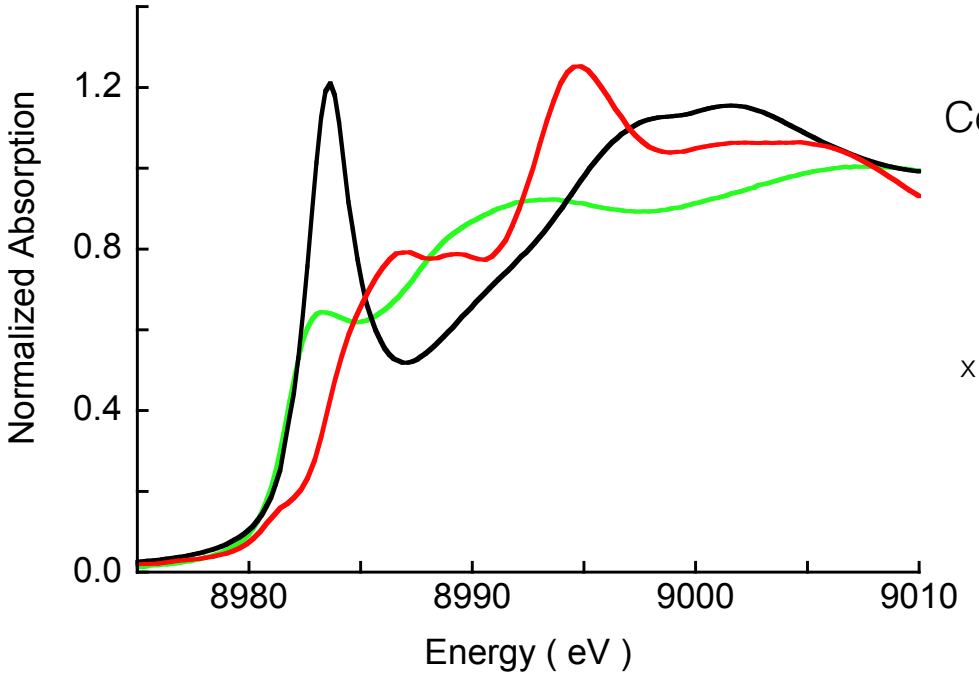
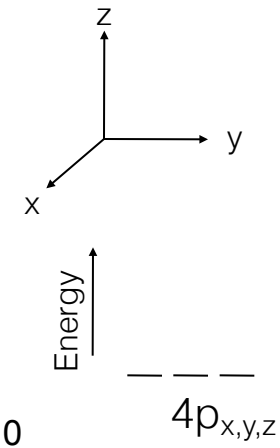
Coordination Number and Geometry

Factors that Affect Metal K-edge Shape and Energy

Coordination Number and Geometry

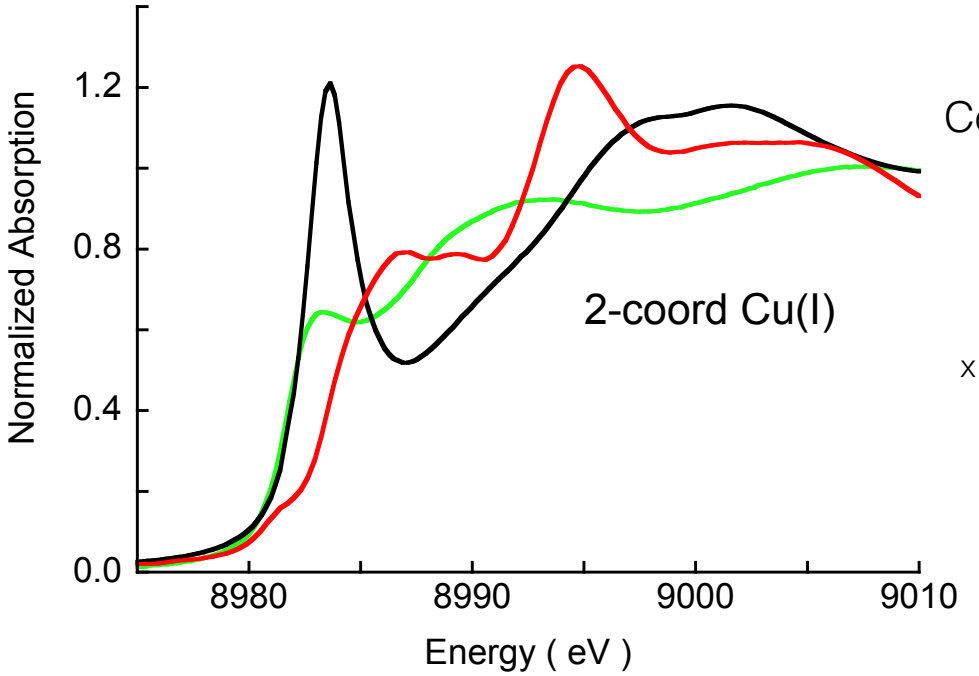


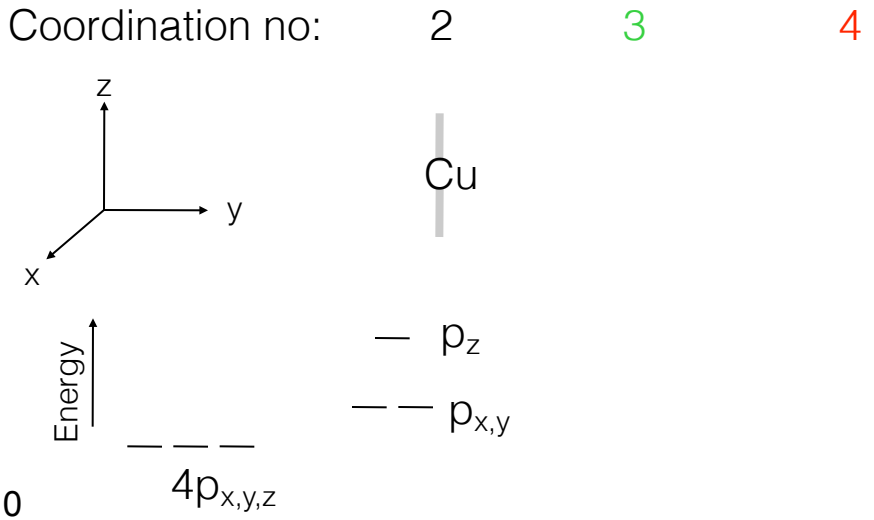
Coordination no: 2 3 4



Factors that Affect Metal K-edge Shape and Energy

Coordination Number and Geometry





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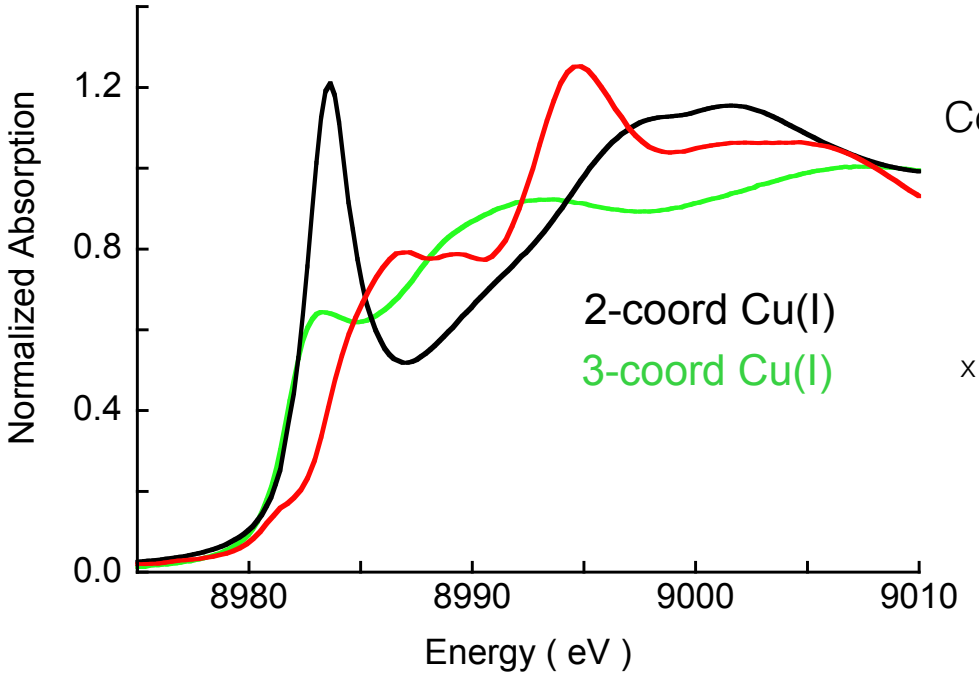

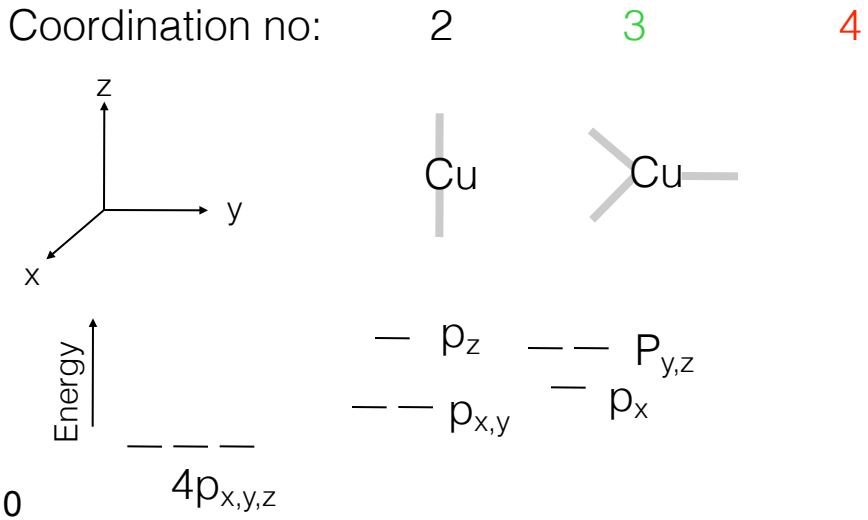

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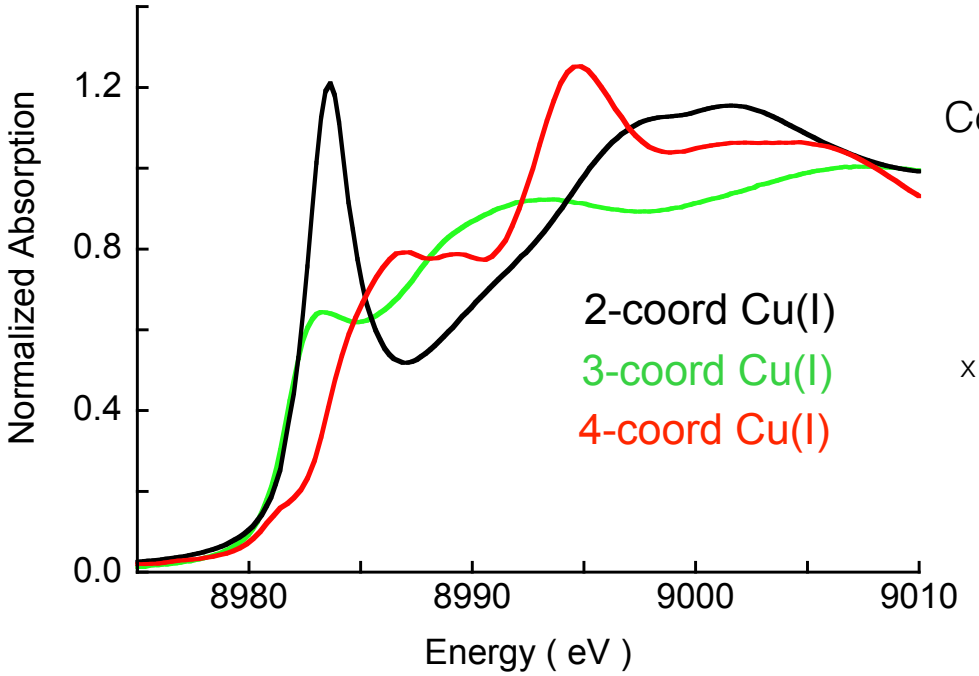

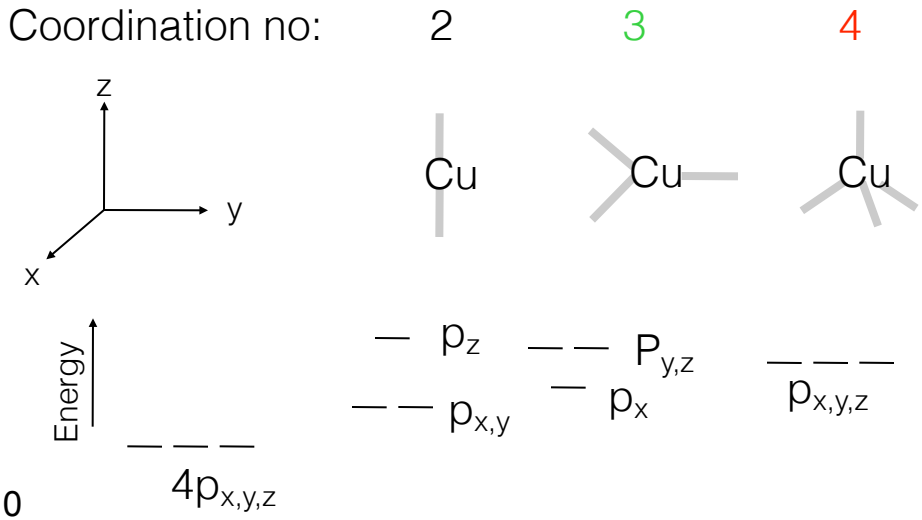

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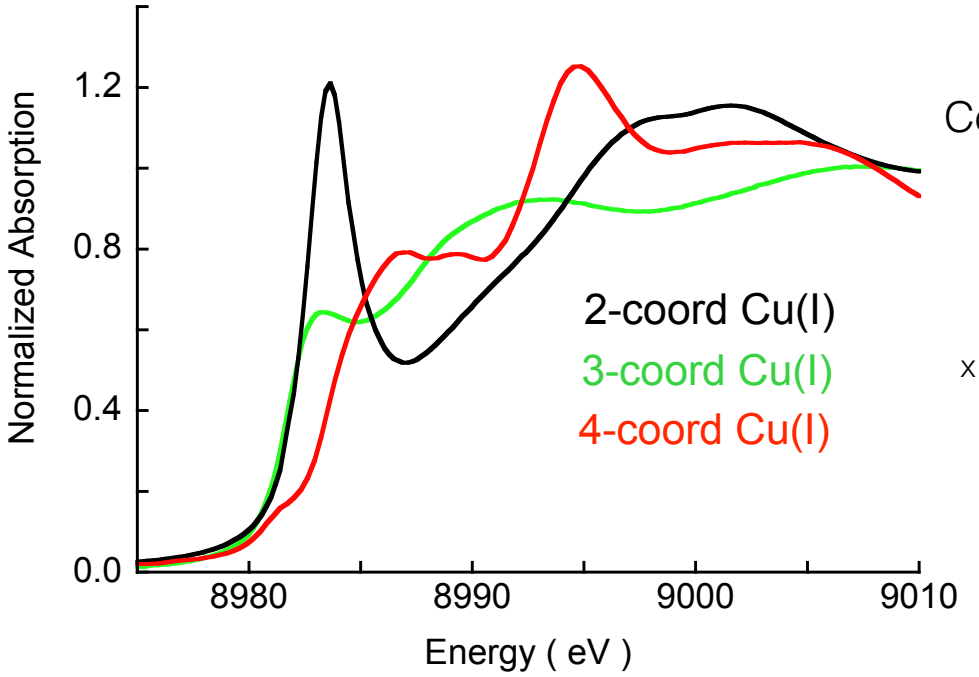

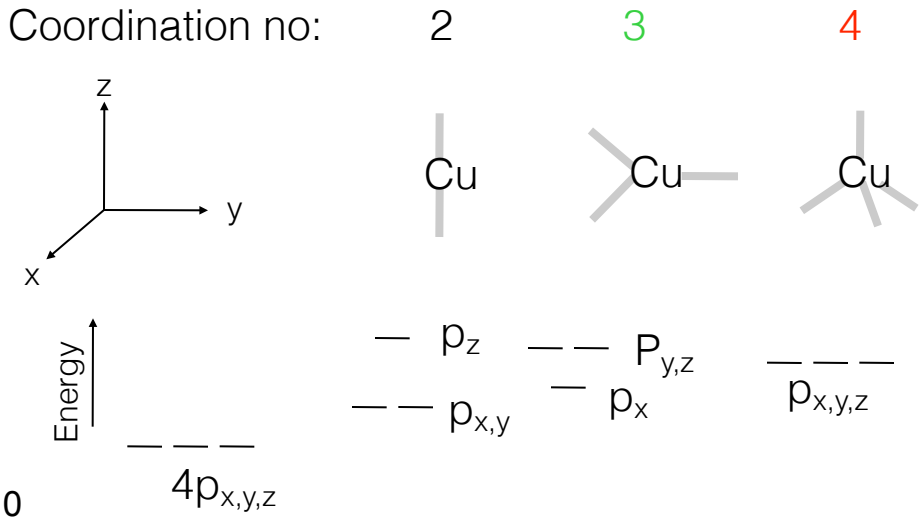

Rising edge has strong contribution from the 1s ⟶ 4p transition.

In special cases where the 4p orbital is low-lying, the energy and intensity of the edge transition can be used to estimate coordination number/geometry

Factors that Affect Metal K-edge Shape and Energy

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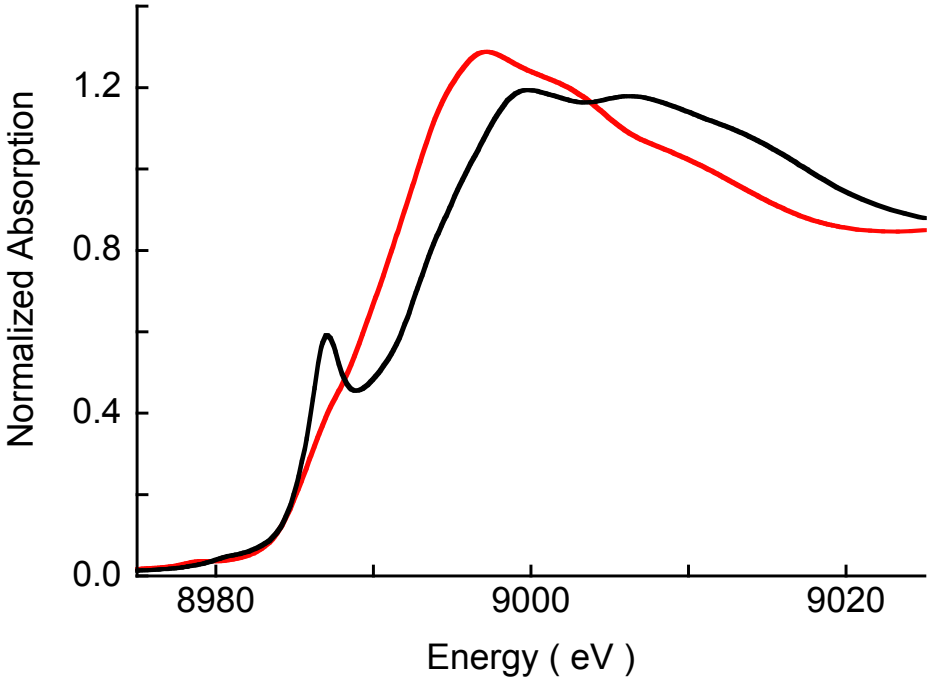
Covalency



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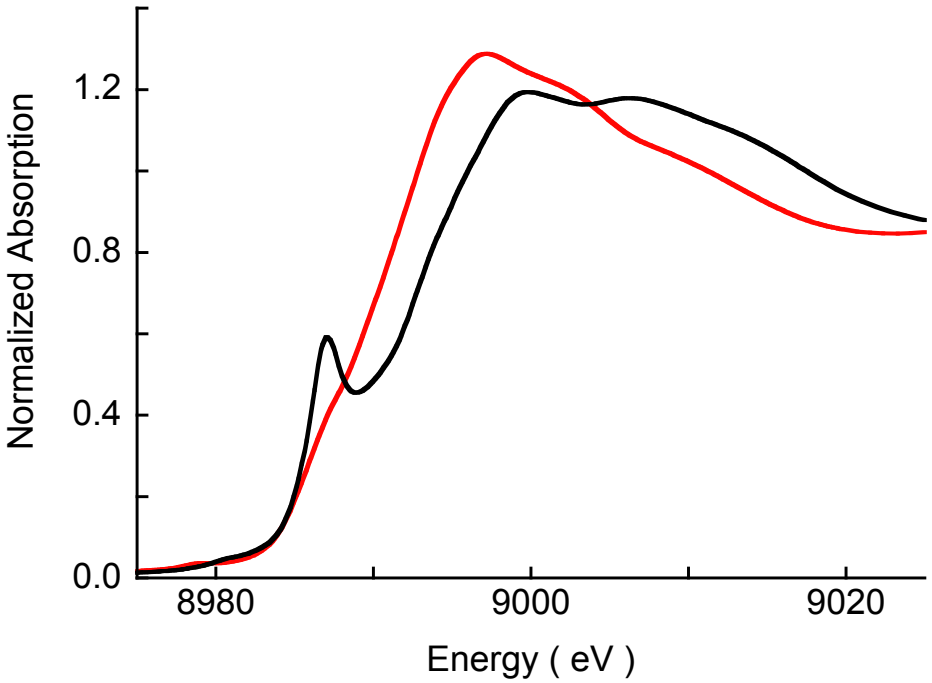

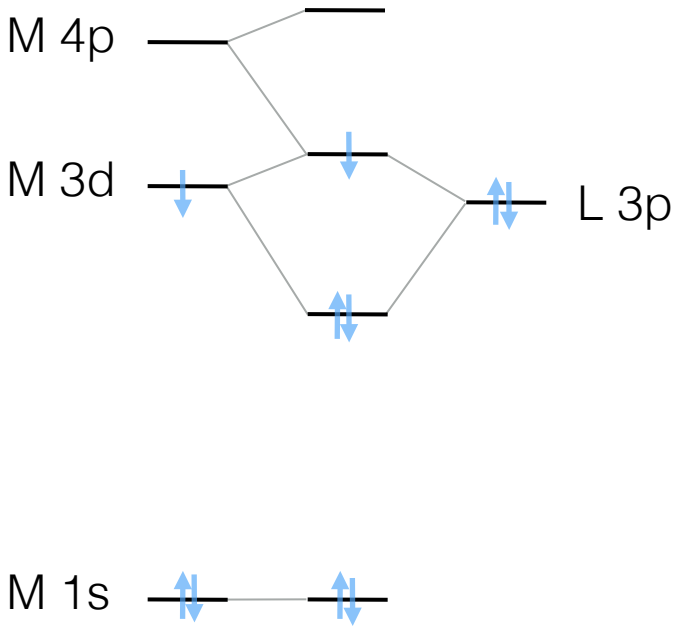

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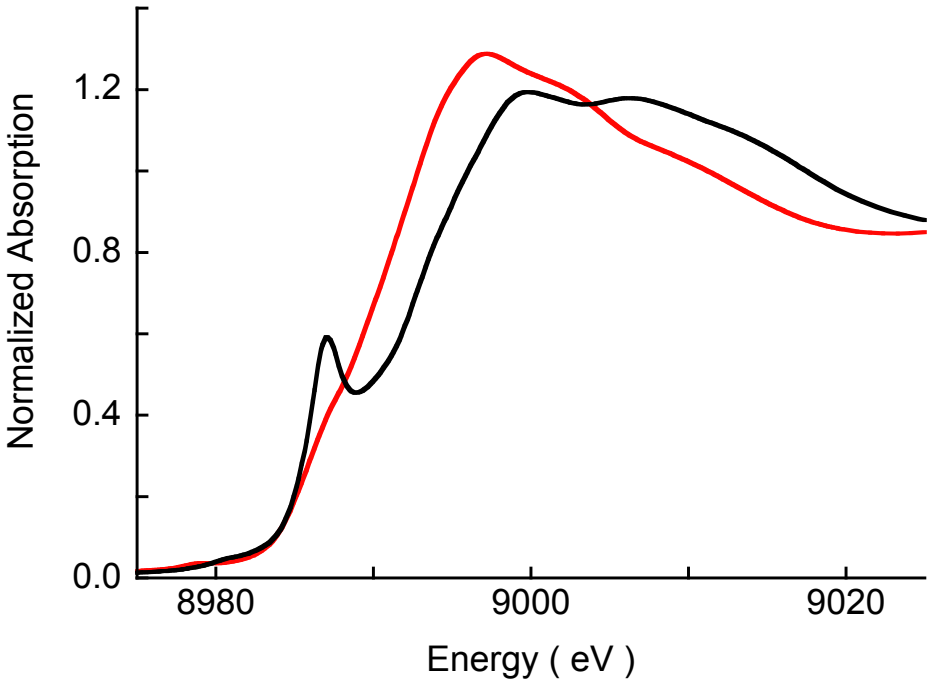

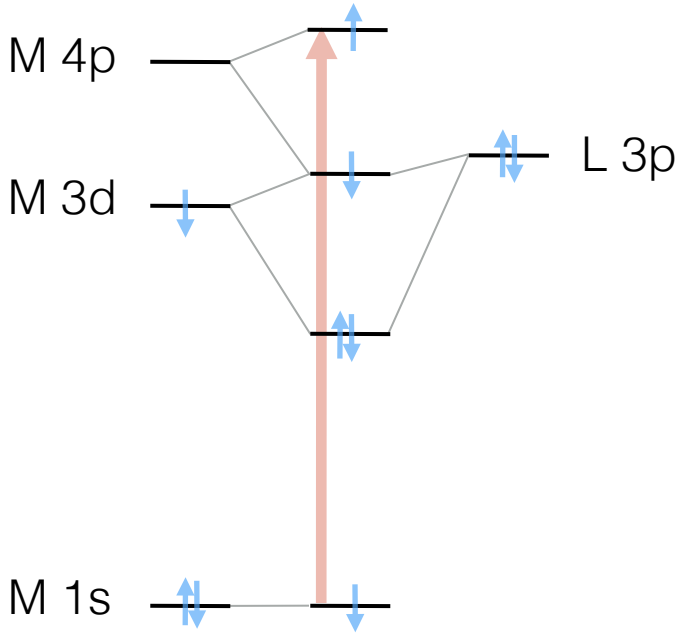

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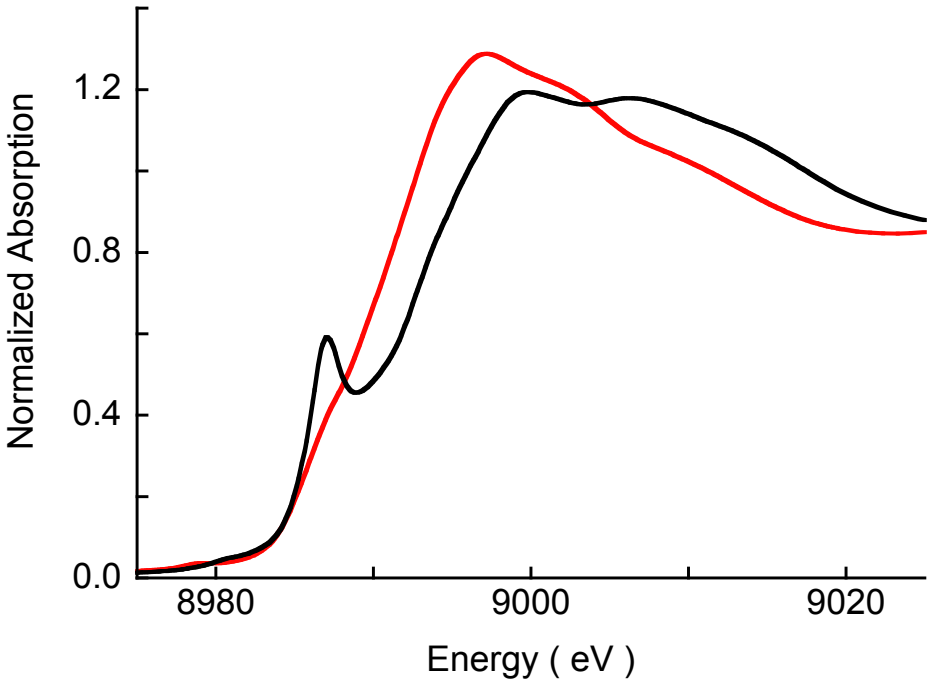

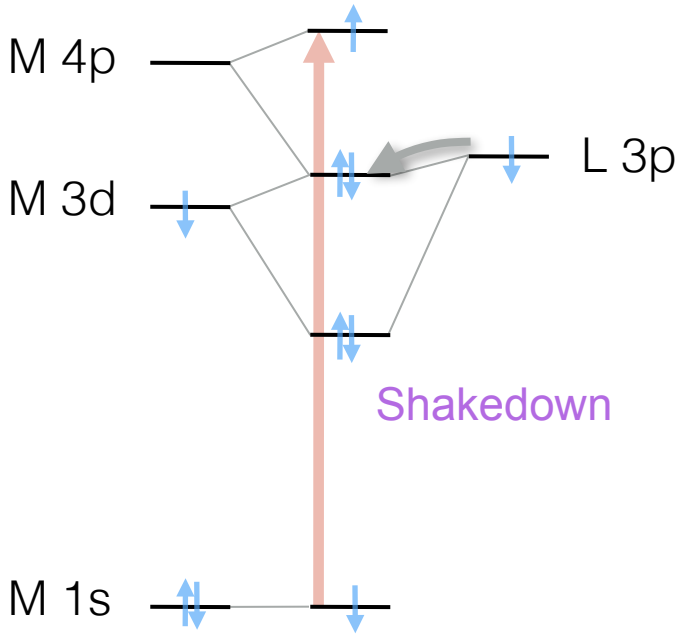

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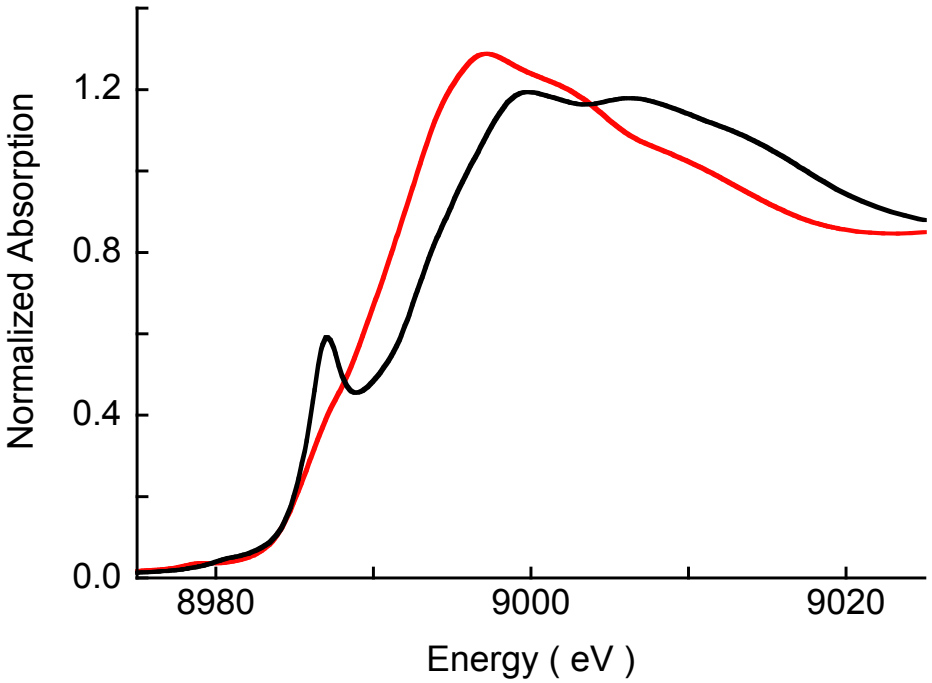
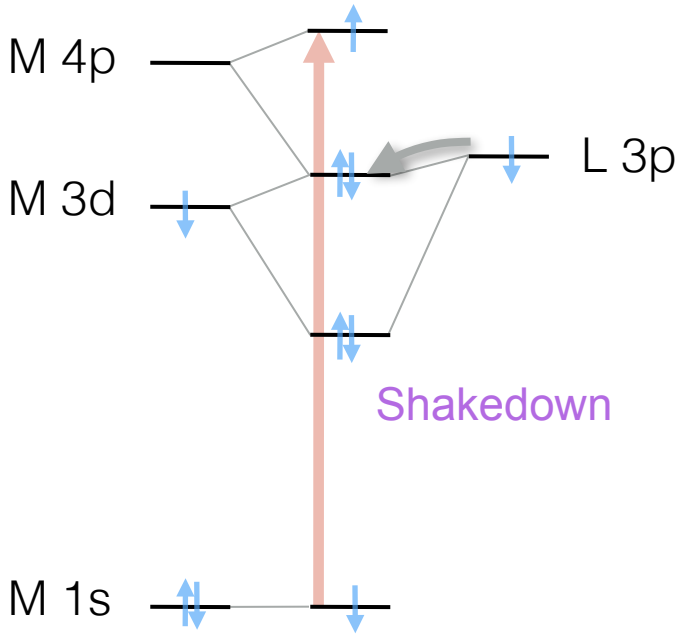


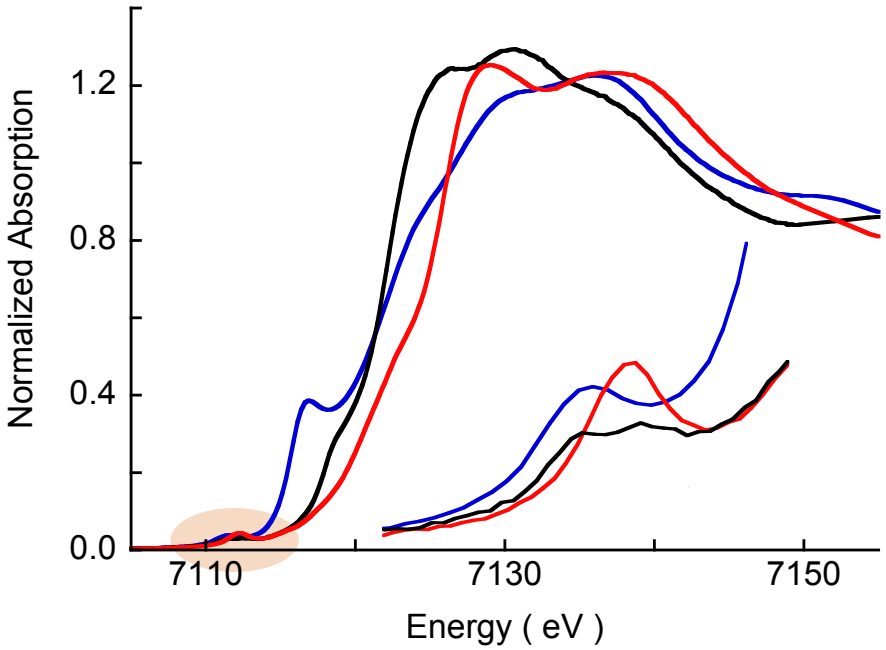


Energy and intensity can be correlated with metal-ligand overlap using the VBCI model. Typically transition present in square-planar complexes.

In comparable systems: Intensity ∝ Covalency Energy ∝ 1/Covalency

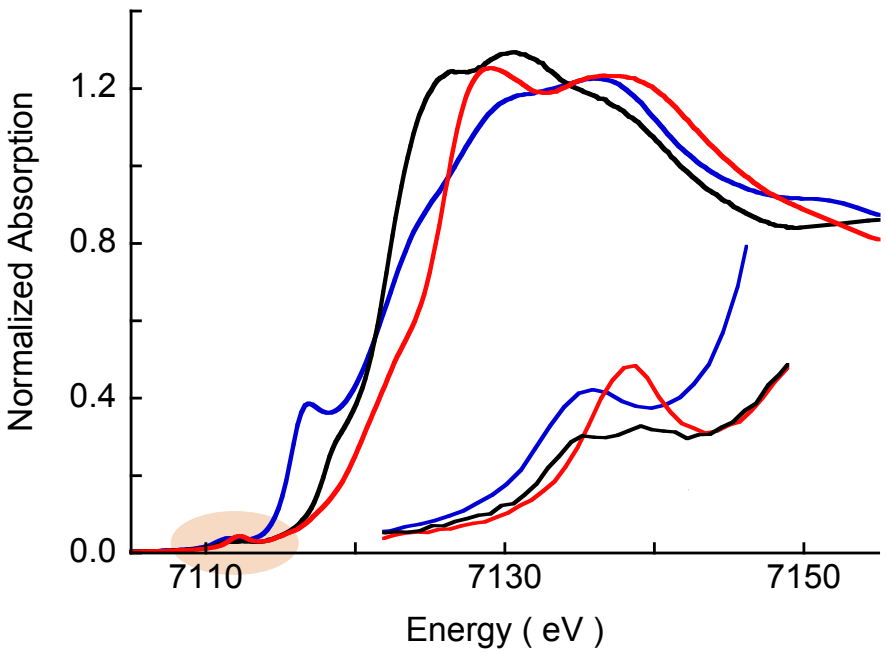
Factors that Affect Metal K-edge Shape and Energy

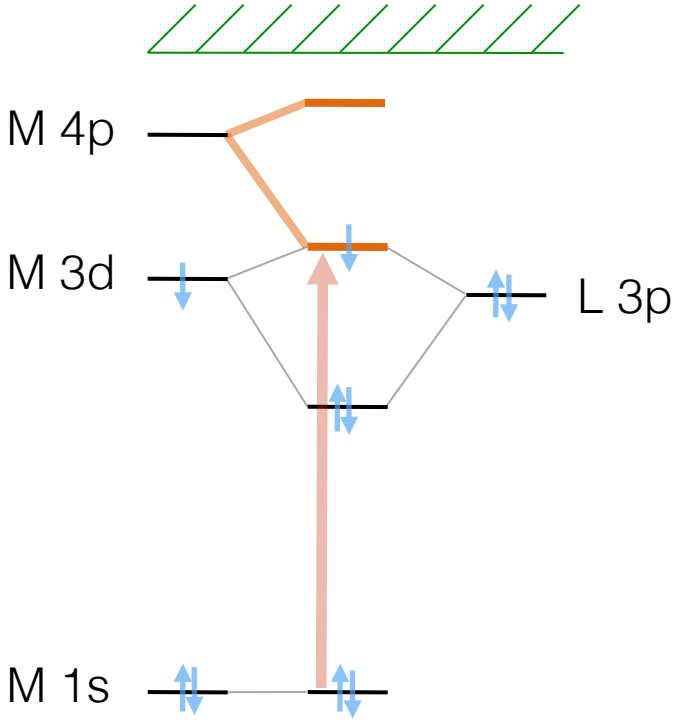
Pre-edge Shape and Energy



Factors that Affect Metal K-edge Shape and Energy

Pre-edge Shape and Energy





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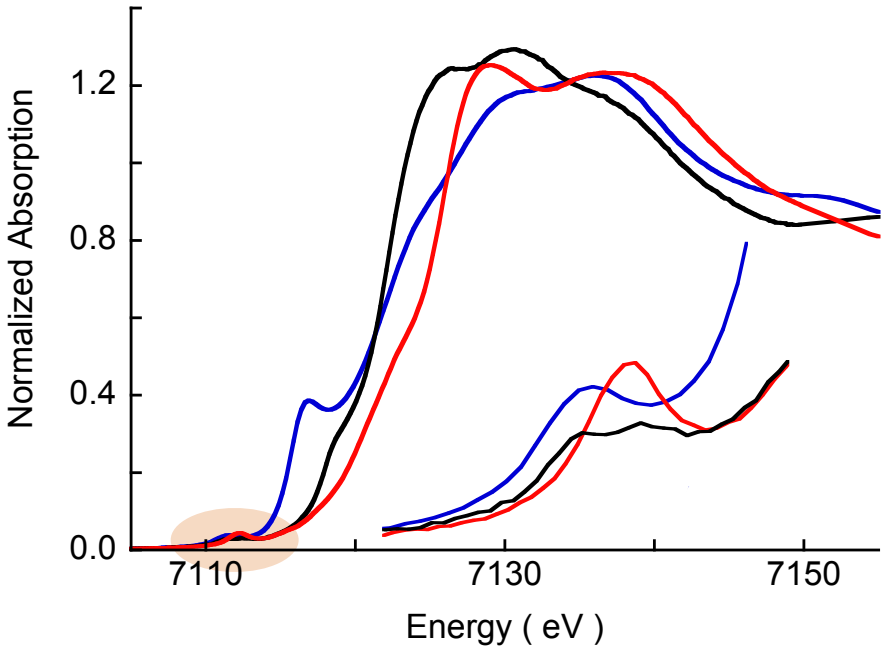
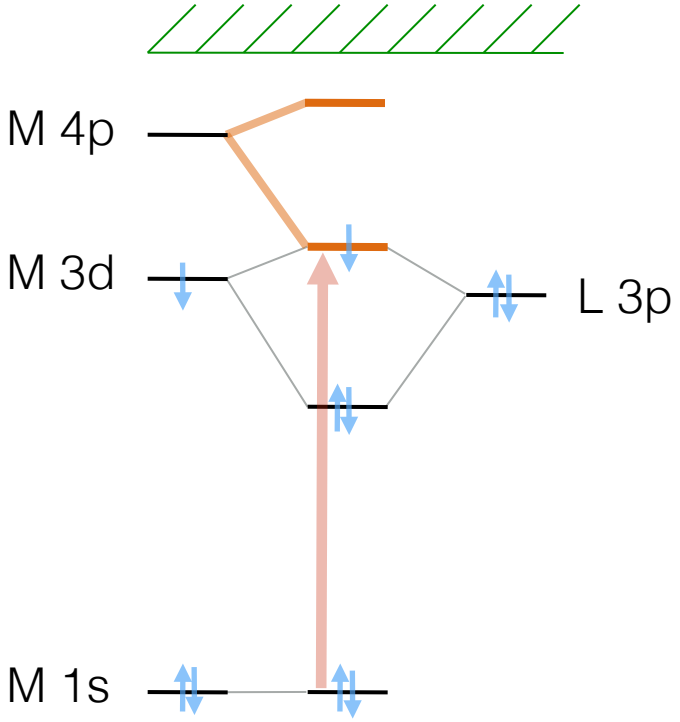
Pre-edge Shape and Energy

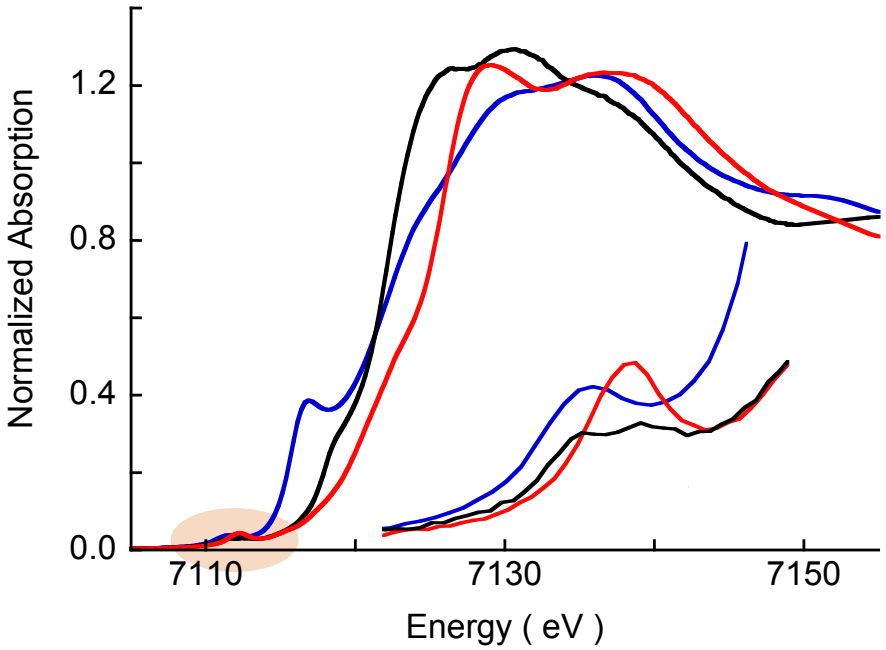


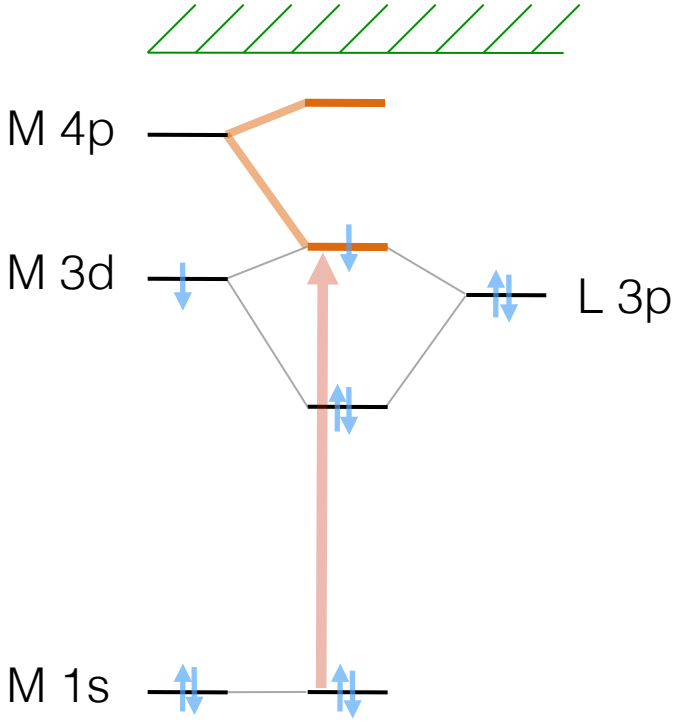


Pre-edge intensity ∝ Deviation from Centrosymmetry ∝ Metal 3d-4p mixing

Factors that Affect Metal K-edge Shape and Energy

Pre-edge Shape and Energy





Pre-edge intensity ∝ Deviation from Centrosymmetry ∝ Metal 3d-4p mixing

Pre-edge intensity pattern is dependent on:

Spin-State b) Oxidation-State c) Ligand-Field splitting d) Multiplet-Effects

Factors that Affect Metal K-edge Shape and Energy

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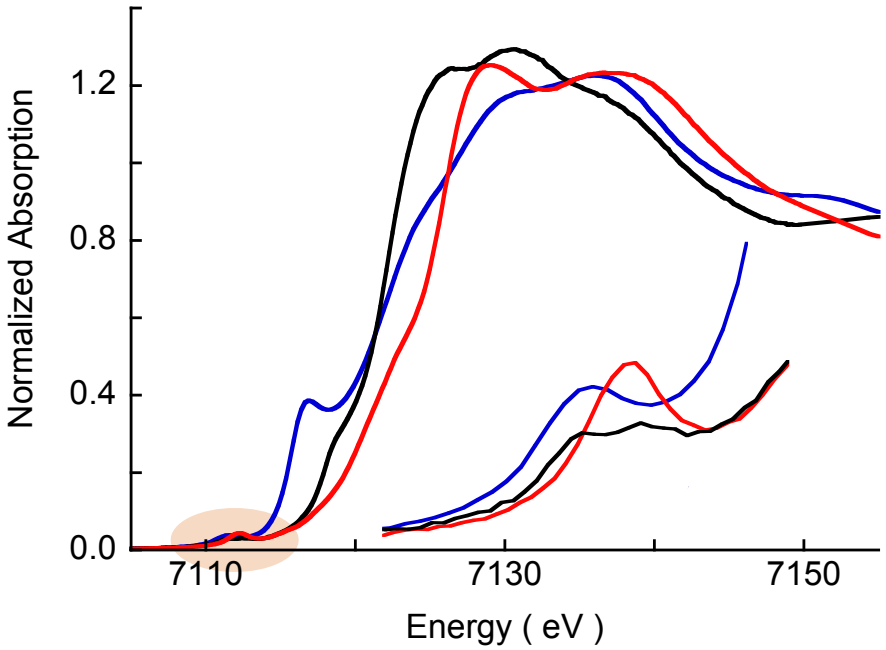

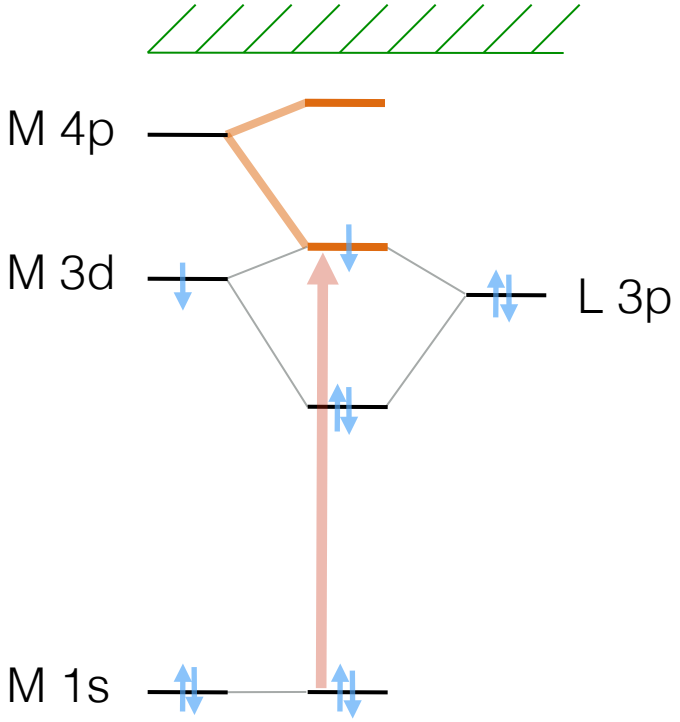

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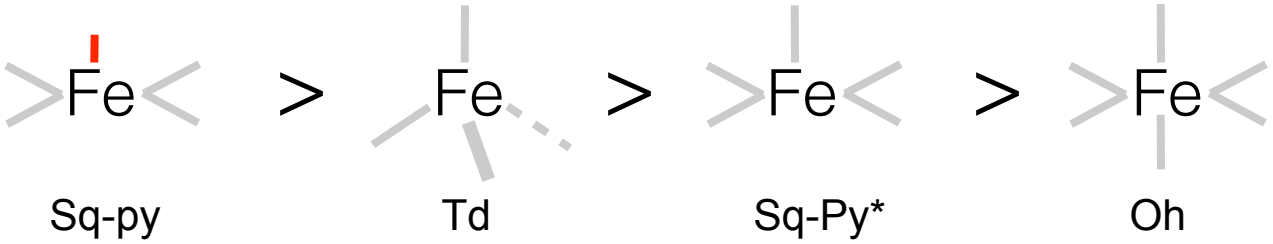

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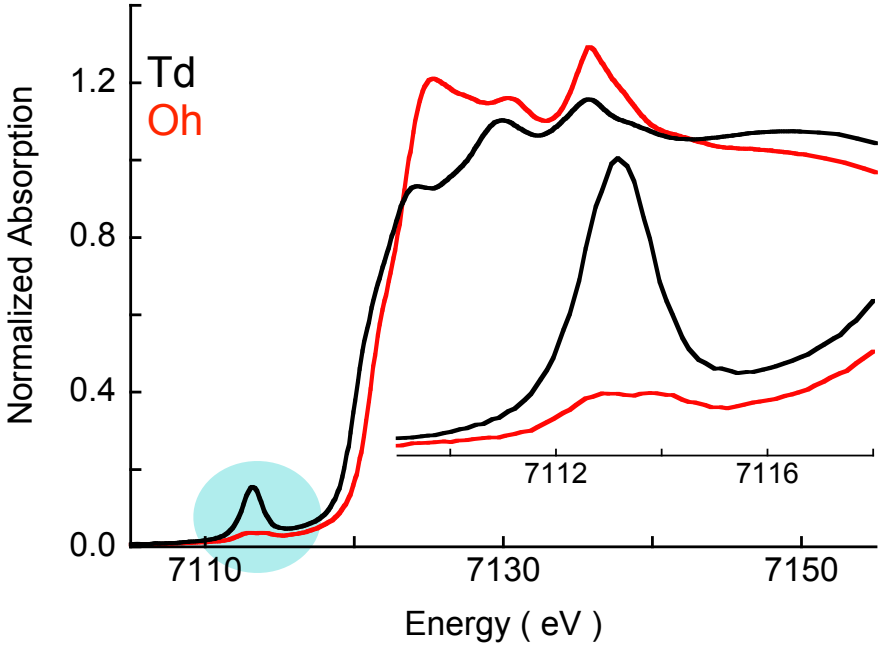

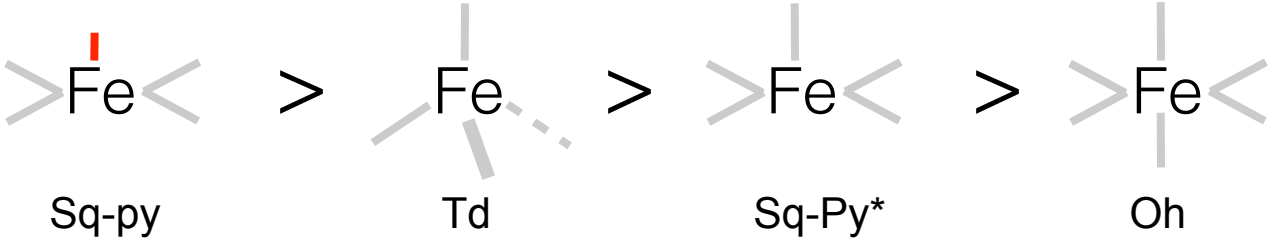

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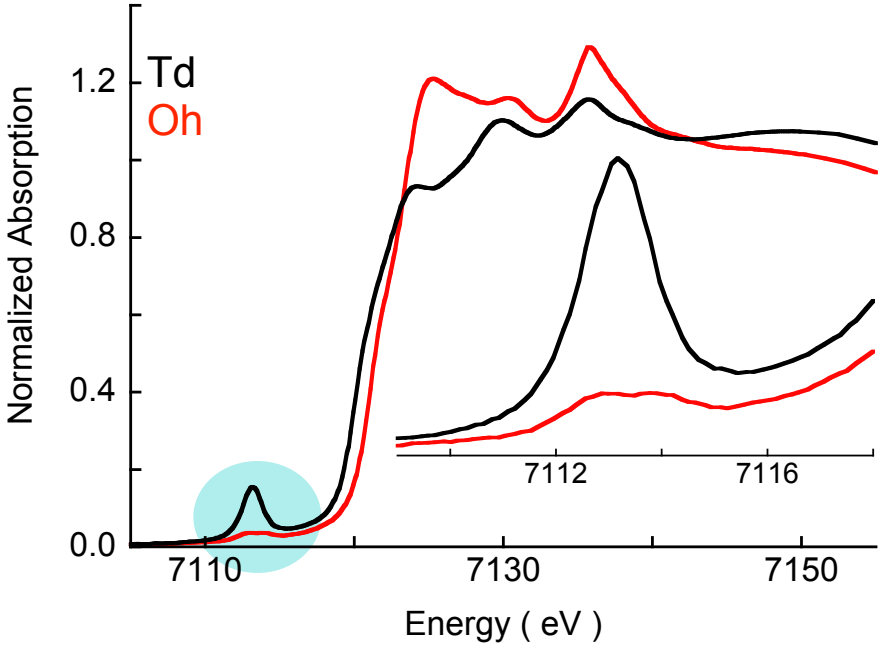
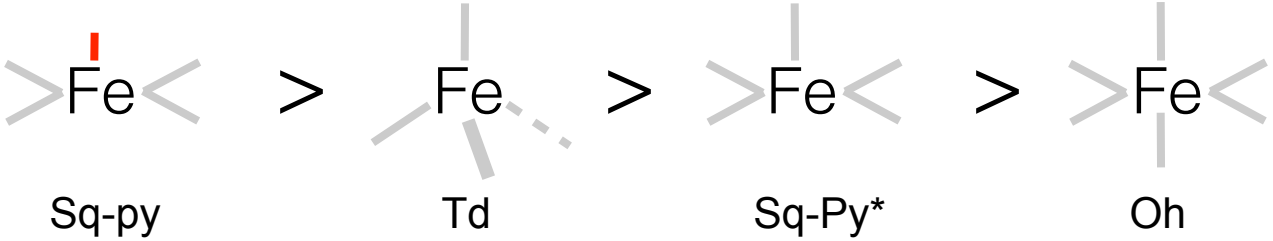


Oh

4p orbitals : t_{1u} symmetry

3d orbitals: t_{2g} and e_g symmetry

No Mixing = Weak pre-edge



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Factors that Affect Metal K-edge Shape and Energy

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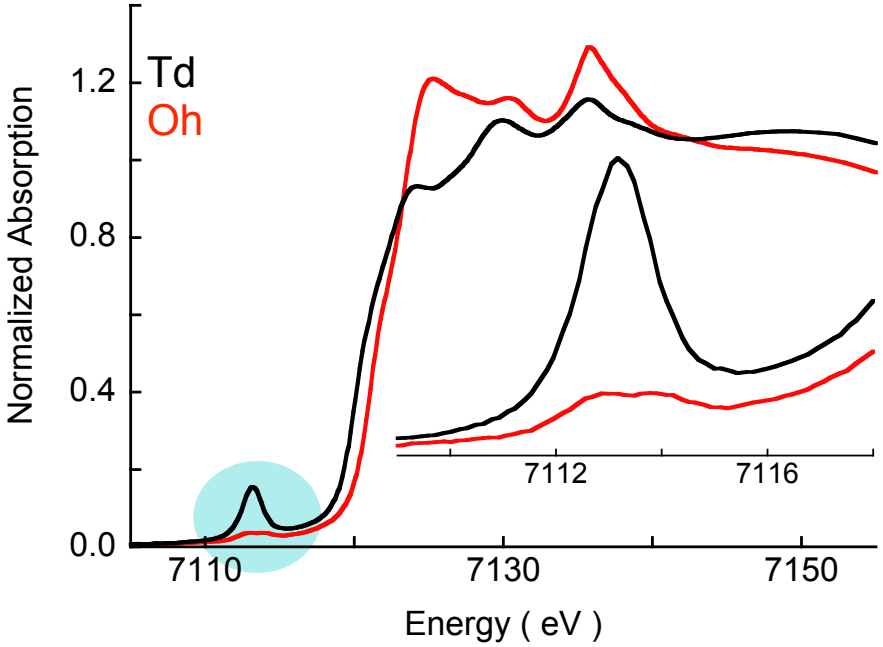

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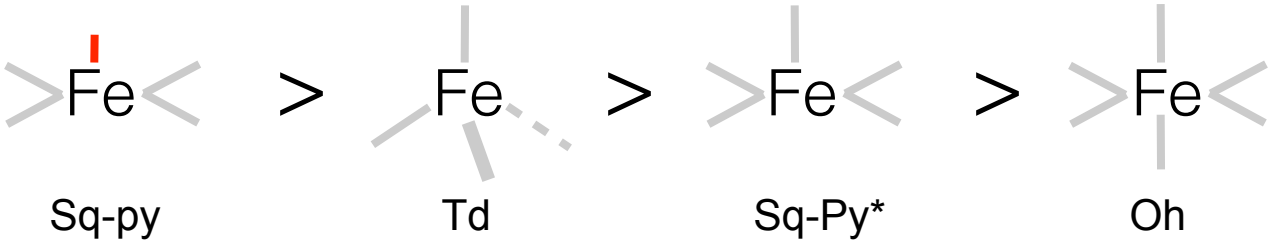
Td
4p orbitals : t_2 symmetry
3d orbitals: t_2 and e symmetry
Mixing = Intense pre-edge

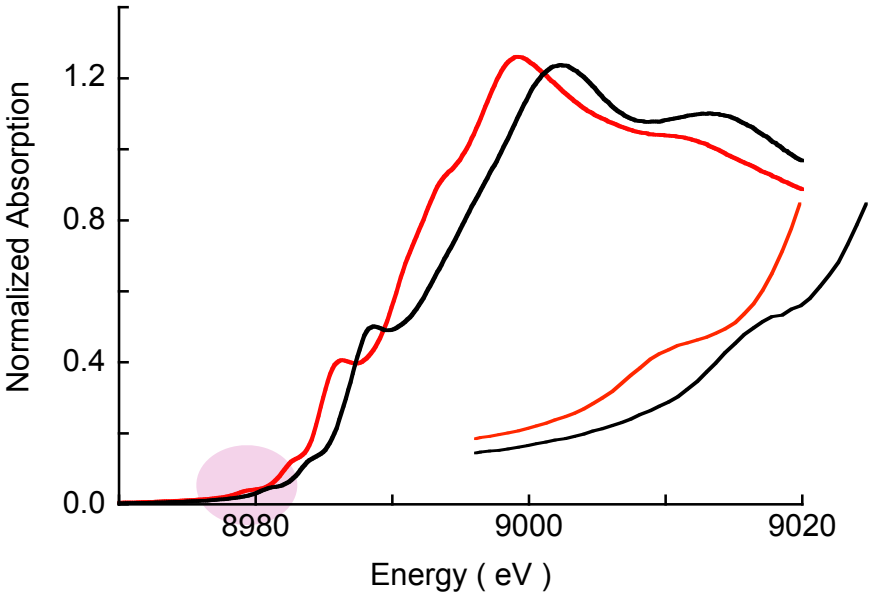


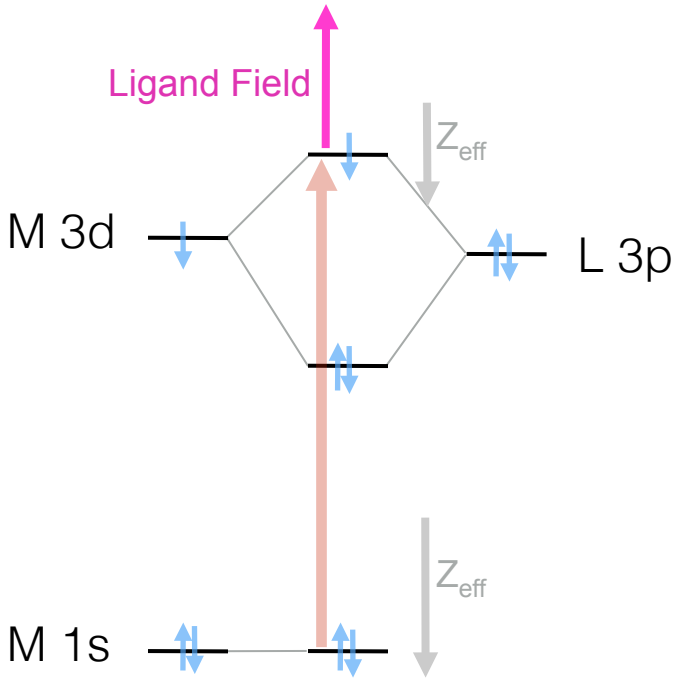
Pre-edge intensity ∝ Deviation from Centrosymmetry ∝ Metal 3d-4p mixing

Factors that Affect Metal K-edge Shape and Energy

SLAC

Pre-edge Energy





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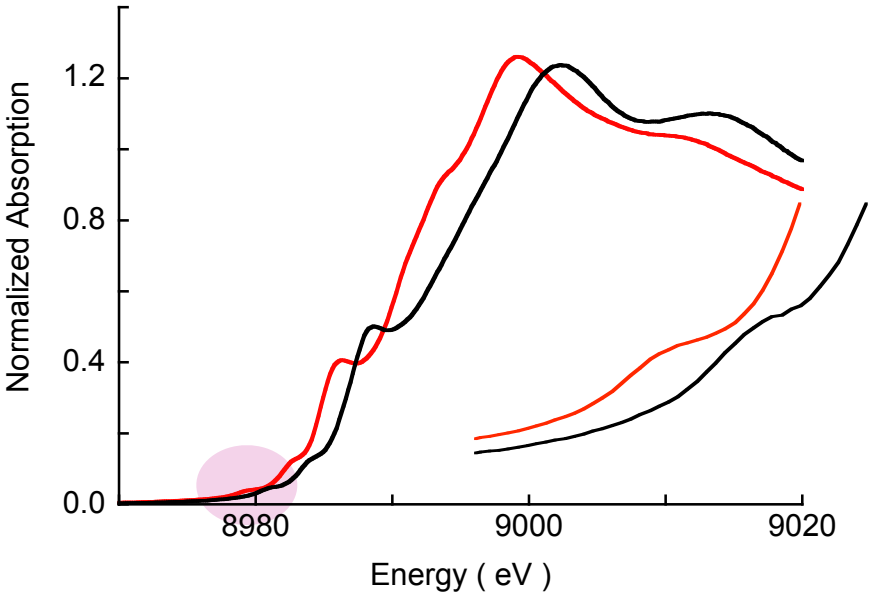
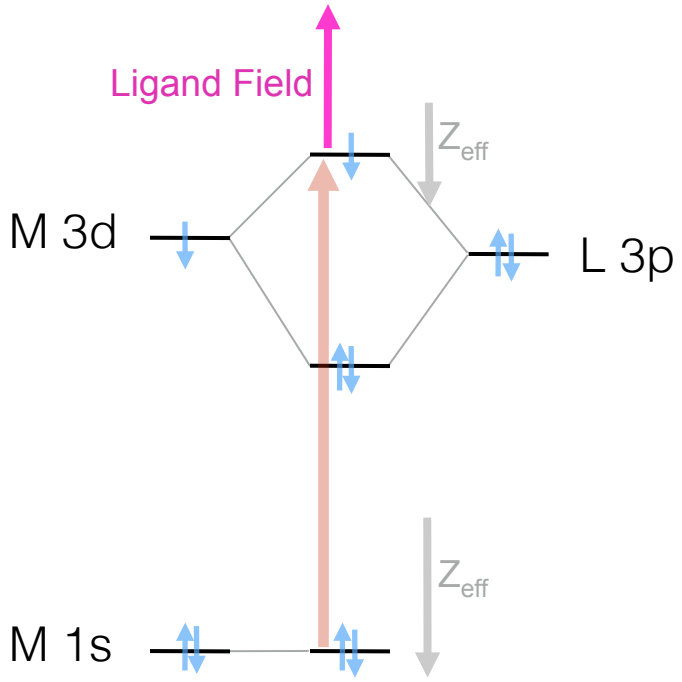
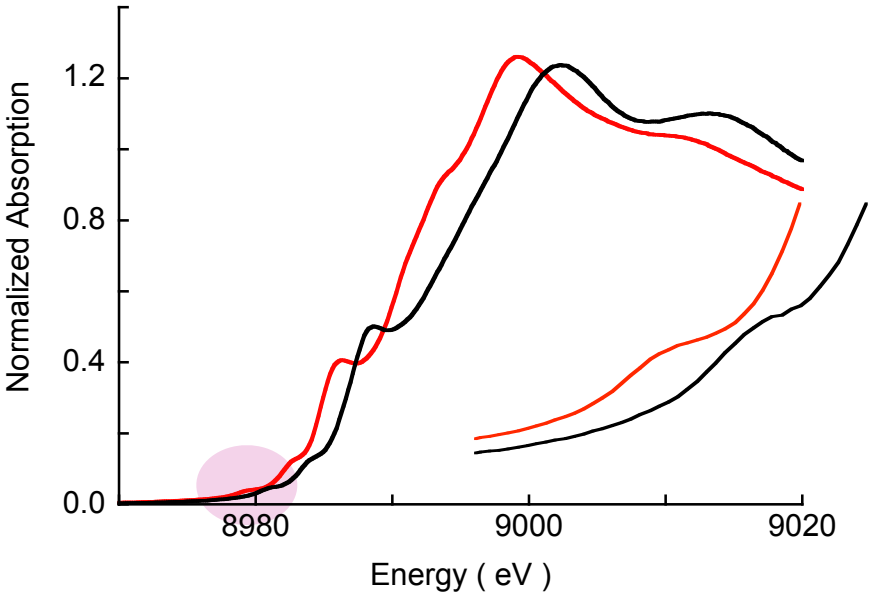


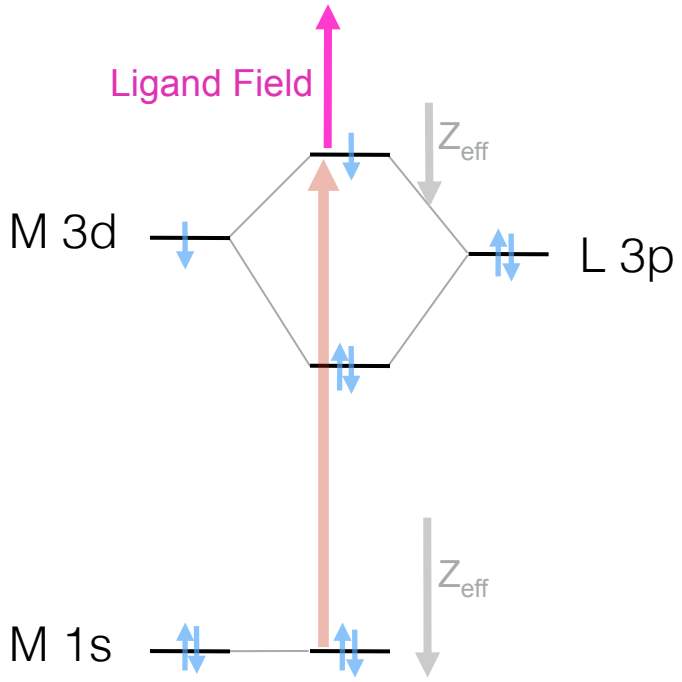


Pre-edge intensity-weighted average energy is modulated by Ligand-Field strength

Factors that Affect Metal K-edge Shape and Energy

Pre-edge Energy



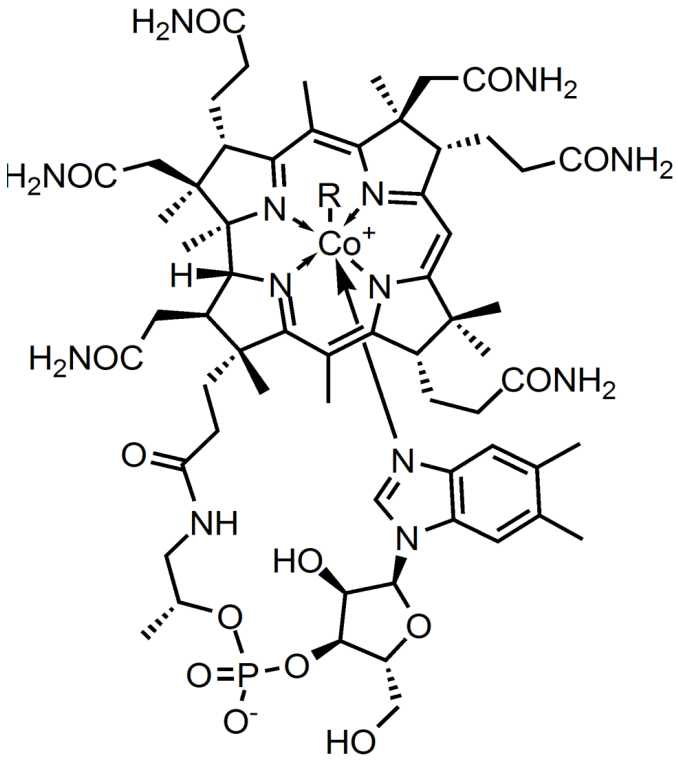


Pre-edge intensity-weighted average energy is modulated by Ligand-Field strength

Z_{eff} or charge on the metal affects the energy of all energy levels equally, therefore has minimal effect on pre-edge energy position

Pre-edge Example 1 : Cobalamin

Vitamin B_{12} derivative: Cobalamin



R = 5'-deoxyadenosyl, Me, OH, CN

Problem: Determination of Co-C bond distance in Me-Cobalamin

Pre-edge Example 1 : Cobalamin

Crystallography consistently gave a long Co-C distance than reasonable.
Question – Could the diffraction data have error from beam-damage/decomposition?

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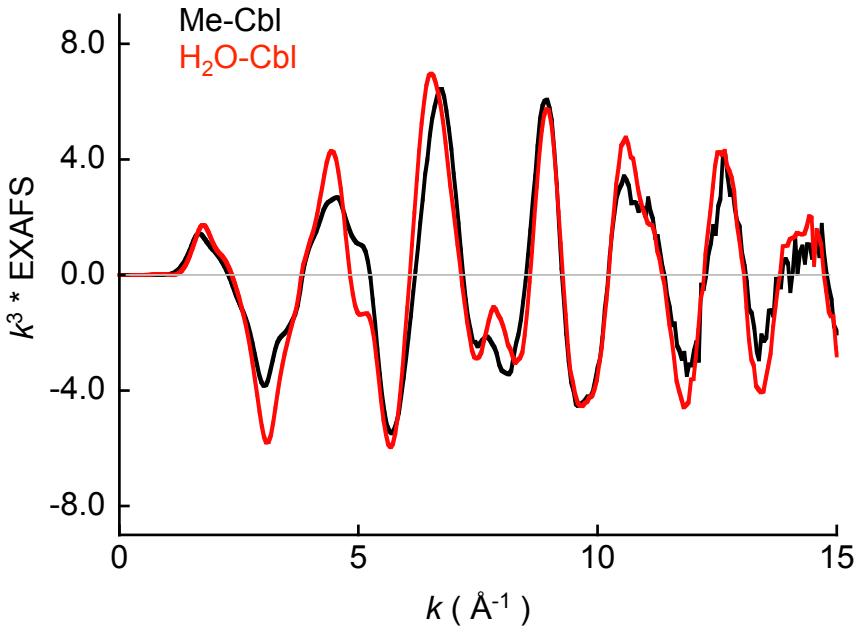

Me-Cbl and H_2O-Cbl have similar EXAFS

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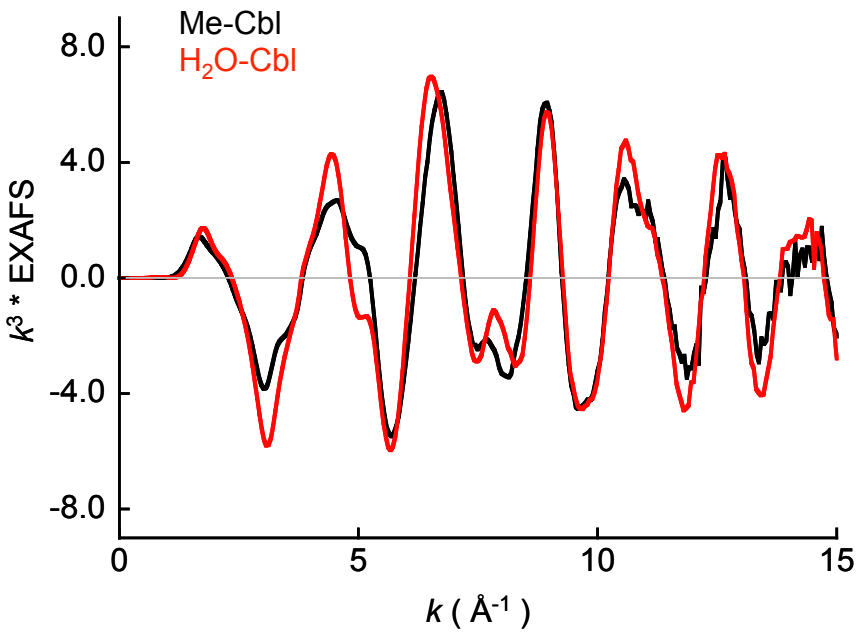

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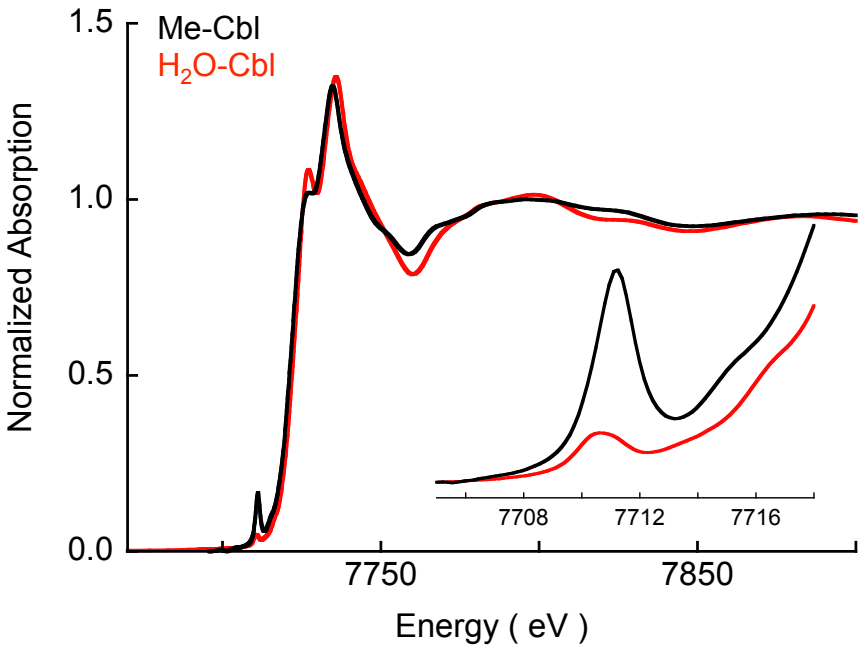

Pre- and rising-edge data distinct

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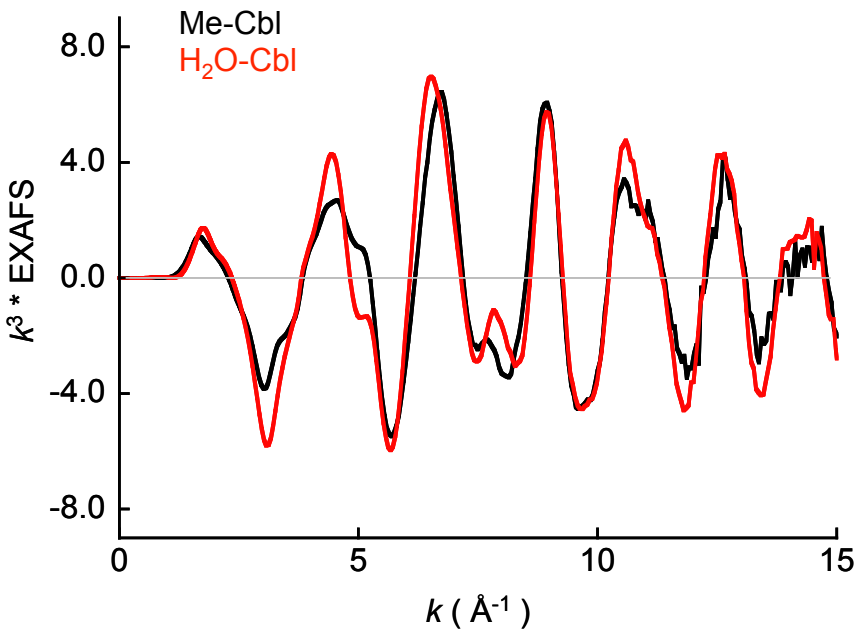

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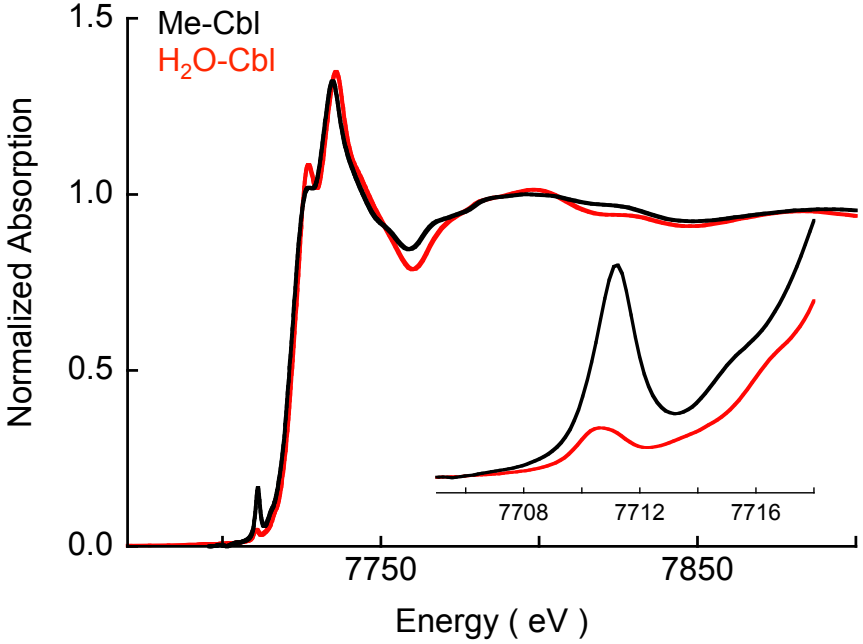
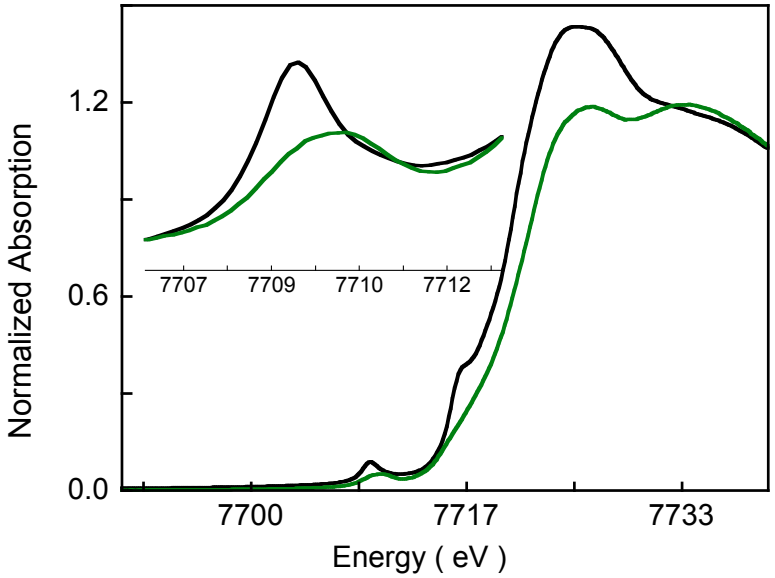


Pre- and rising-edge data distinct

Near-edge data were used to show a) crystal structure was erroneous b) determine the Me-Co distance to atomic resolution.

Pre-edge Quiz

Match the Spectra and Assign Oxidation State



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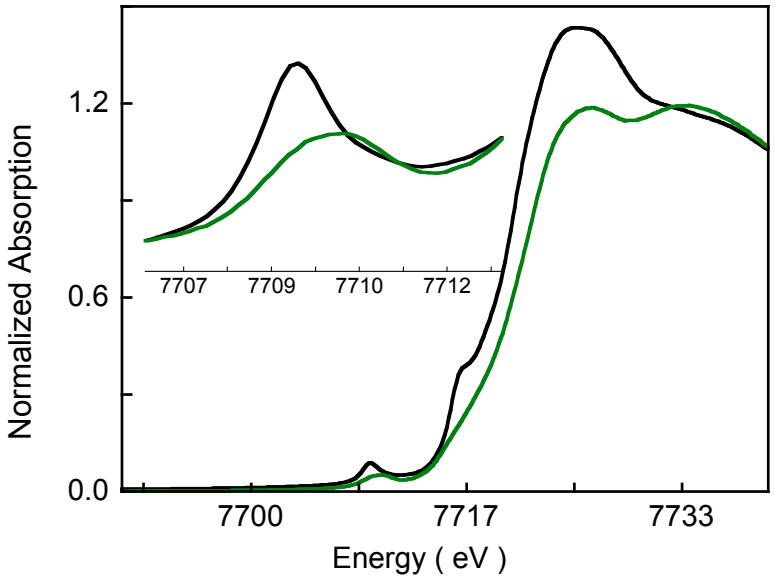
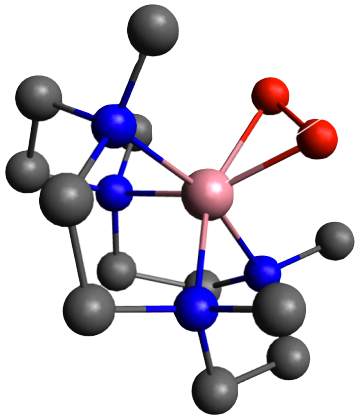
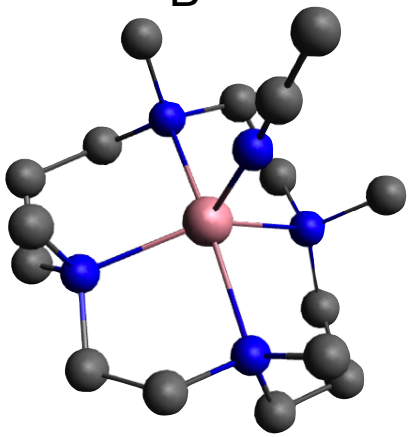


A



B



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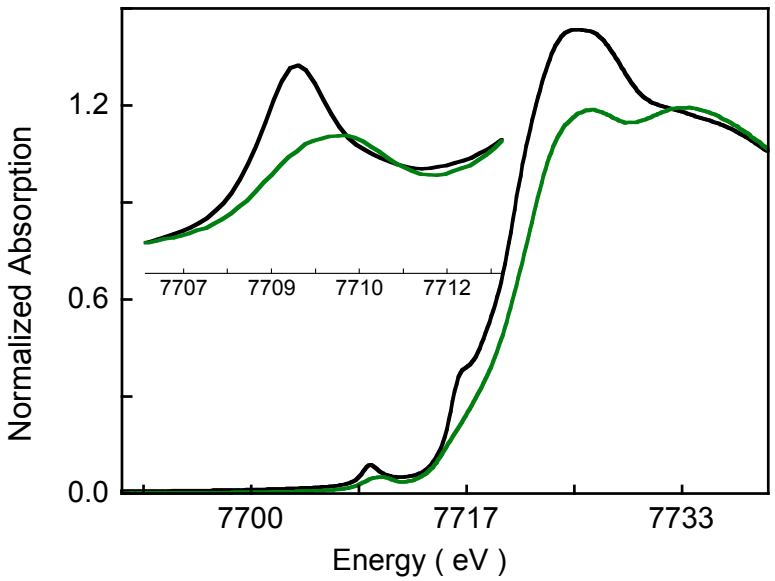

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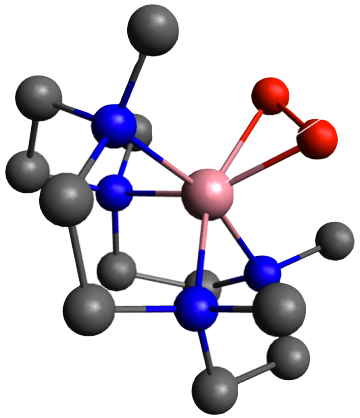
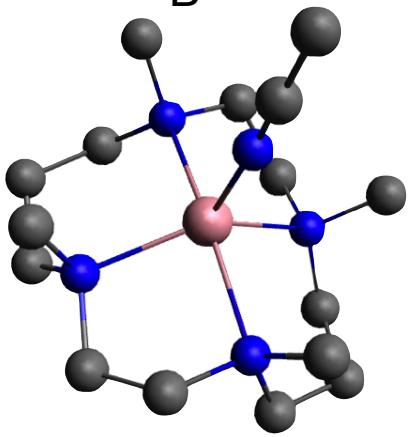


6-coordinate

B



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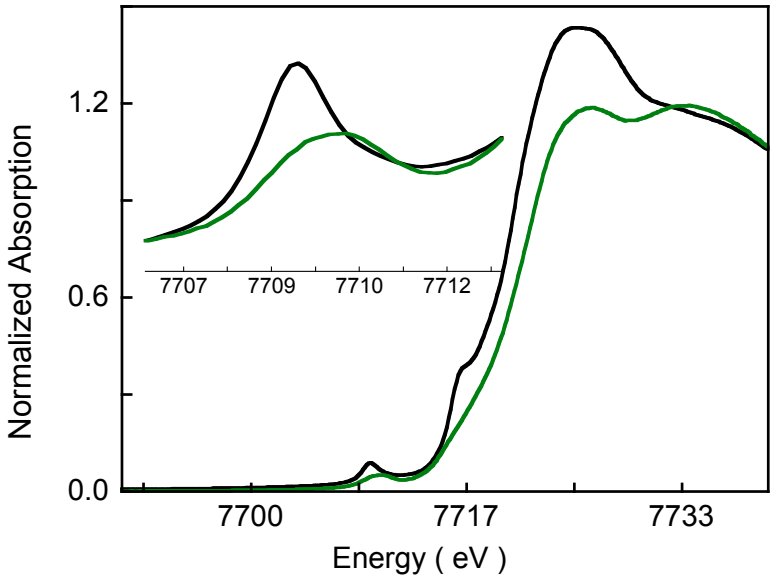

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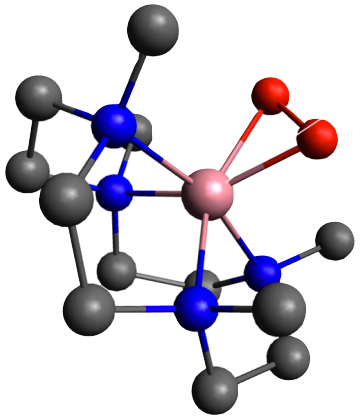

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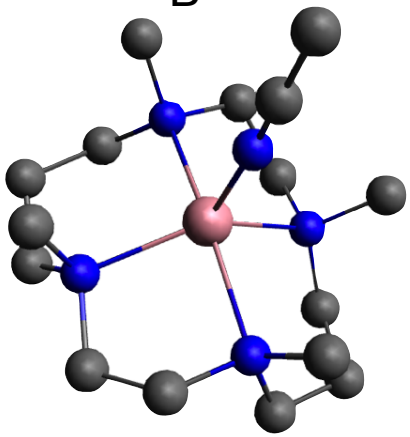

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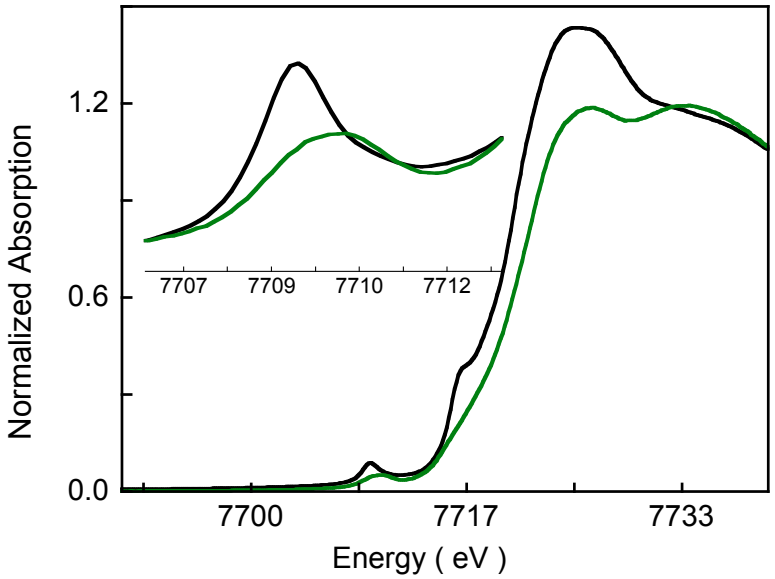
Which deviates more from centrosymmetry?

Which system has least 4p mixing?

Can you guess the oxidation state of A and B?

Pre-edge Quiz

Match the Spectra and Assign Oxidation State



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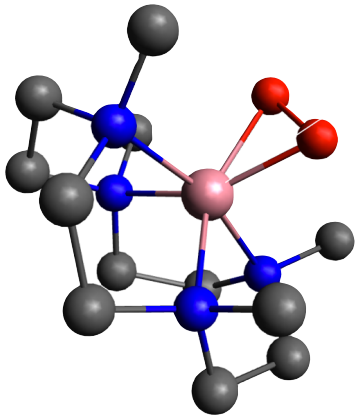

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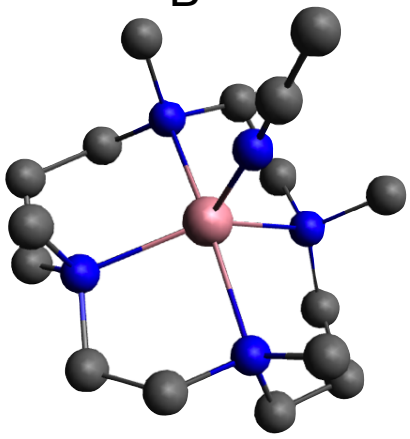

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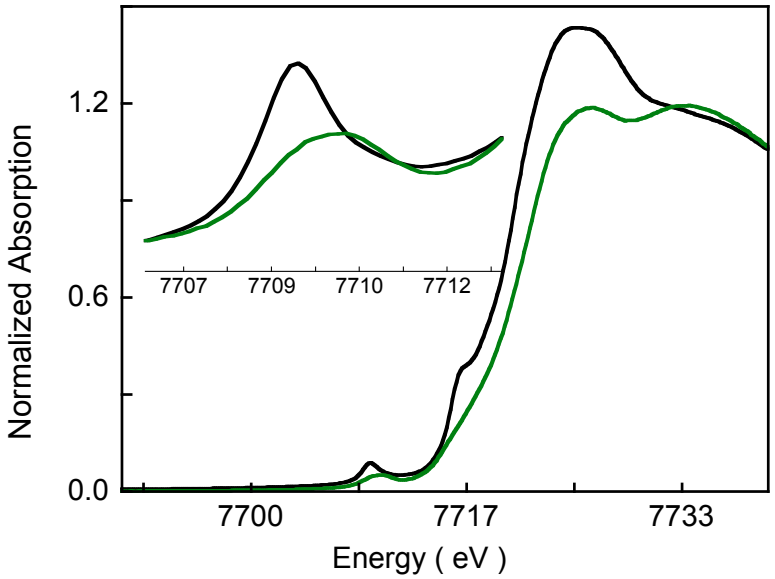

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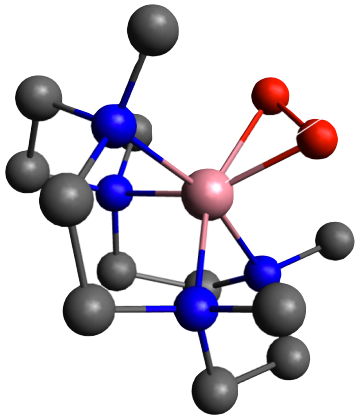

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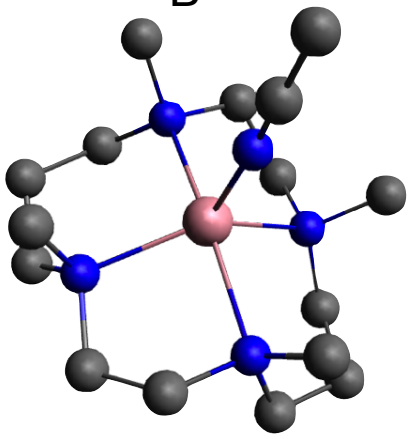

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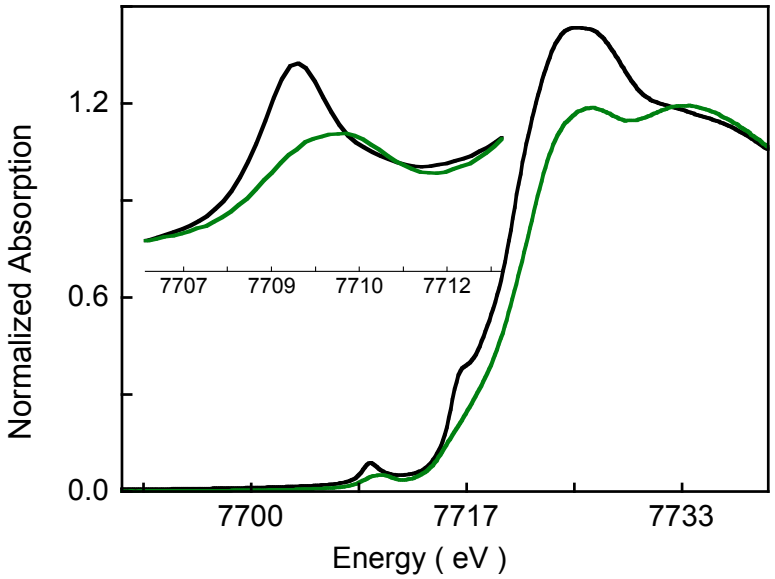

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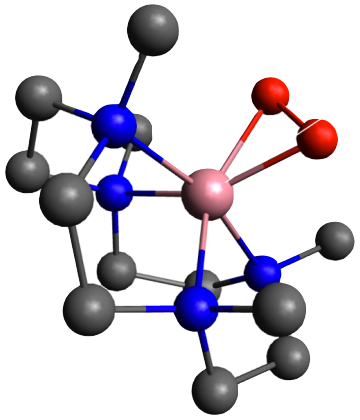

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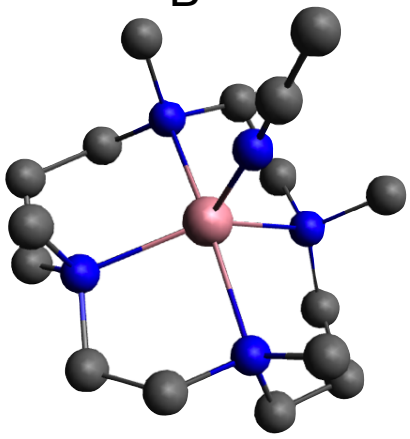

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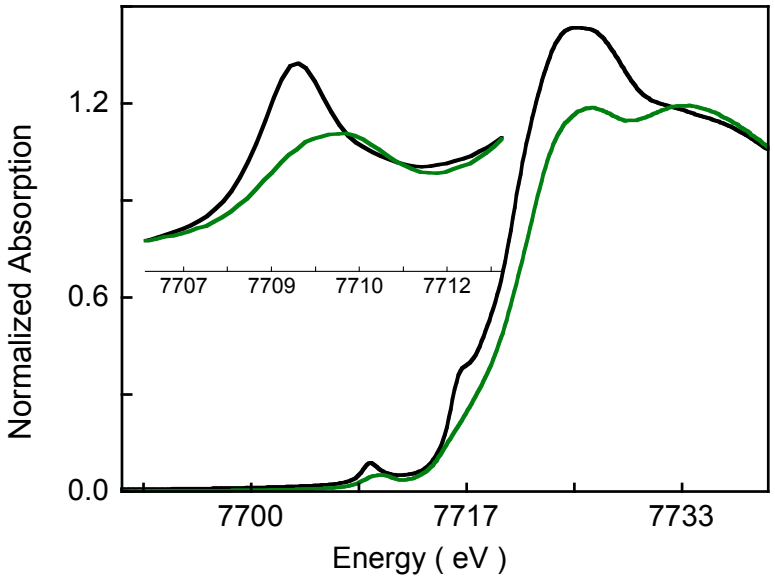

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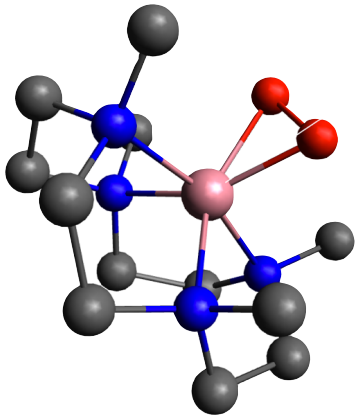

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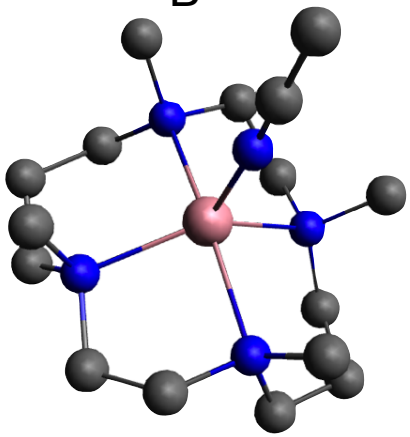

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A , green spectrum, Co^{3+} B, black spectrum, Co^{2+}

What About EXAFS ??

EXAFS Data

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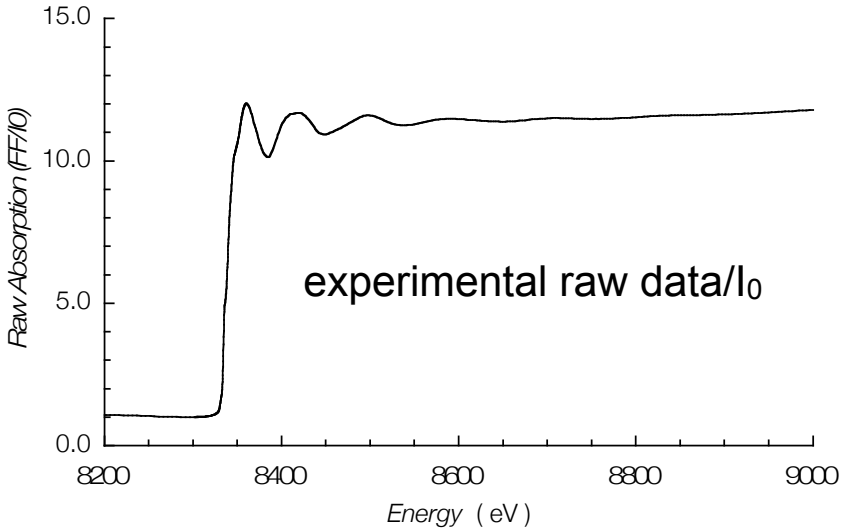
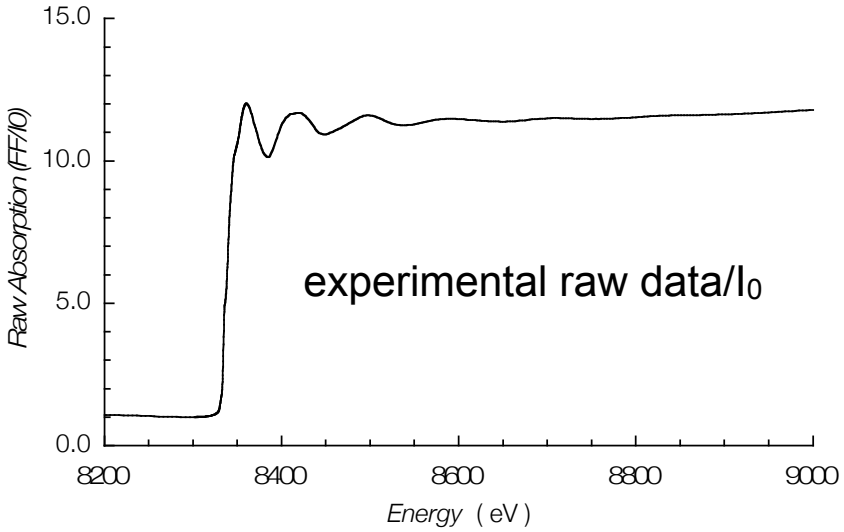

15.0
10.0
5.0
0.0
Raw Absorption (FF/I0)
experimental raw data/I0
8200
8400
8600
8800
9000
Energy (eV)

EXAFS Data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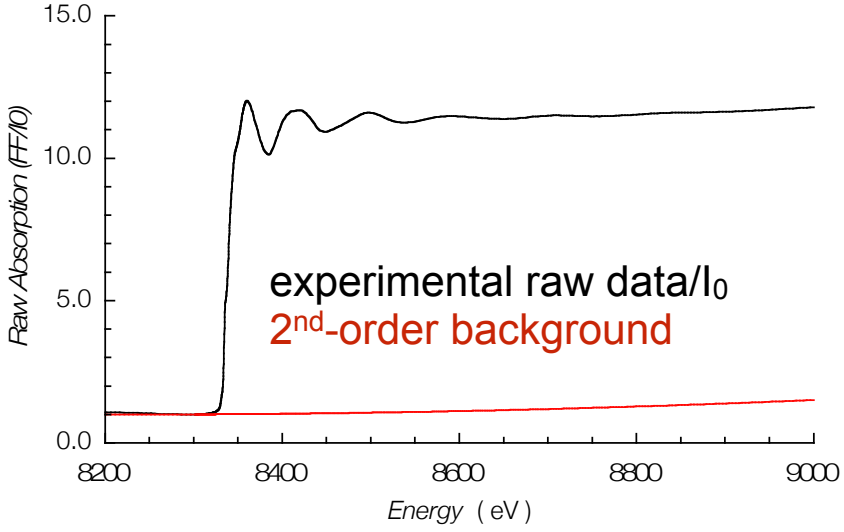
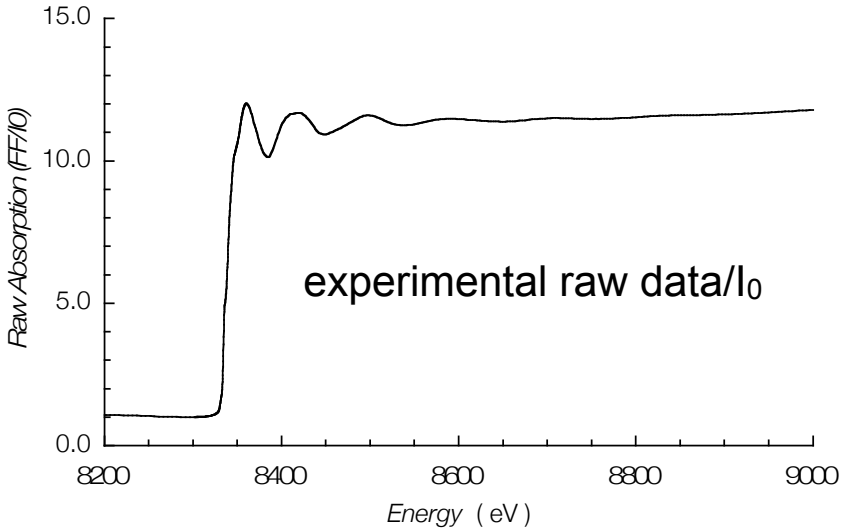
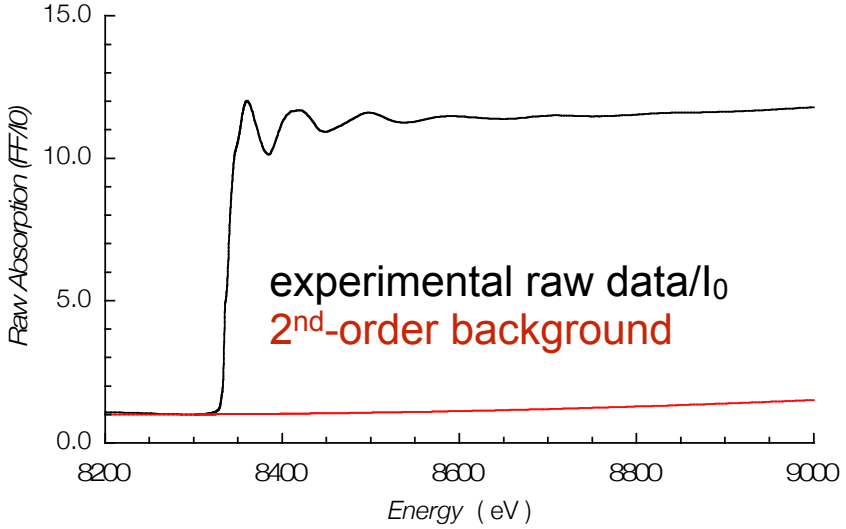
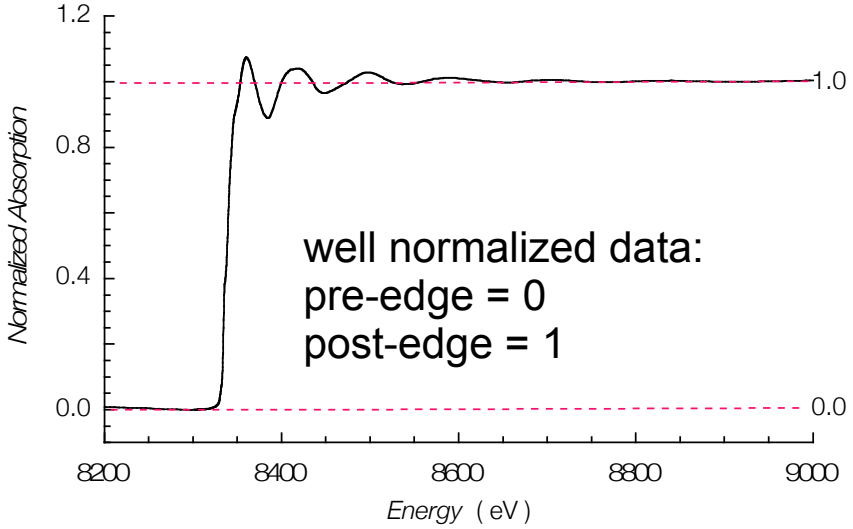
Energy (eV)
experimental raw data/I0


15.0
10.0
5.0
0.0
Raw Absorption (FF/I0)
8200
8400
8600
8800
9000
Energy (eV)
experimental raw data/I0
2nd-order background

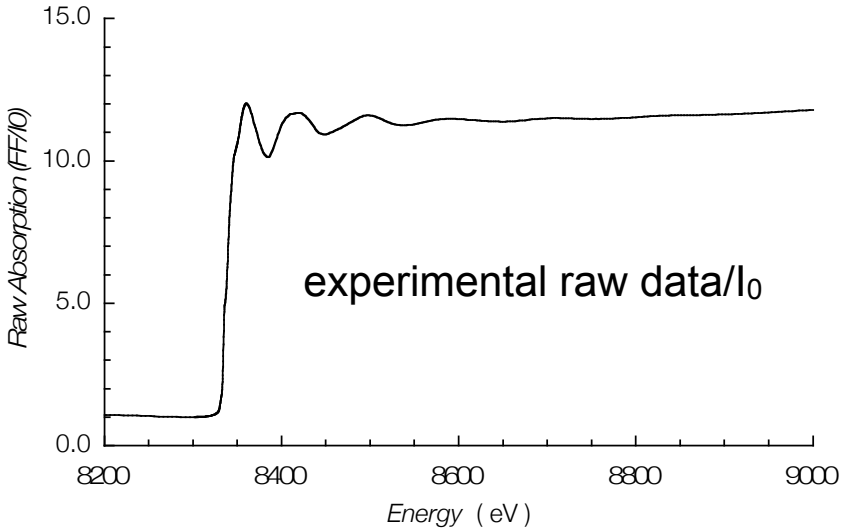
EXAFS Data

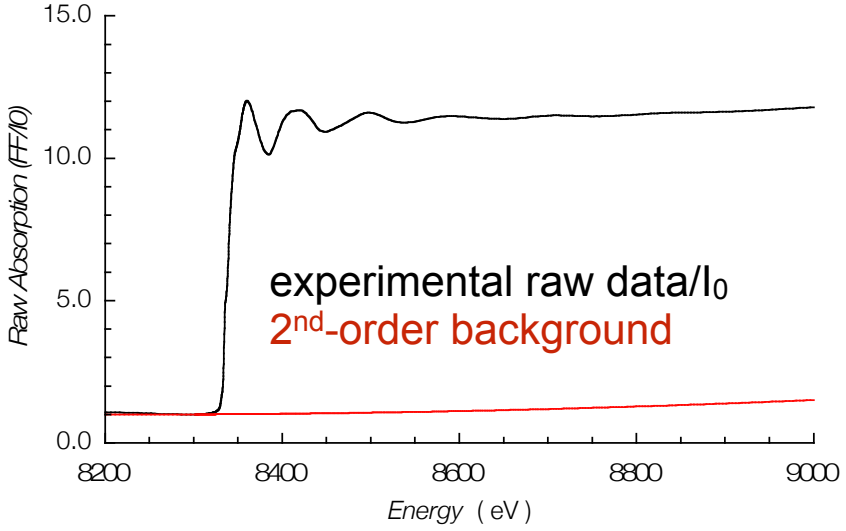

15.0
10.0
5.0
0.0
Raw Absorption (FF/I0)
8200
8400
8600
8800
9000
Energy (eV)
experimental raw data/I0

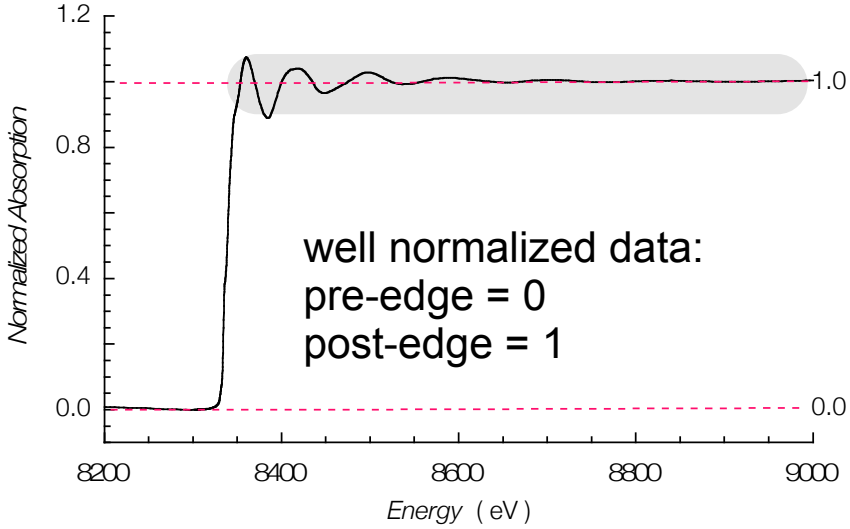

15.0
10.0
5.0
0.0
Raw Absorption (FF/I0)
8200
8400
8600
8800
9000
Energy (eV)
experimental raw data/I0
2nd-order background

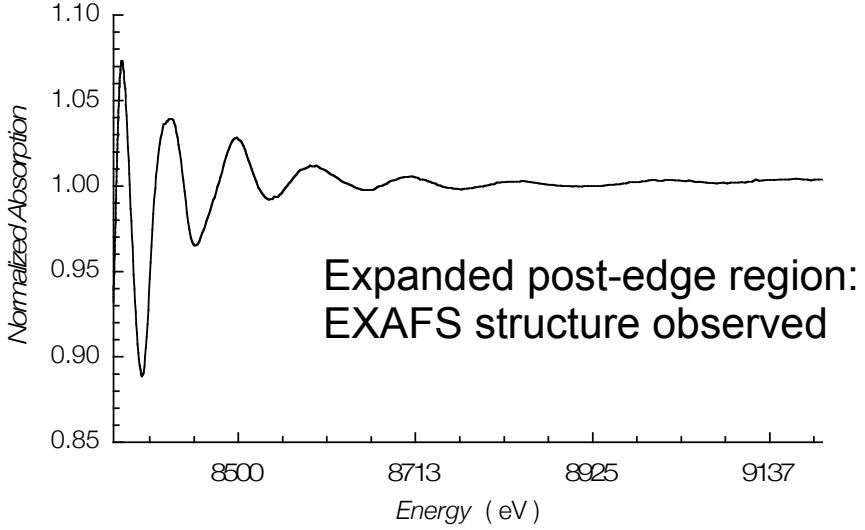

1.2
0.8
0.4
0.0
Normalized Absorption
1.0
0.0
8200
8400
8600
8800
9000
Energy (eV)
well normalized data:
pre-edge = 0
post-edge = 1

EXAFS Data


15.0
10.0
5.0
0.0
Raw Absorption (FF/I0)
8200
8400
8600
8800
9000
Energy (eV)
experimental raw data/I0

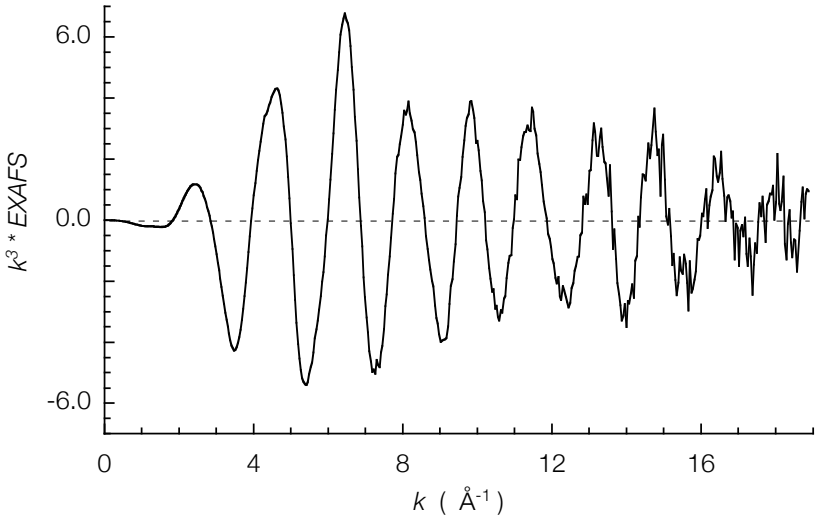

15.0
10.0
5.0
0.0
Raw Absorption (FF/I0)
8200
8400
8600
8800
9000
Energy (eV)
experimental raw data/I0
2nd-order background


1.2
0.8
0.4
0.0
Normalized Absorption
1.0
0.0
8200
8400
8600
8800
9000
Energy (eV)
well normalized data:
pre-edge = 0
post-edge = 1


1.10
1.05
1.00
0.95
0.90
0.85
Normalized Absorption
8500
8713
8925
9137
Energy (eV)
Expanded post-edge region:
EXAFS structure observed

EXAFS Data

EXAFS Data & Fourier Transforms

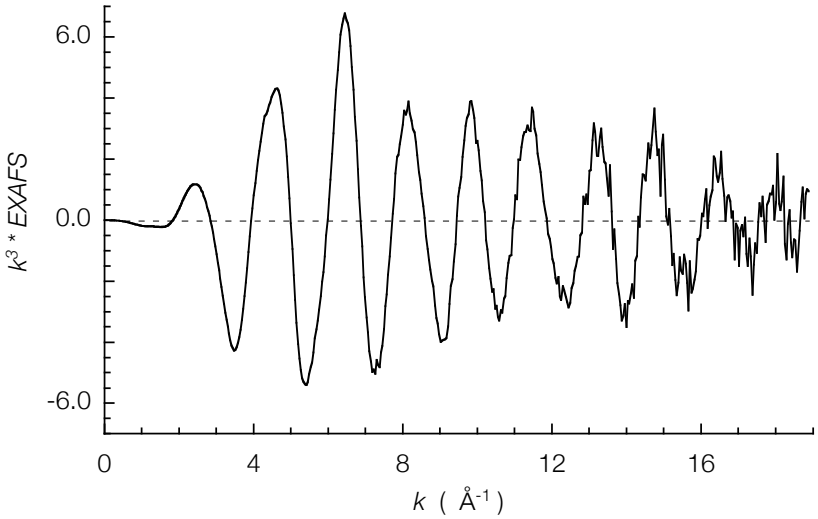


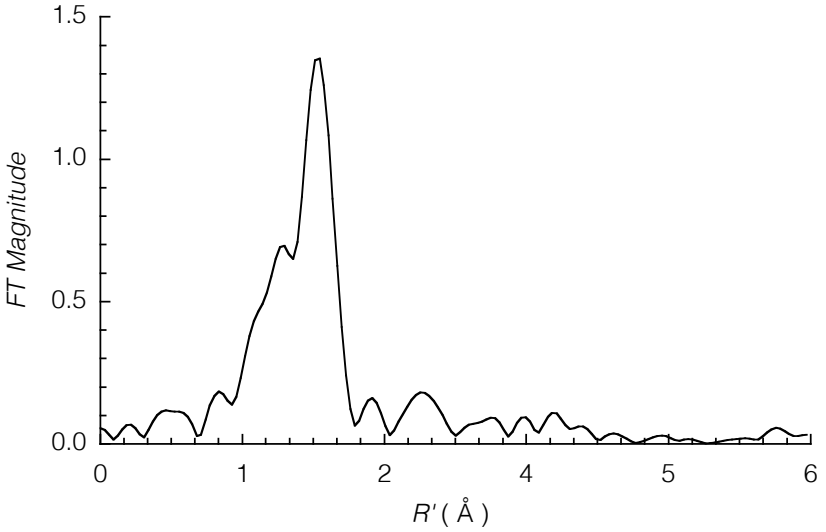
The final EXAFS is multiplied by k^3 to compensate for the rapid attenuation with energy.

Less intuitive about local structure.

EXAFS Data

EXAFS Data & Fourier Transforms





The final EXAFS is multiplied by k^3 to compensate for the rapid attenuation with energy.

Less intuitive about local structure.

Fourier Transform (FT) is performed over a chosen k range to yield the radial distribution.

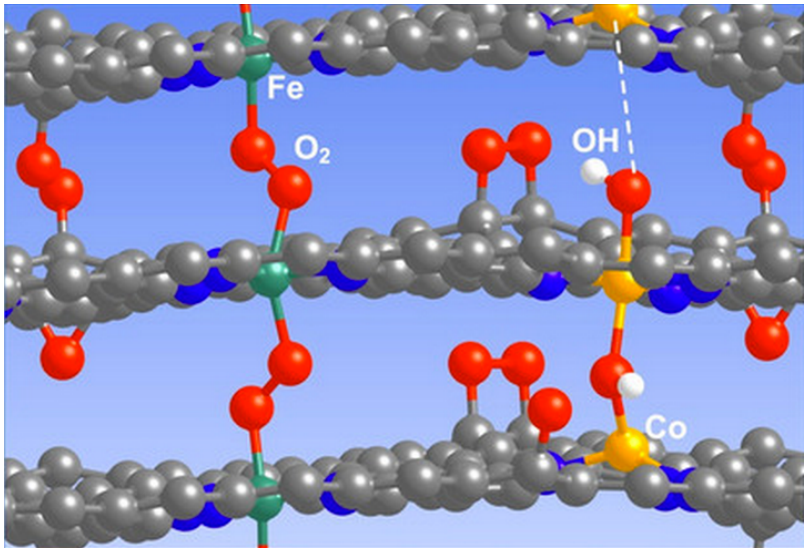
Can be directly correlated to M-L distances

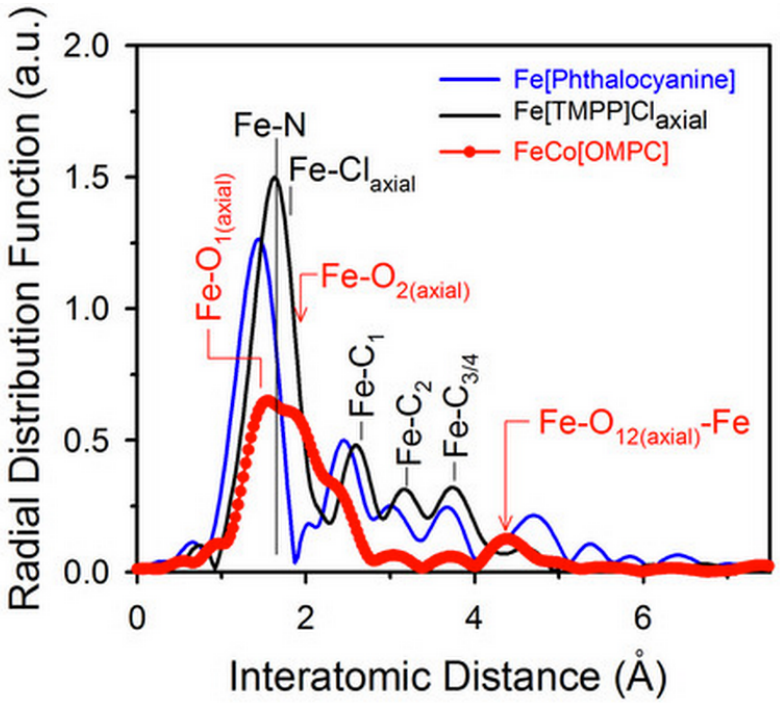
EXAFS : Information Content

How Many of What Type of atoms are at What Distance from the Absorbing Element

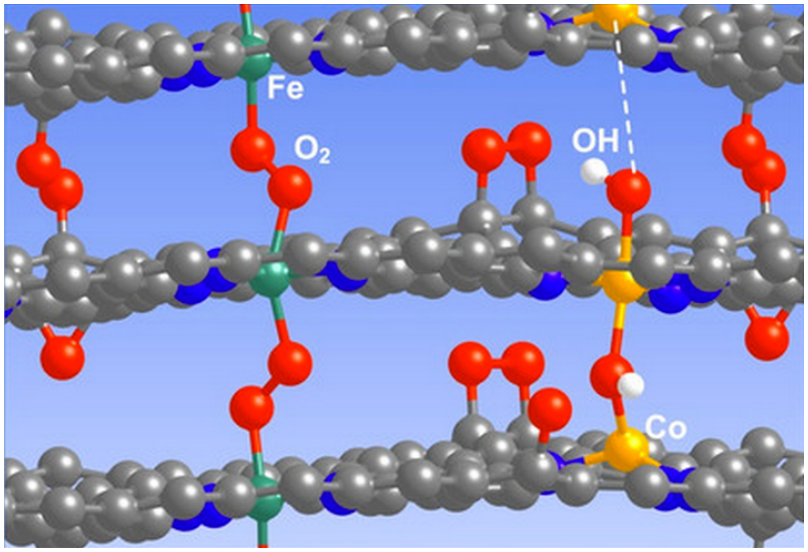
Distances	± 0.02 Å
Coordination Nos.	± 20-25 %
Angle Information	In cases tending to linearity
Distance Range	5 Å
Scattering Atom	ΔZ ± 1 (Z=6-17), ΔZ ± 3 (Z=20-35)
Contamination Allowed	Maximum 15%
Typical sample concentration	500μM or more

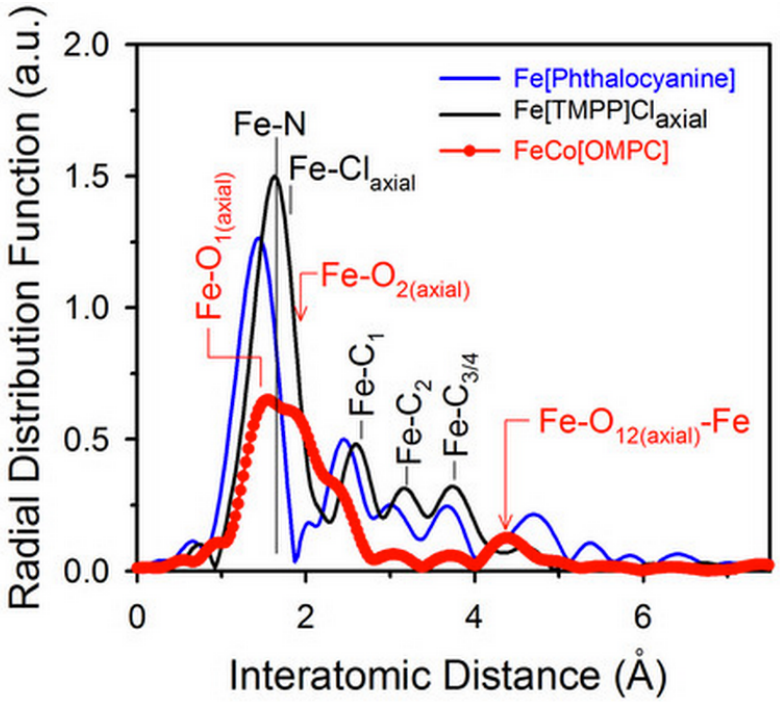
Ordered Systems





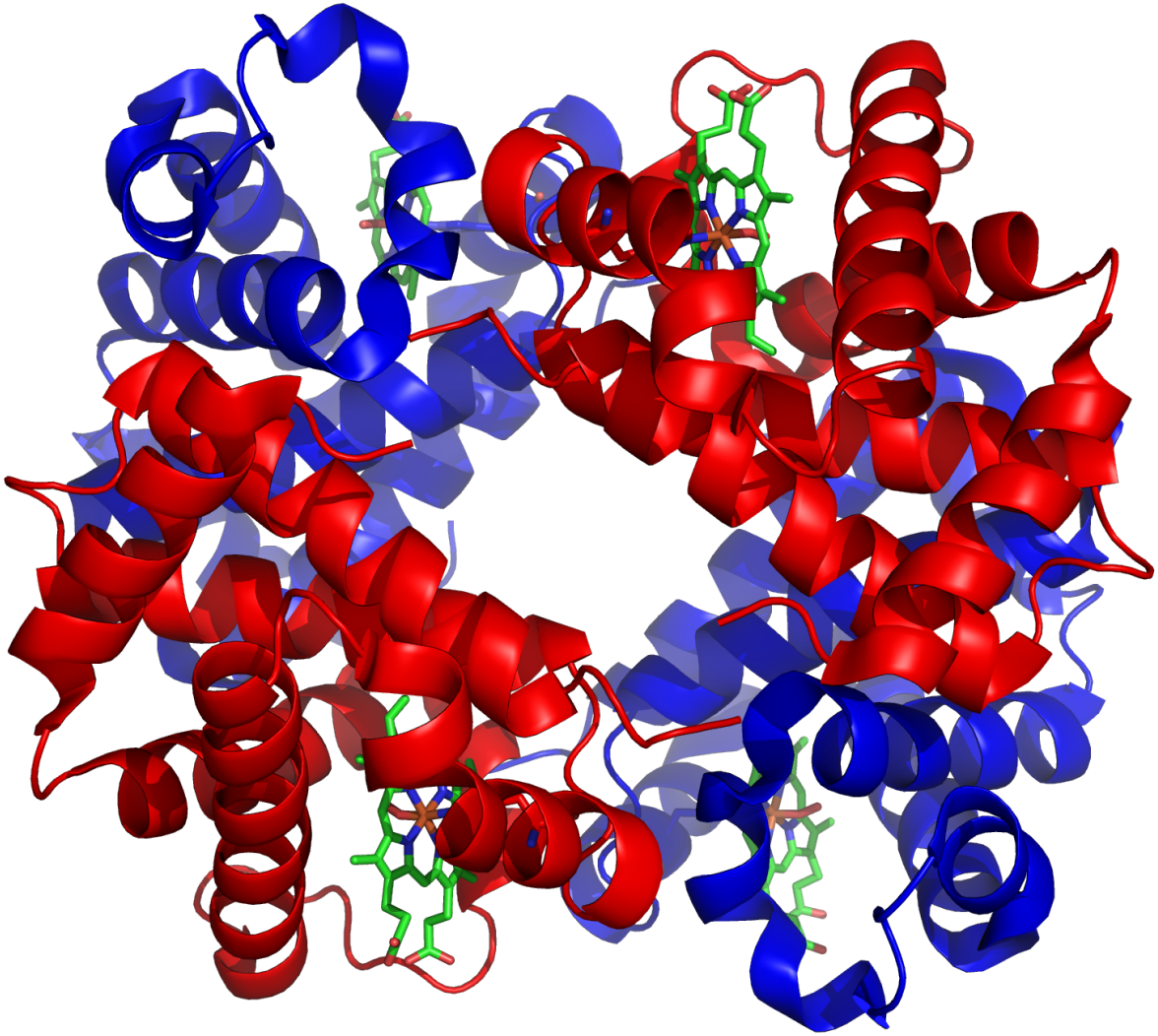
Ordered Systems





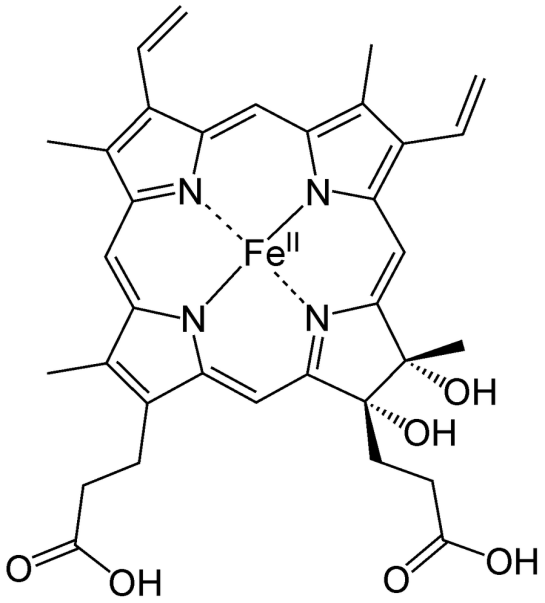
- EXAFS more ordered (data to high *k*)
- Shell by Shell analysis is popular and FT based fitting is helpful.
- Rigid structure - similarity between related systems.

Biological Systems

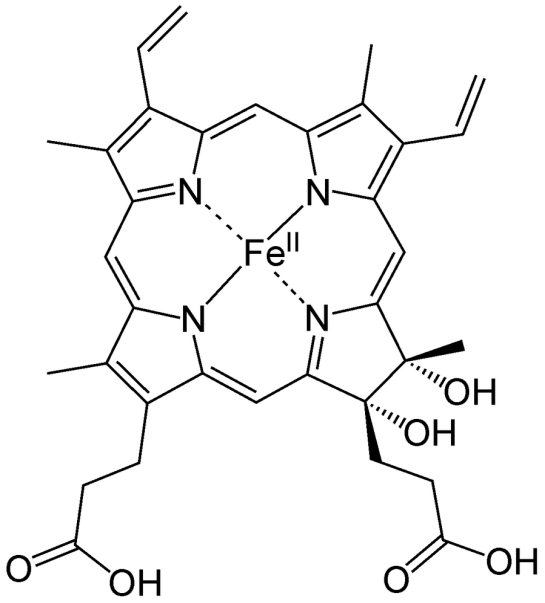


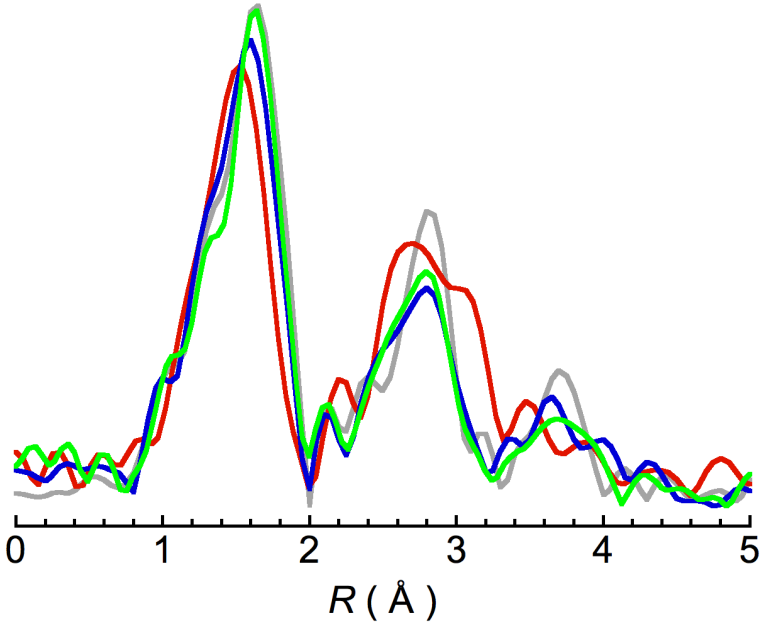
Biological Systems

Biological Systems

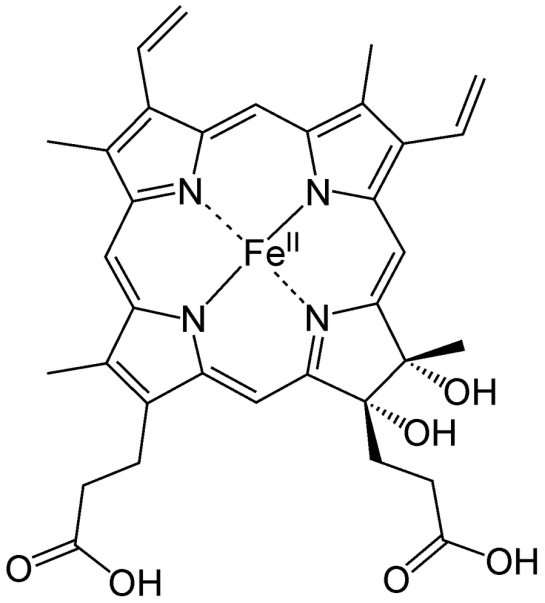


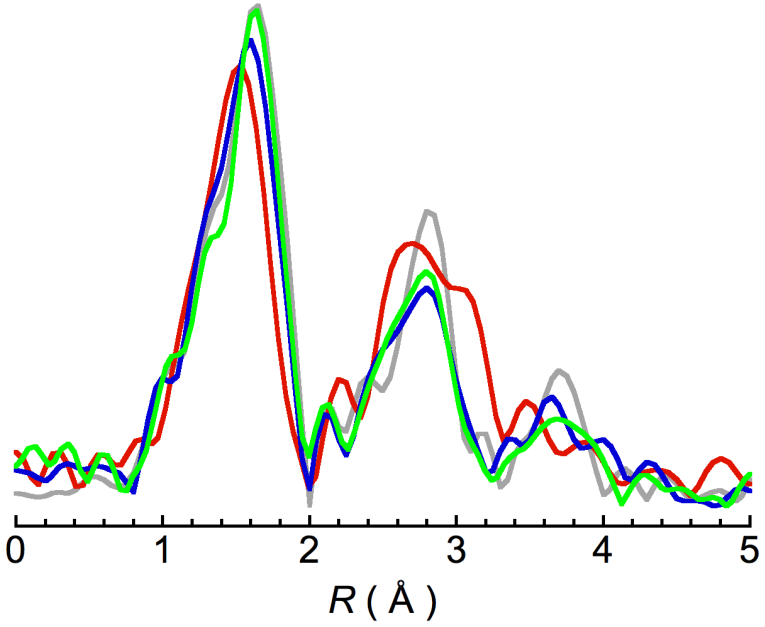
Biological Systems





Biological Systems





- EXAFS are more disordered (data to k=11-15 Å).
- Complete EXAFS analysis necessary for meaningful interpretation.
- Confidence mostly in first shell & second shell metal coordination.

Experimental Considerations

Experimental Considerations

- Sample Requirement
  - ~1 mM in metal, 100 uL in volume, 20-30% glycerol/glassing agent.
  - 0.1-1 mM for heavy metals Z > Cu, ~2mM for Z < Fe.
  - Duplicates for photoreducing systems.

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- Beamline Specification
  - Liq He cryostat (10-15K) : must
  - 30+ element Ge Detector: critical
  - BL equipped with fast shutters, beam filters, ease of detuning: critical
  - Automated data measurement: required

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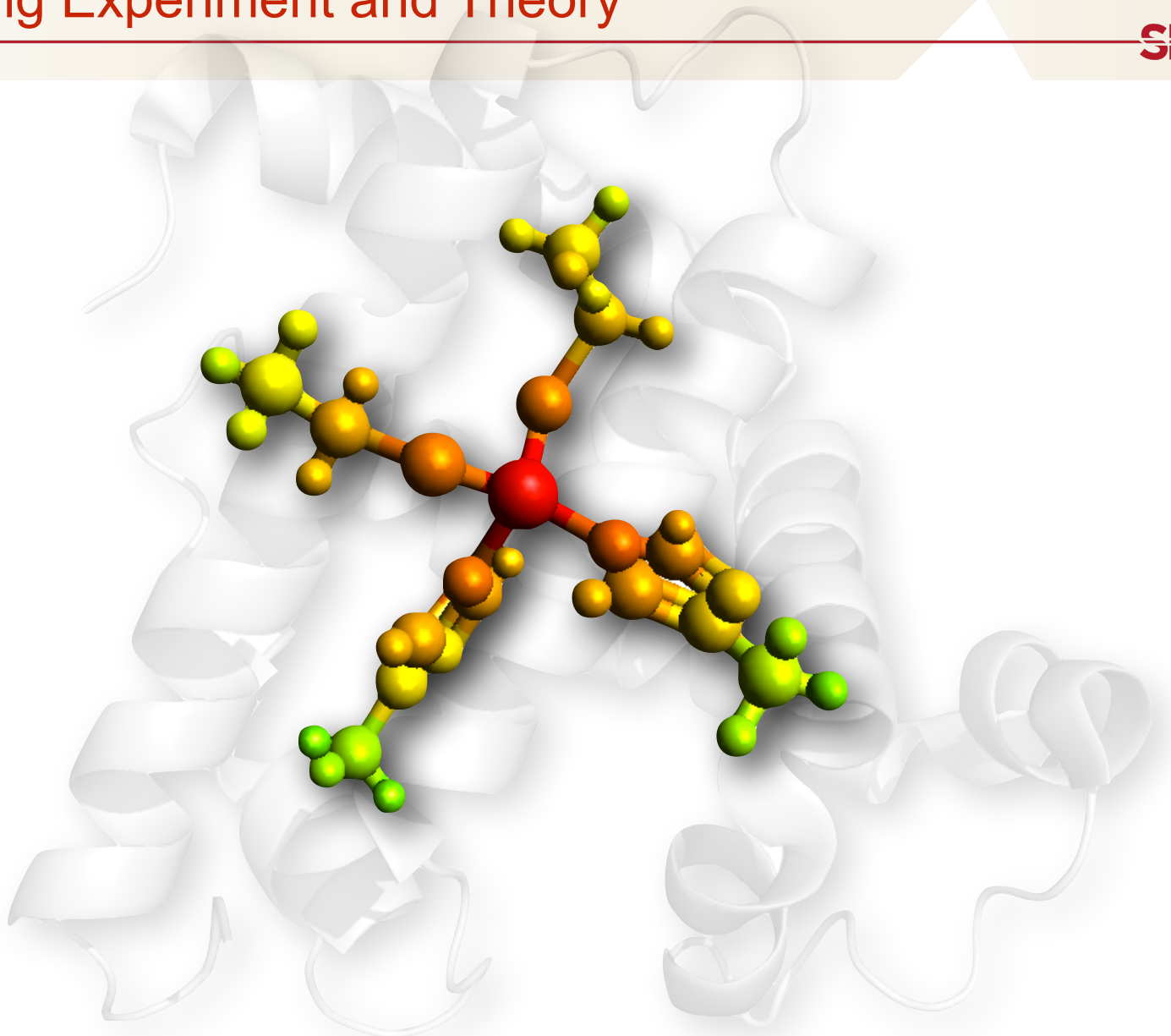
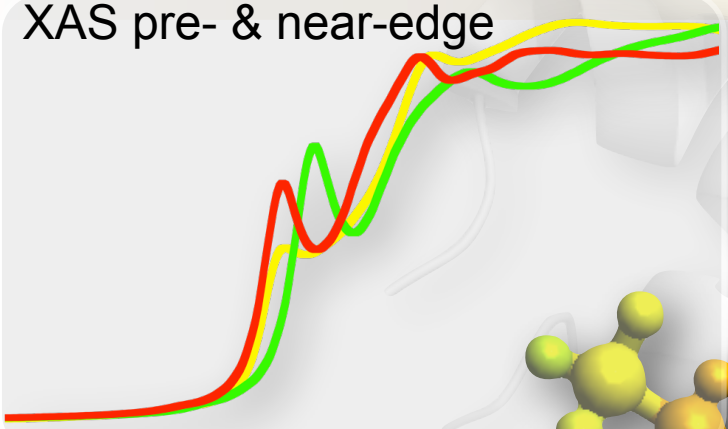
- Measurement Time
  - Time : 5-15 hours (per-sample, excluding duplicates)
  - Reproducibility : At least once

Combining Experiment and Theory

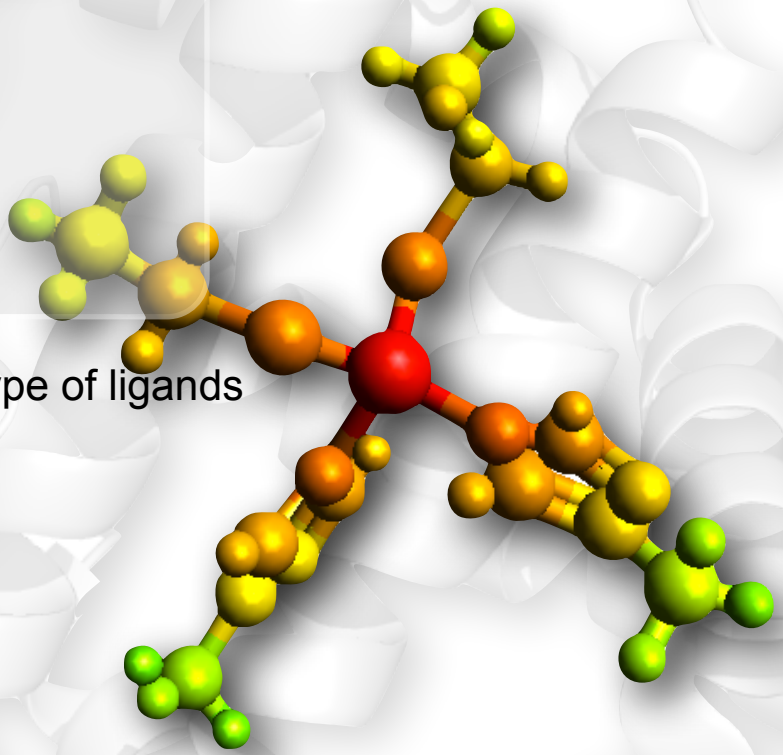


Combining Experiment and Theory

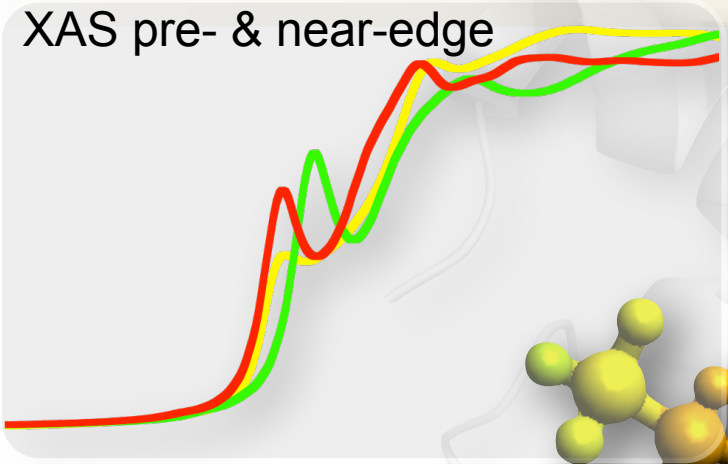
XAS pre- & near-edge

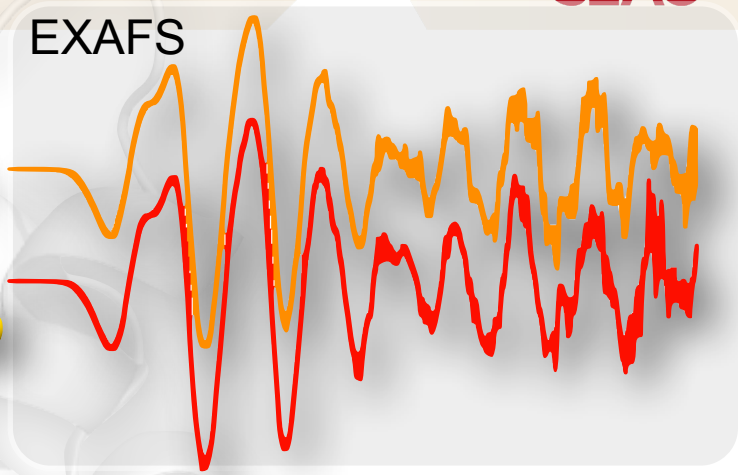


Electronic information:
covalency, bond strength, type of ligands



Combining Experiment and Theory





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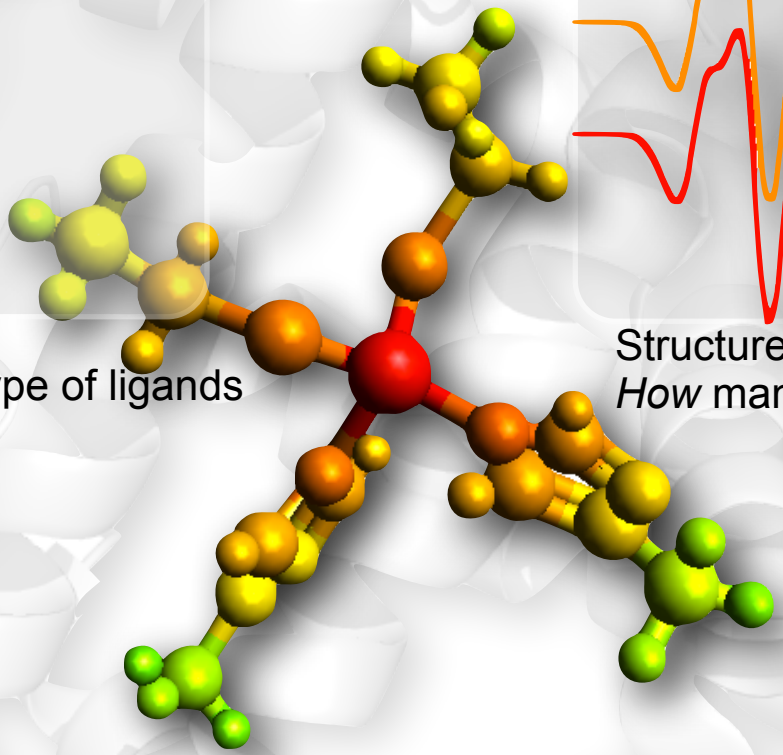
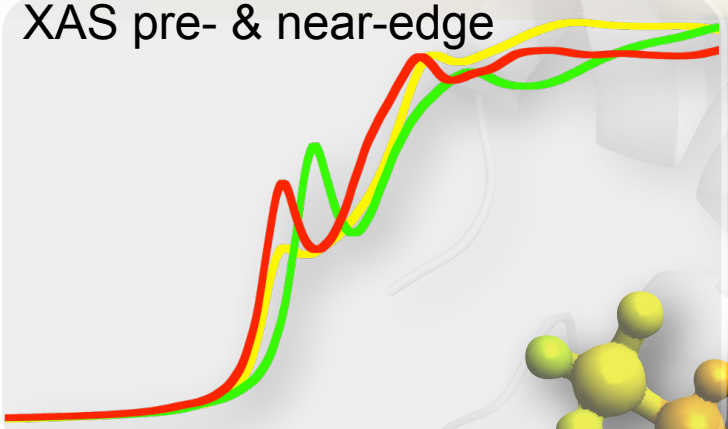


Structure: Information on Ligands
How many, *What* type, *How* far.

Combining Experiment and Theory

SLAC

XAS pre- & near-edge



Electronic information:
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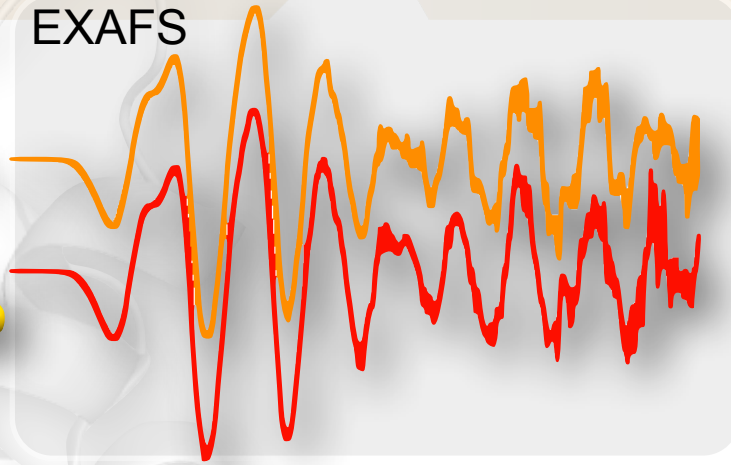
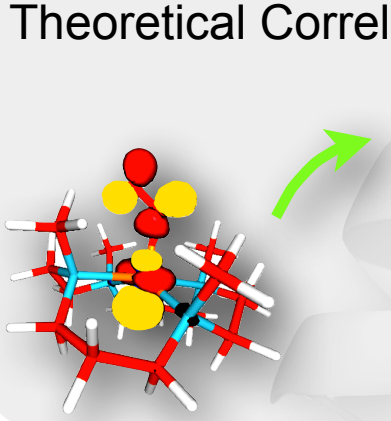
EXAFS



Structure: Information on Ligands
How many, *What* type, *How* far.

Theoretical Correlation

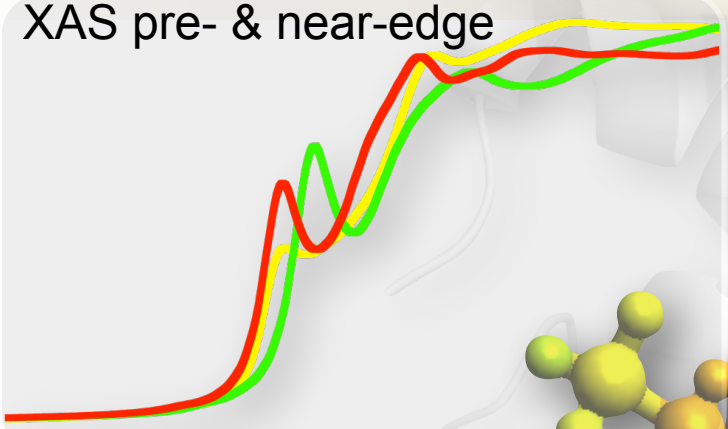


Detailed Electronic Information

Combining Experiment and Theory

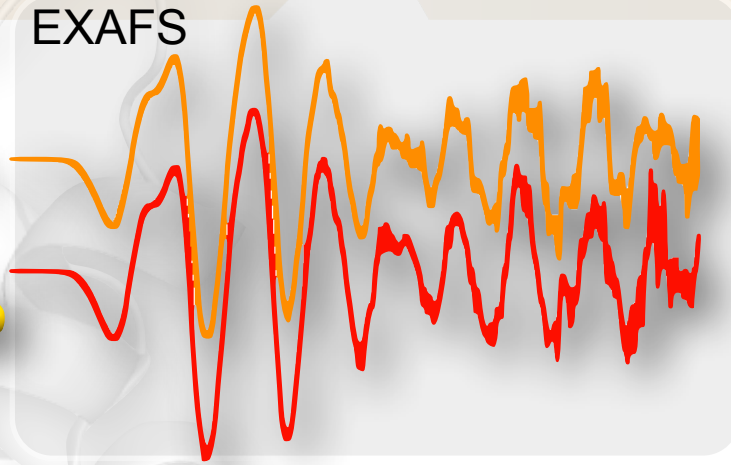
SLAC

XAS pre- & near-edge



Electronic information:
covalency, bond strength, type of ligands

EXAFS



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How many, *What* type, *How* far.

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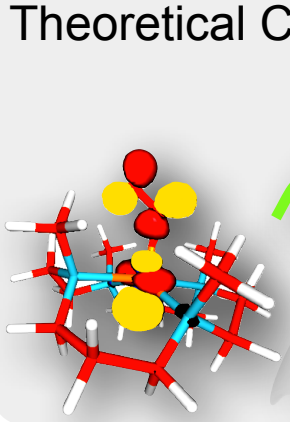
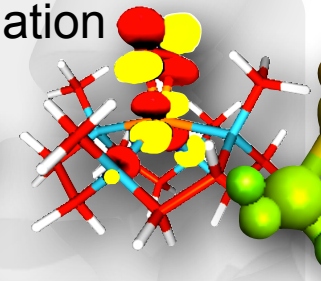
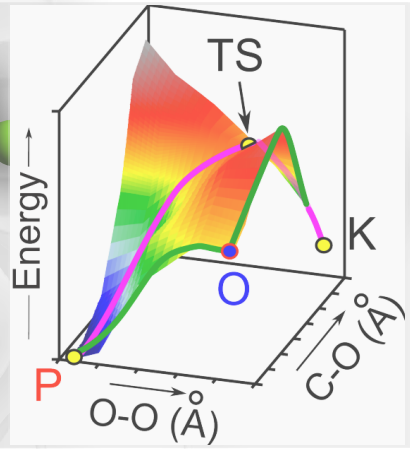




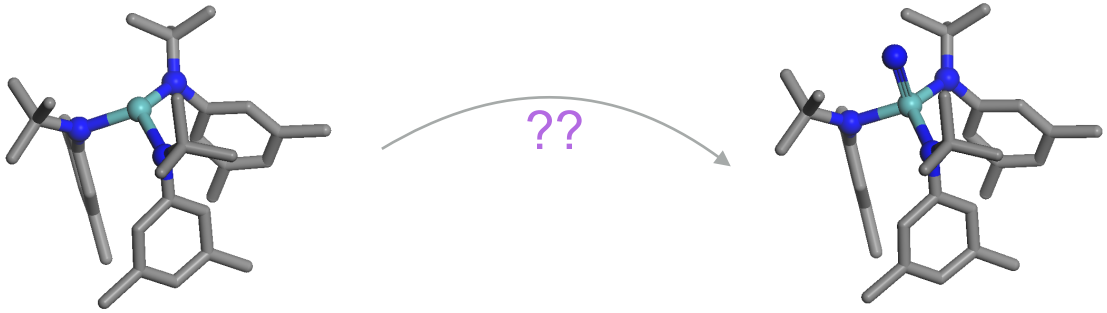


Detailed Electronic Information

Structure Function Correlation

Application of XAS to Homogeneous Catalysis 1

A low temperature solution intermediate: N_2 cleavage by a Mo(III) complex



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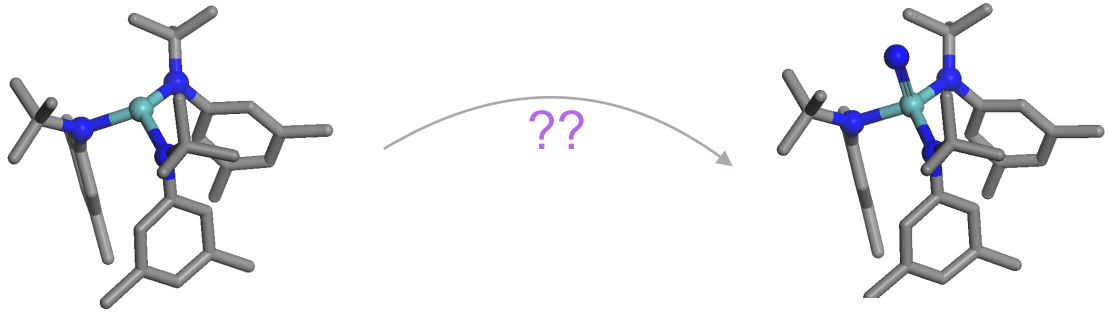
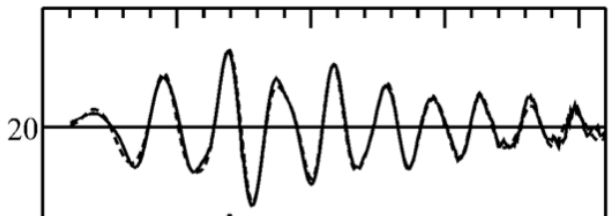
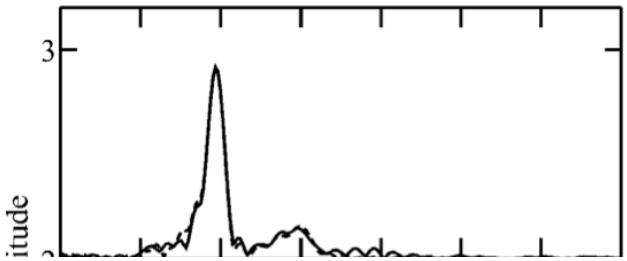
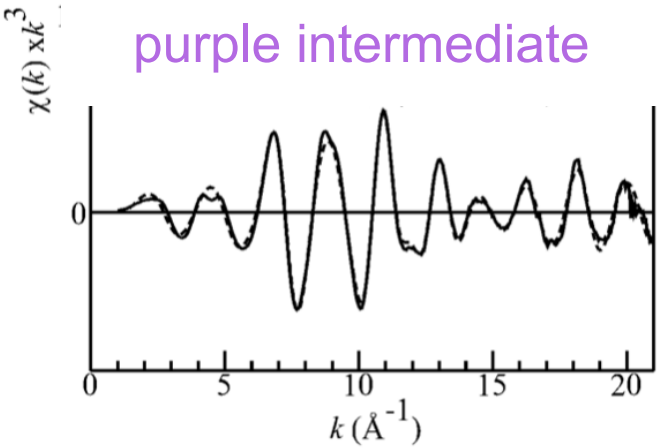


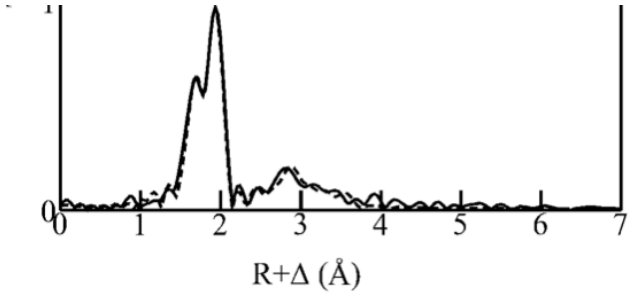




purple intermediate

purple intermediate





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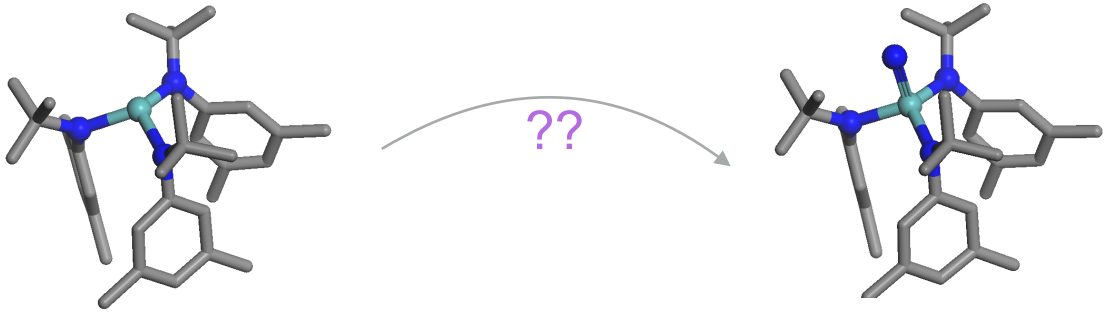
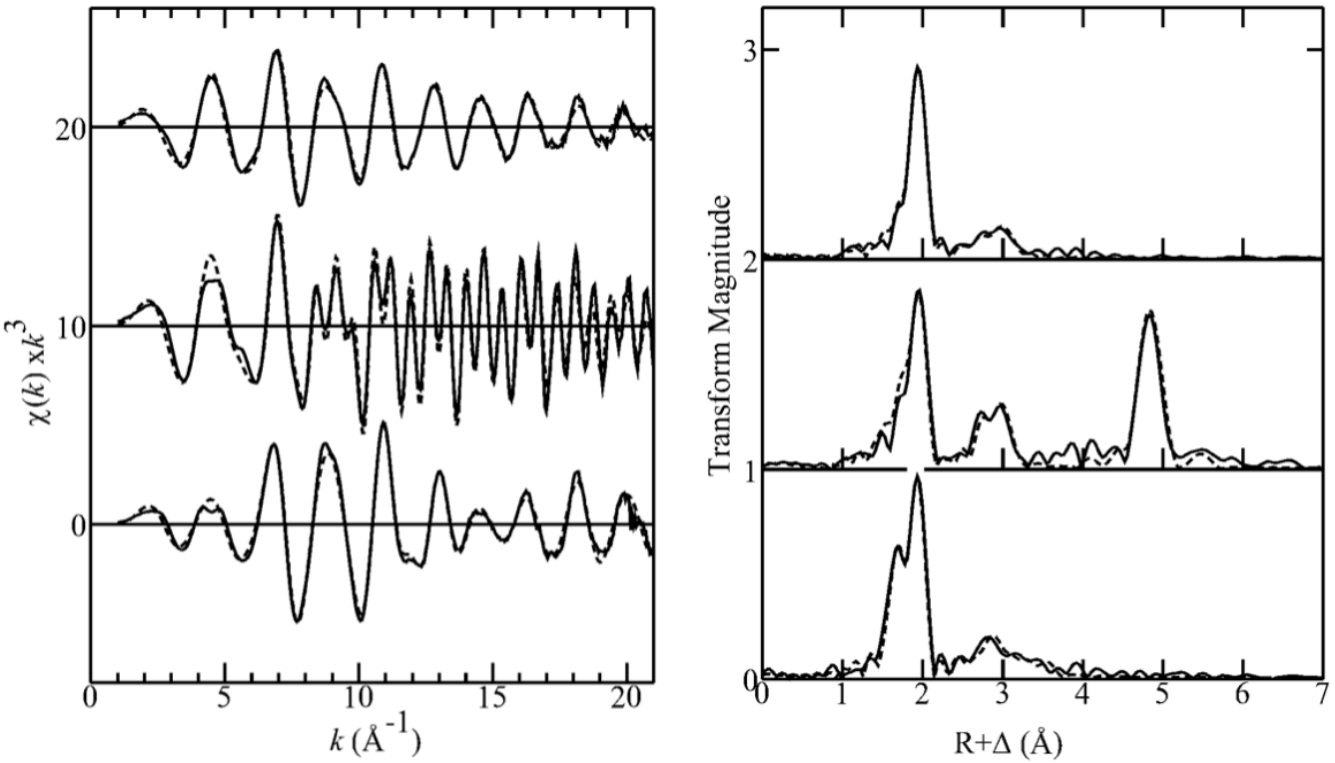
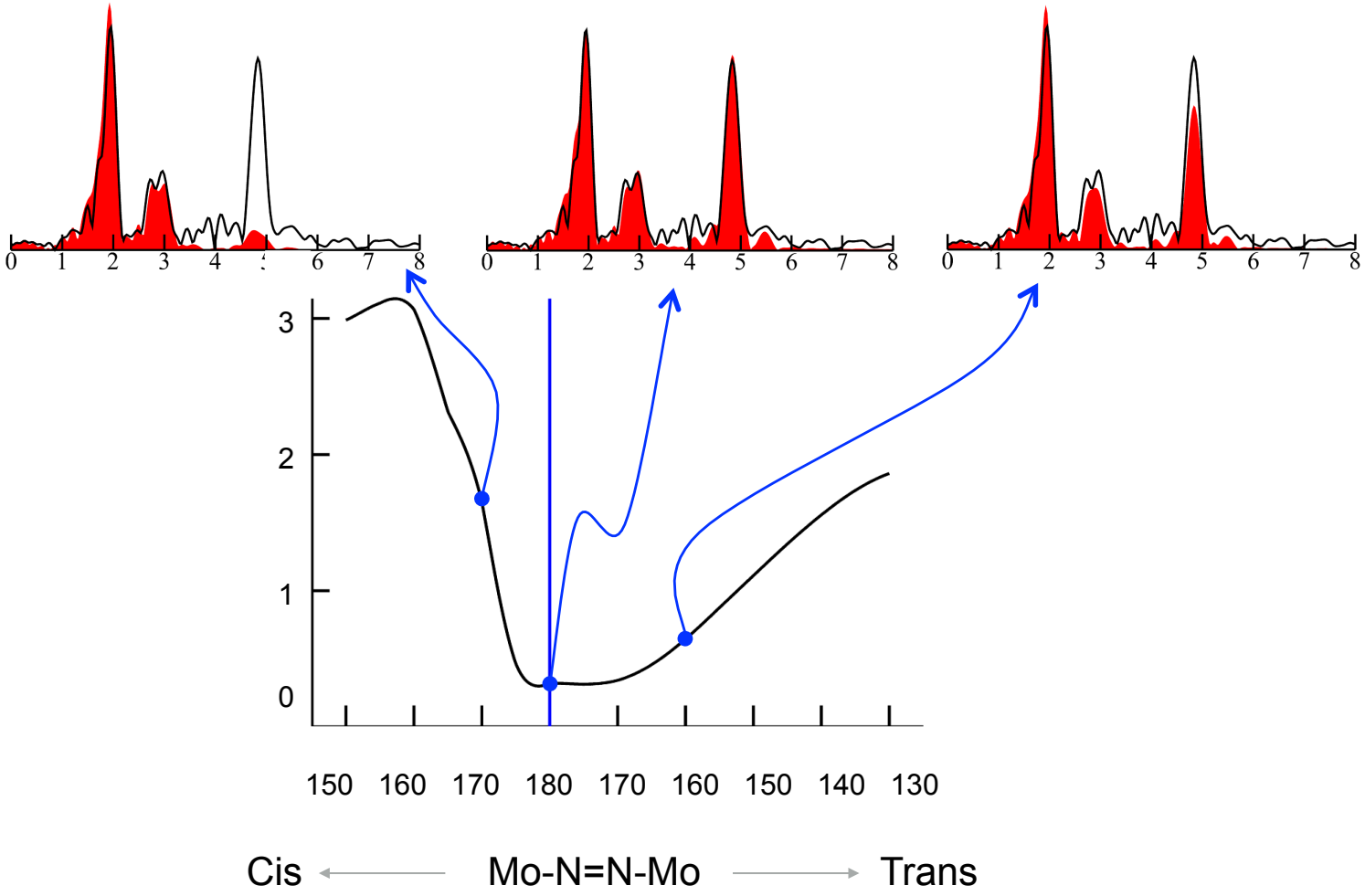




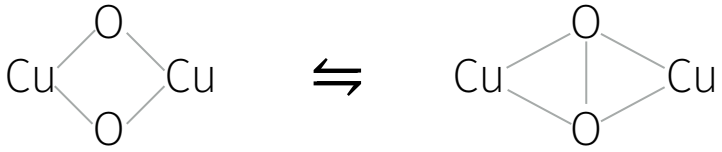
Homogeneous Catalysis 1

Linear Mo_2N_2 (Mo-N=N-Mo)bond in the intermediate ~5 Å feature



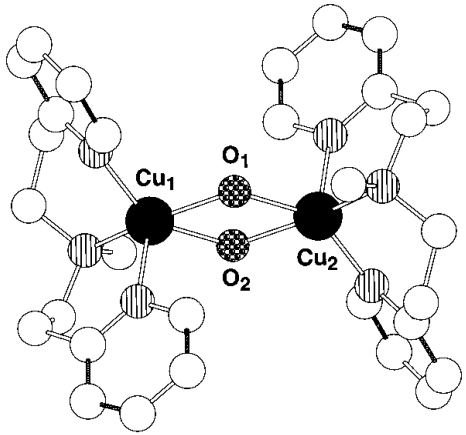
Application of XAS to Homogeneous Catalysis 2

Disproving small molecule crystal structure



Cu-Cu 2.9 Å , O-O 2.3 Å

Cu-Cu 3.6 Å , O-O 1.41 Å



Cu-Cu 3.4 Å , O-O 1.66 Å

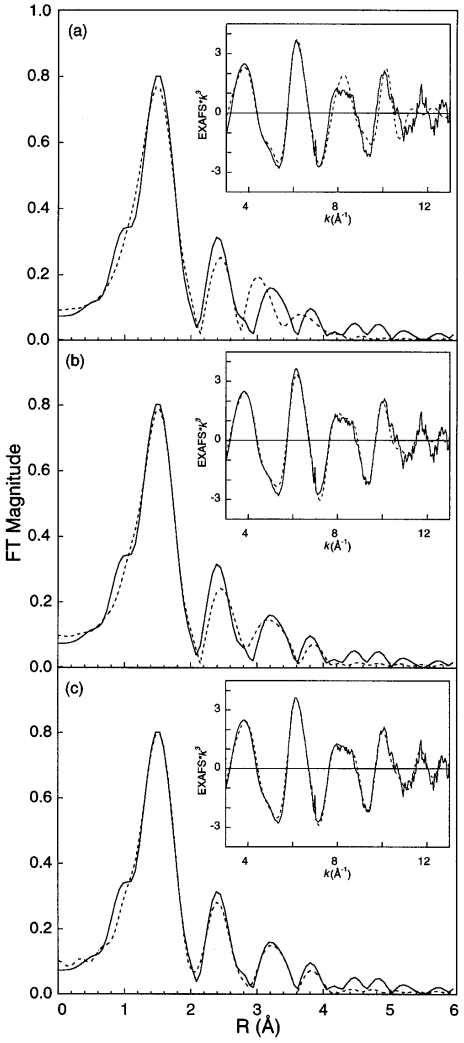
catalytically important conversion

$[\{Cu(MePY_2)\}_2O_2](BArF)_2$

Intermediate trapped during fast interconversion of the two species?

Structurally/Functionally distinct?

Homogeneous Catalysis 2

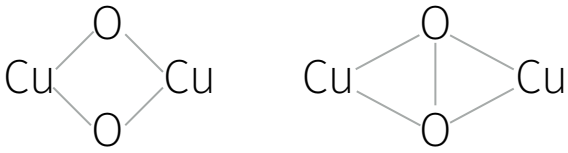


Data and fits for $[\{Cu(MePY2)\}_2O_2]^{2+}$

(a) the crystallographic Cu-Cu distance of 3.45 Å

(b) 100% 3.6 Å Cu-Cu

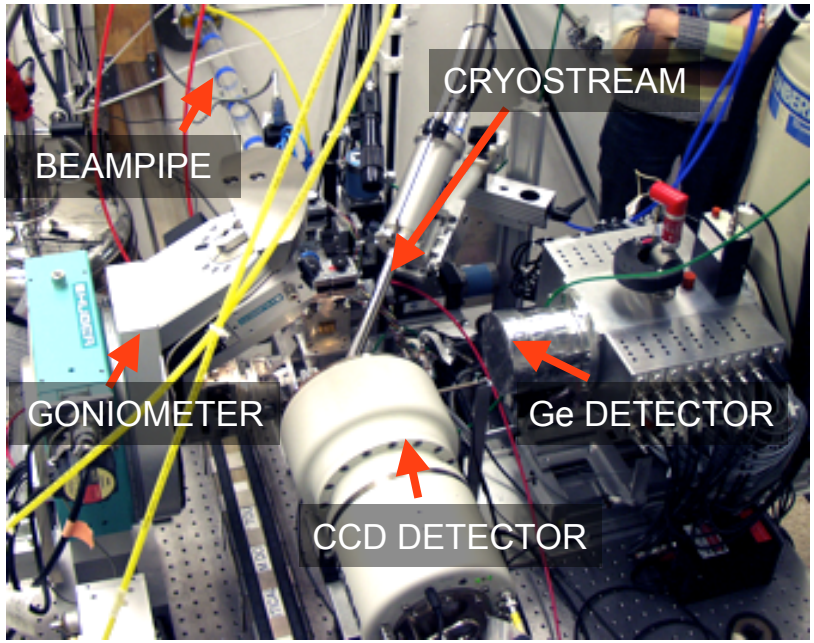
(c) a mixture of 80% 3.6 Å Cu-Cu and 20% 2.8 Å Cu-Cu.

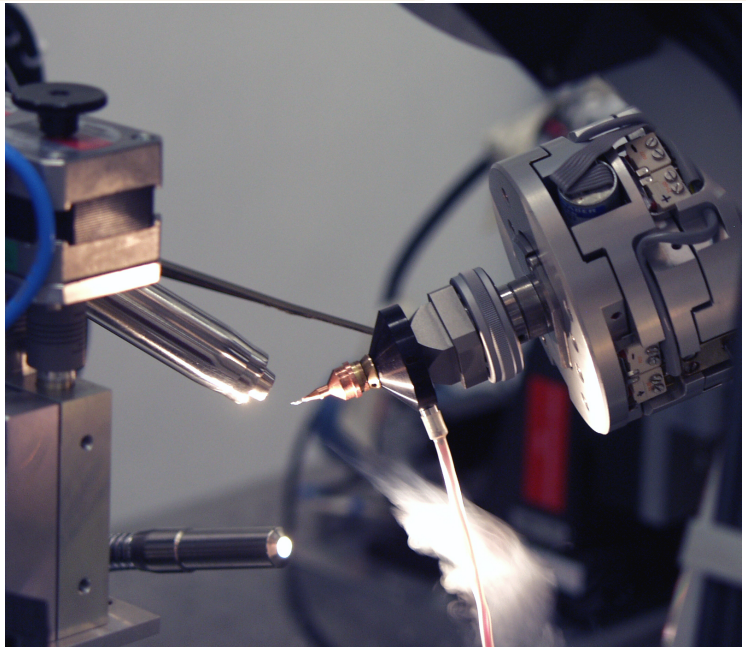


mixture of species

Small molecule crystallography can be incorrect

SSRL Beamline 9-3





- Sequential measurement of X-ray diffraction and X-ray absorption is possible.
- Sample requirements: Single crystals for polarized measurements : ~100 μm.
- Smaller proteins with heavier transition metals (higher than Ni) ~50 μm.
- Multiple crystals for standard XAS measurements.

Isotropic XAS

Isotropic XAS

Small Sample Requirement

- Multiple crystals from small starting volume (~5 uL): solution XAS ~100 uL (~ 1mM).

Applicable to Imperfect Crystals

- Twins, multiples, poorly diffracting, cracked etc. several crystals on loop to increase signal

Isotropic XAS

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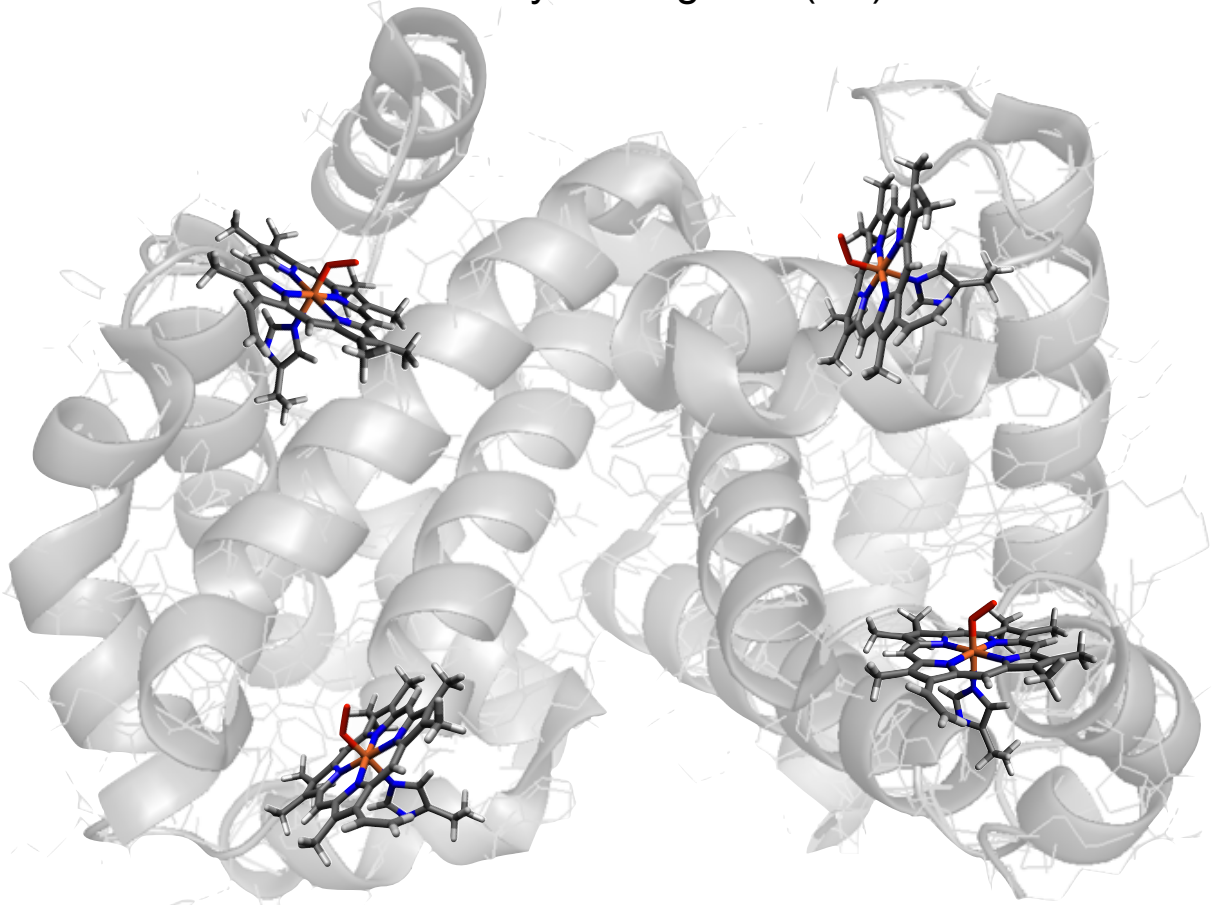
Direct Comparison to Crystallography

- Solution EXAFS may vary from crystallography due to changes in H-bonding or due
- to crystal packing effects. Singe crystal XAS is a direct in-state comparison.

- Monitor photoreduction in single crystals and correlate to photo-damage in crystallography.

Oxyhemoglobin

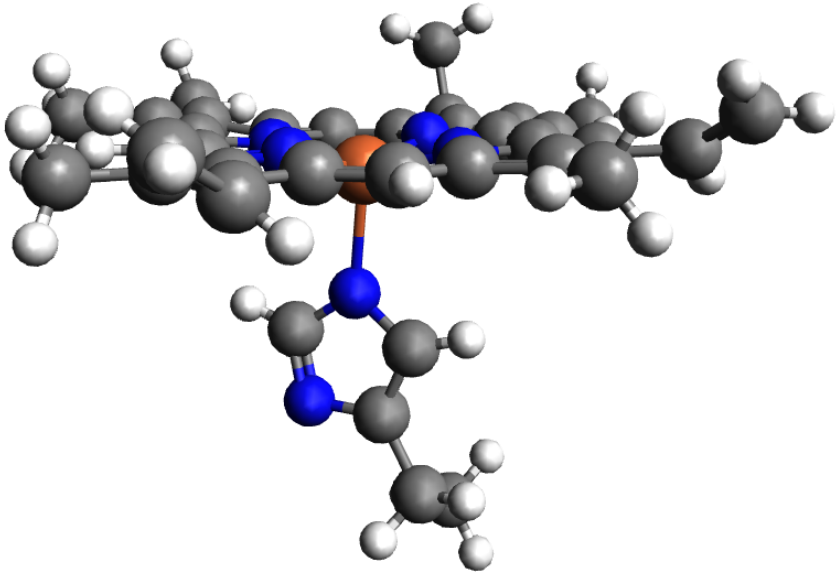
Human interaction with O_2 Mediated by Hemoglobin (Hb)



- Fe containing O_2 transport protein
- Contains an Fe-porphyrin (heme)
- Present in all vertebrates
- Binds upto 4 O_2 molecule per Hb

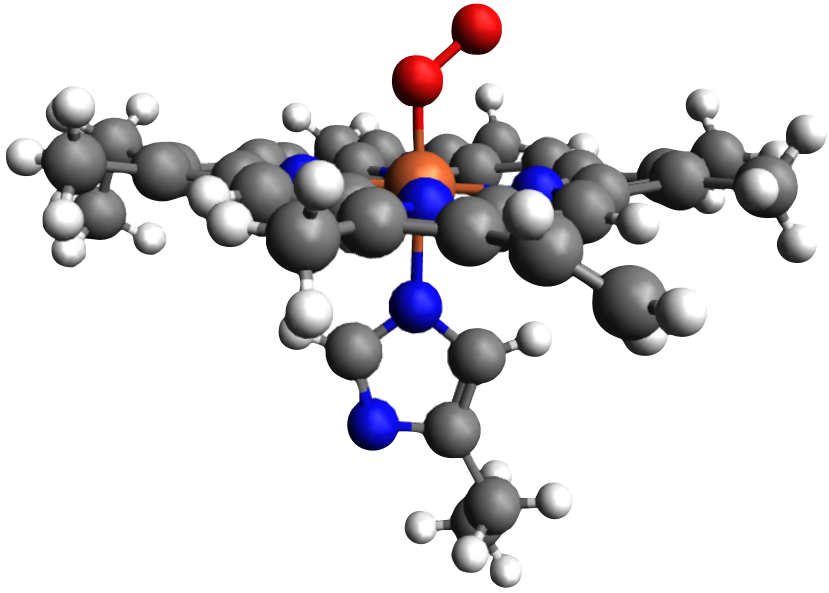
Oxyhemoglobin

Fe^{2+}(Protoporphyrin IX)(His) + O_2



Fe^{2+} + O_2
(S=2, 4 unpaired e^-) ($^3\Sigma_g^-$ S=1, 2 unpaired e^-)

Fe-O_2 (Protoporphyrin IX) (His)



Fe-O_2 (Protoporphyrin IX) (His)
(S=0, 0 unpaired e^-)

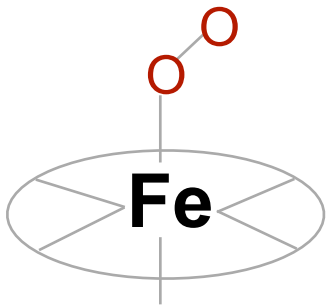
24 electrons undergo spin-pairing in the 4 subunits to form oxyhemoglobin!!

Electronic Structure

Ferrous

Fe^{2+} O_2

S=0, S=0

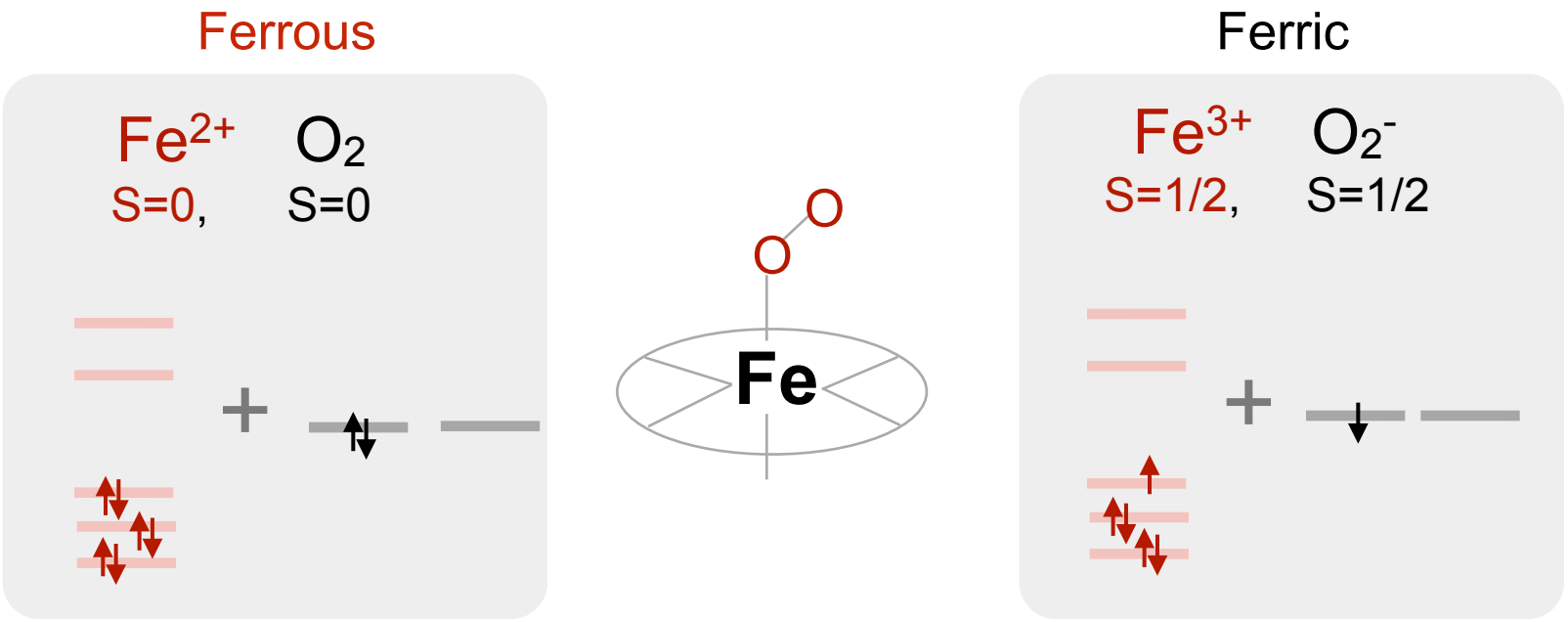


Ferric

Fe^{3+} O_2^-

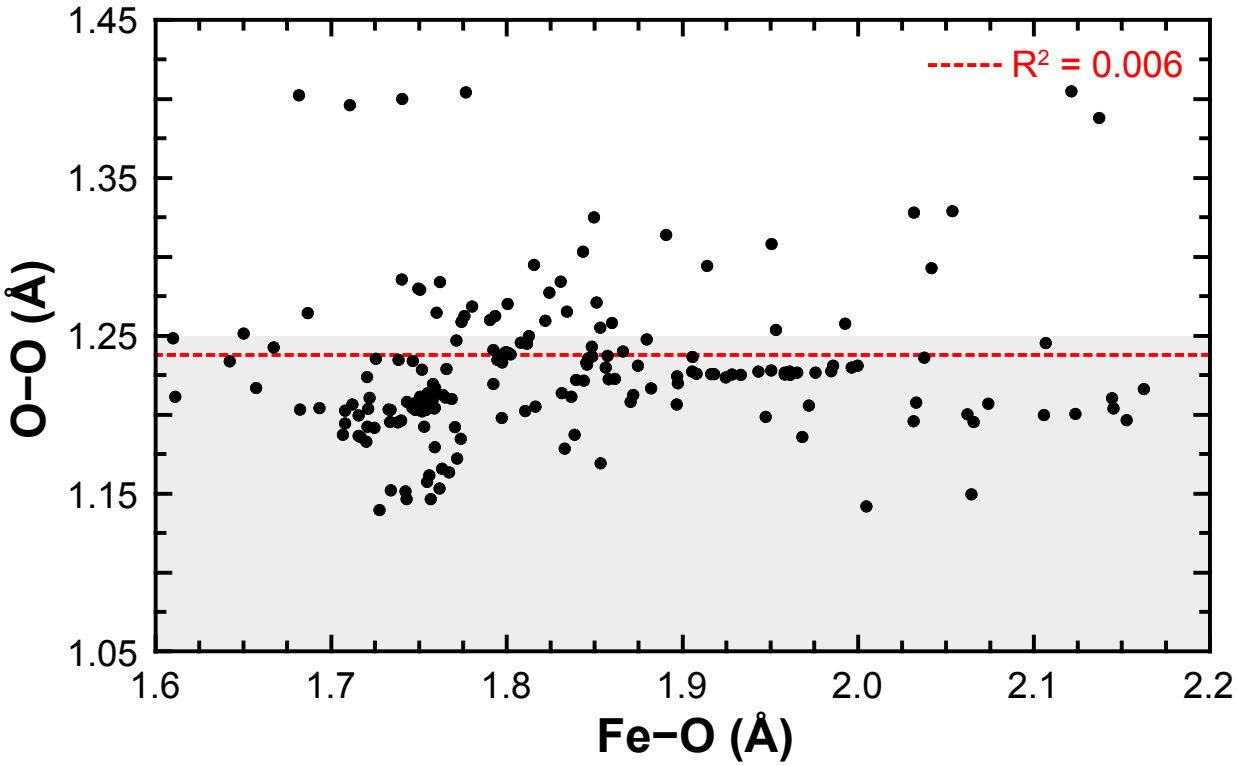
S=1/2, S=1/2

Electronic Structure



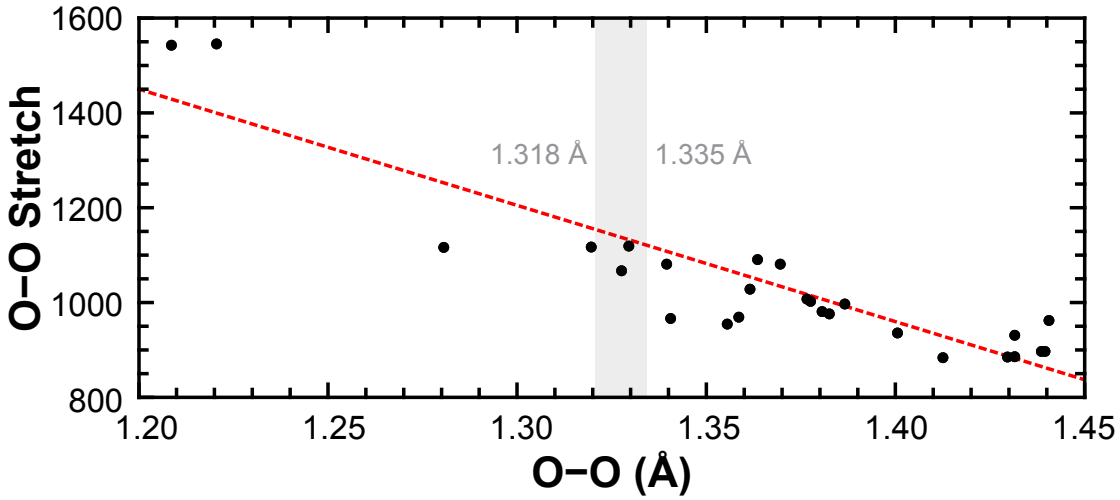
Is the metal center Reduced (Ferrous) or Oxidized (Ferric)?

Crystallography



- O-O Bond Distance indicates Reduced (Ferrous).
- Why is there a large spread in Fe-O ?

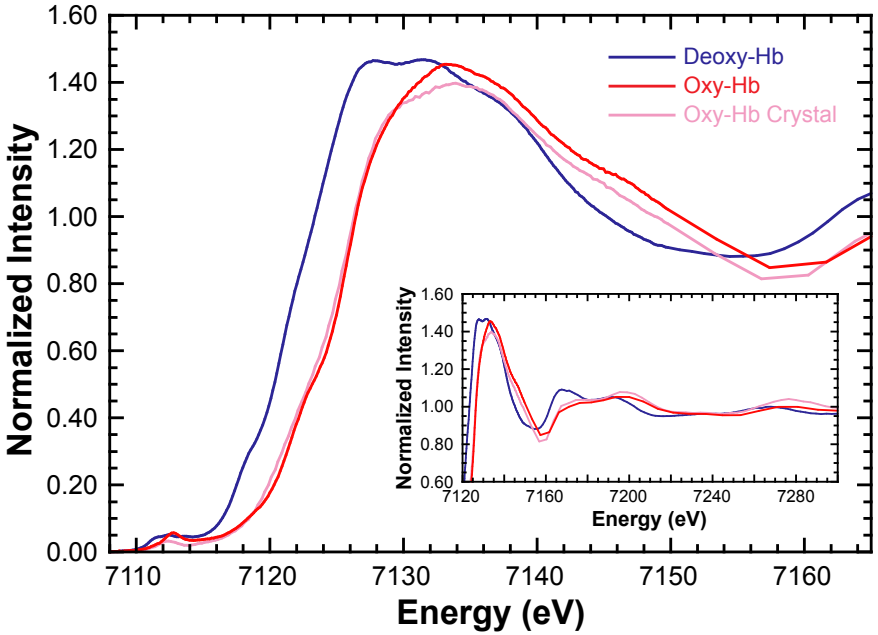
Solution Spectroscopy

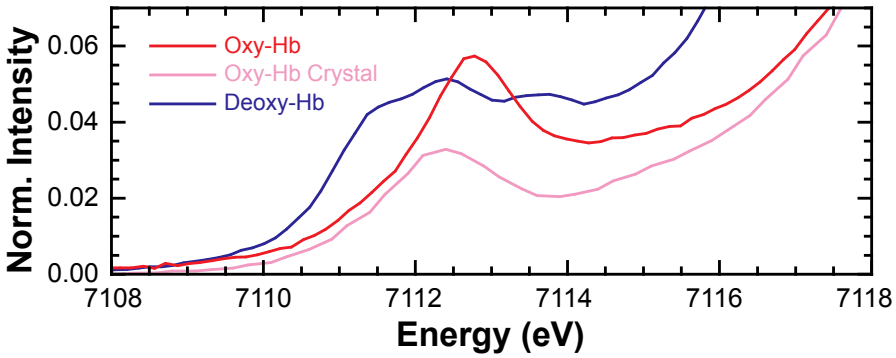


- O-O Bond Distance derived from spectroscopy (rRaman) indicates Oxidized (Ferric).

Discrepancy between solution spectroscopy and x-ray crystallography??

Solution & Crystal XAS

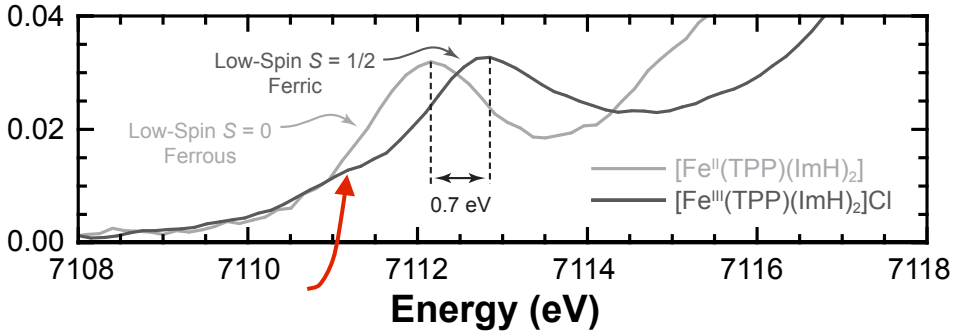




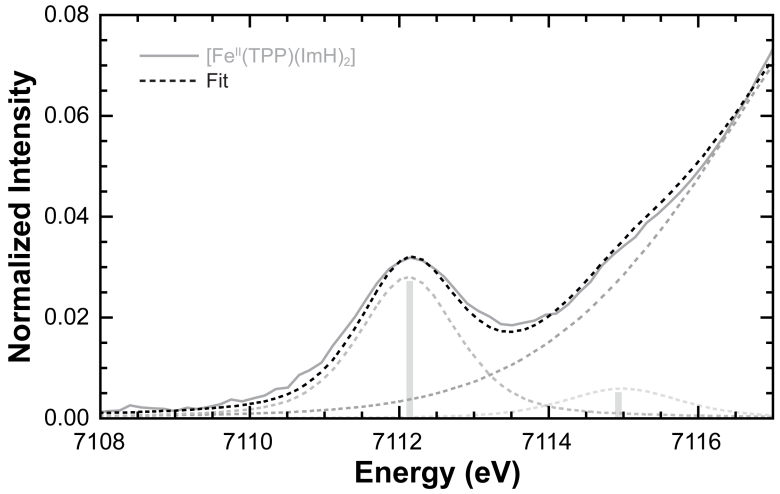
- Crystal near-edge similar to solution.
- Structure analysis shows very similar O_2 bound geometry.
- Fe K-edge and pre-edge distinctly different from starting material - deoxyHb
- *Curiously* - Fe K-pre-edge for oxyHb in solution and crystalline forms are different.
- Since geometric structure is similar, does this point to electronic changes?

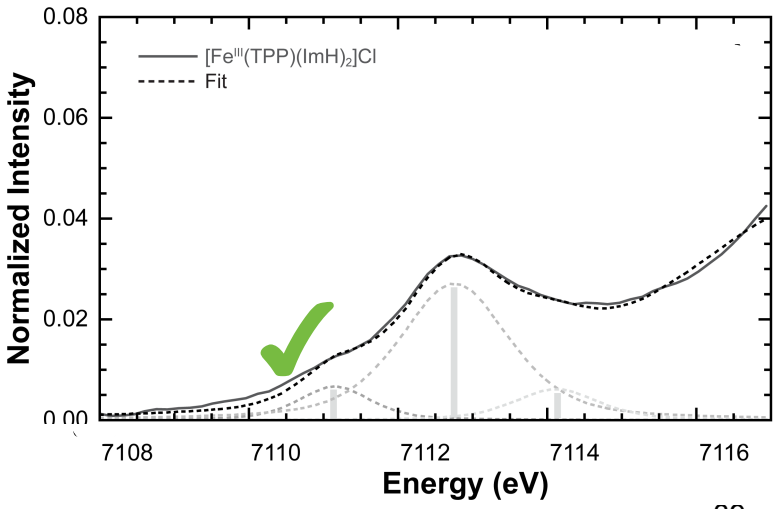
Model Comparison

What do small molecule models with $Fe^{3+}O_2^-$ and $Fe^{2+}O_2$ look like?

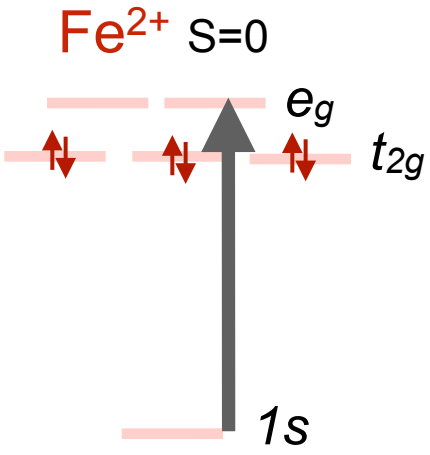


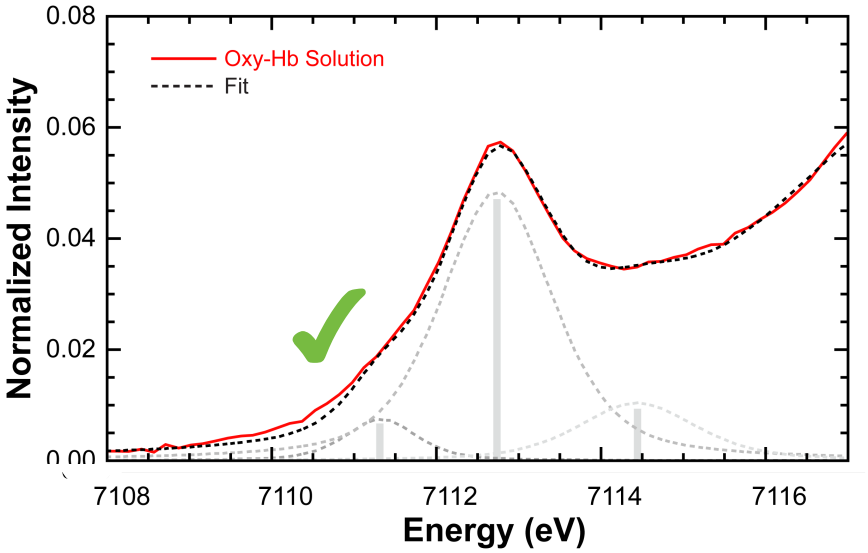
pre-pre-edge feature ??

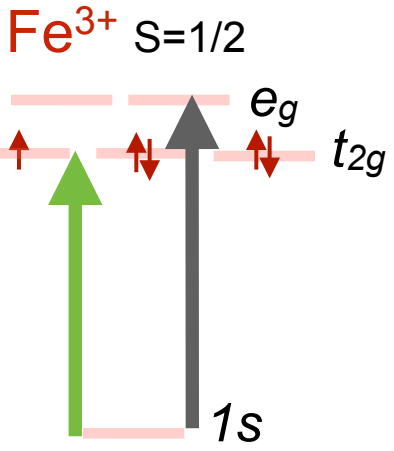


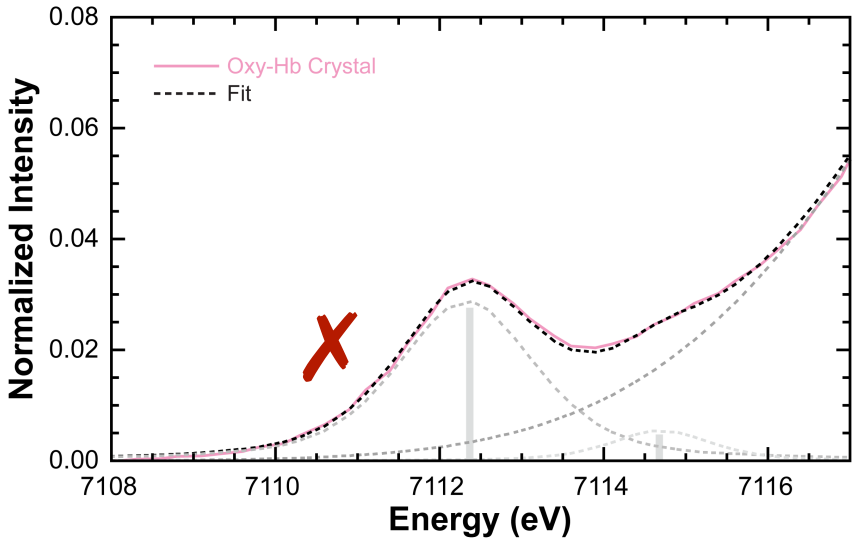


Solution & Crystal Pre-edge

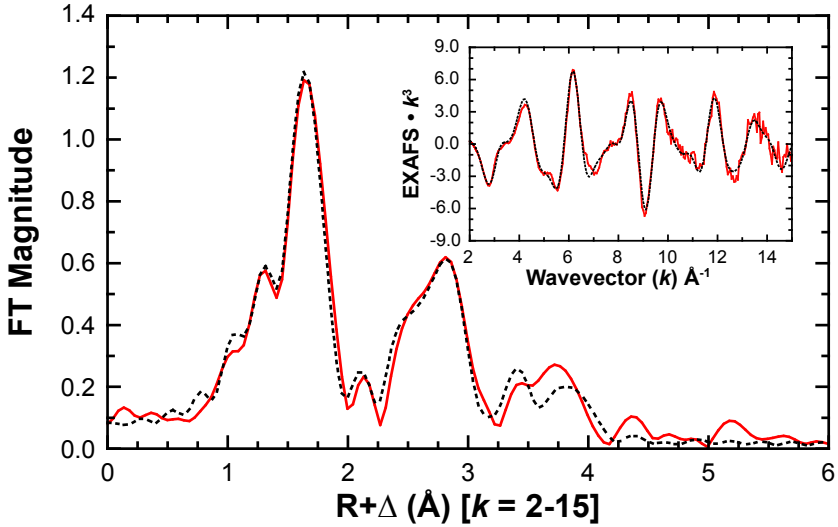


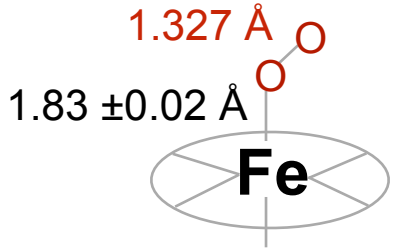


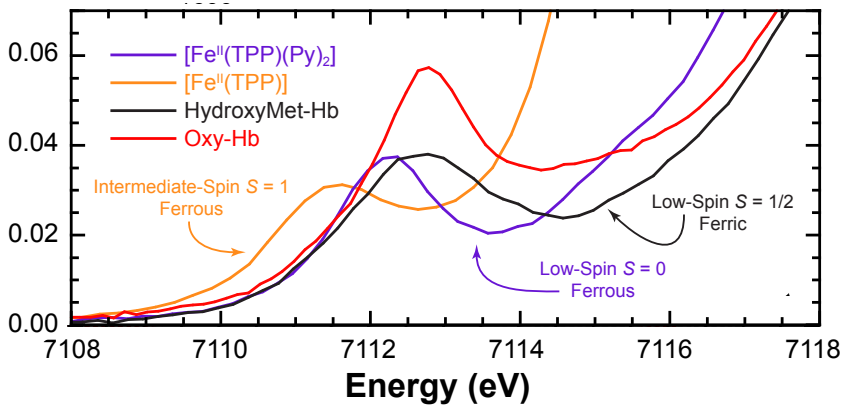


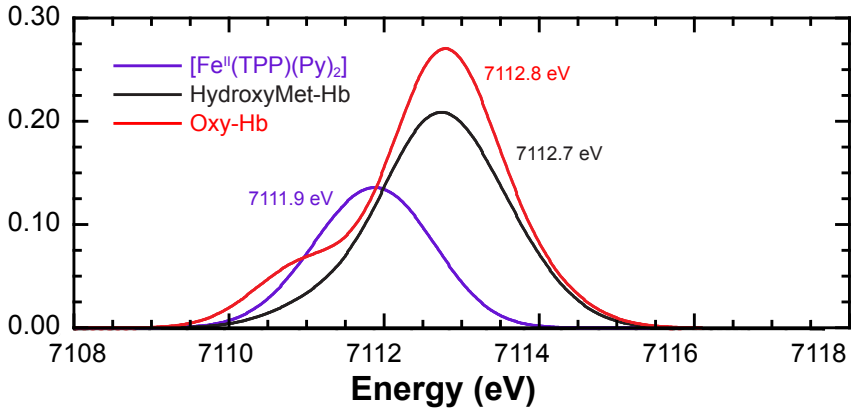


Solution EXAFS and DFT



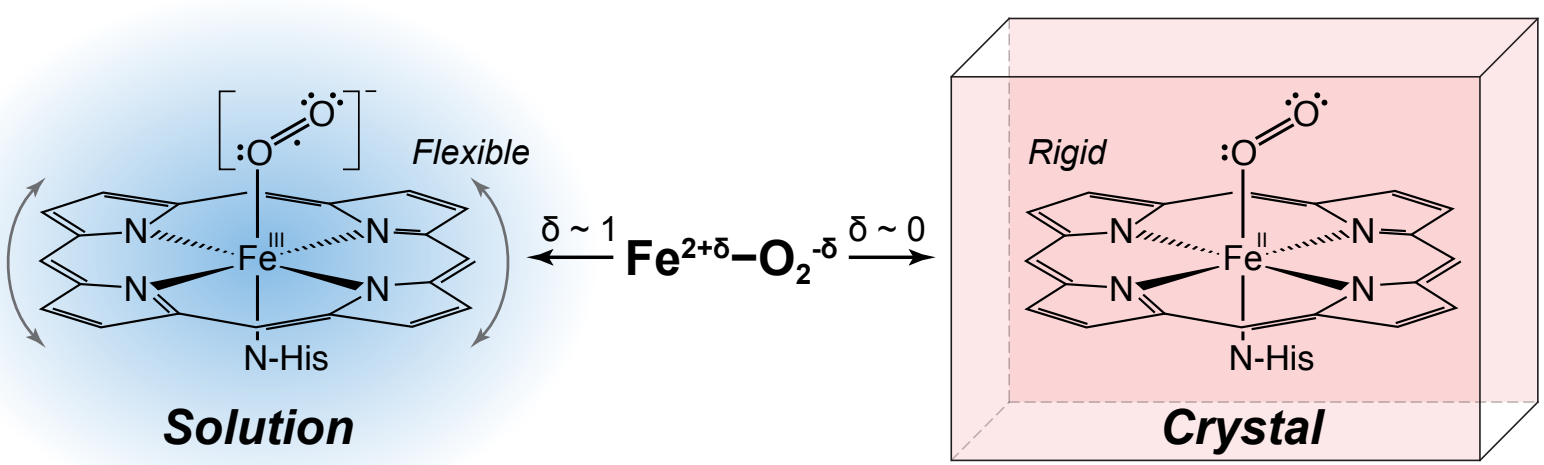






- DFT reproduces EXAFS distances and the differences in pre-edge.

Electronic Structure Interpretation

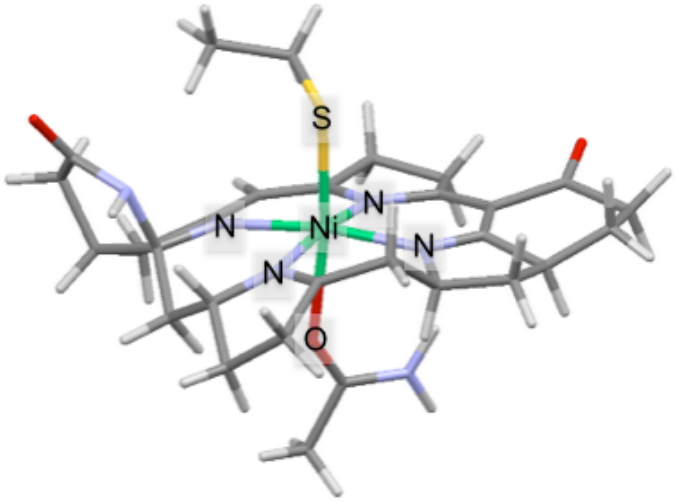


- Differences in Crystallography and Solution Spectroscopy Real.
- Electronic structure of oxyhemoglobin consists of both the ferrous and ferric components.
- Ferric dominates in solution and Ferrous dominates in crystal form.

Electronic Structure of Intermediate in MCR

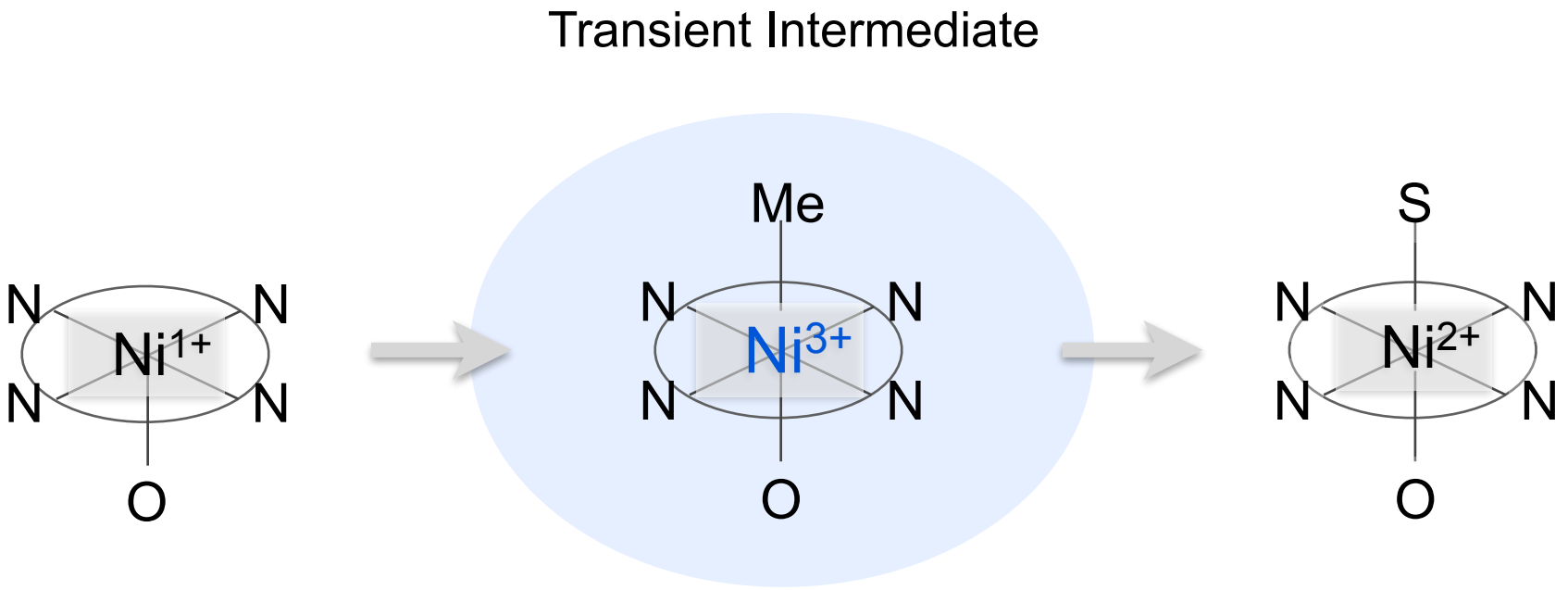
Methyl-Coenzyme M Reductase catalyzes the final step of CH_4 formation in anaerobic archaea

Me-SCoM + H-SCoB → CoM-S-S-CoB + CH_4



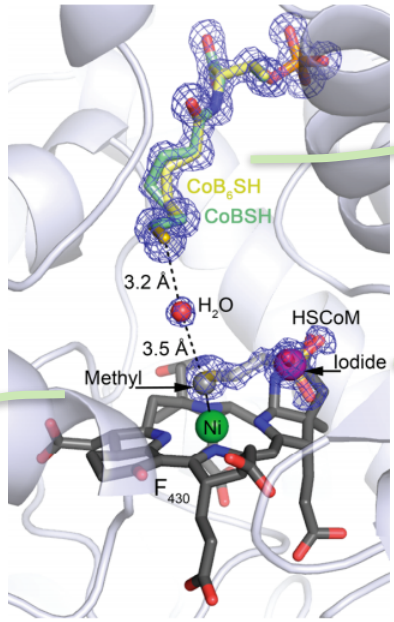
Very important from biological energy perspective - generates 1b tonnes of Methane annually!

Spectroscopically Evaluated Intermediate



An unprecedented Ni(III) site is formed during catalysis. This intermediate is proposed to have an organometallic Ni-C bond.

Crystallography -Challenging



60:40 mixture of coenzyme B and B_6

50:50 mixture of coenzyme M and Methyl

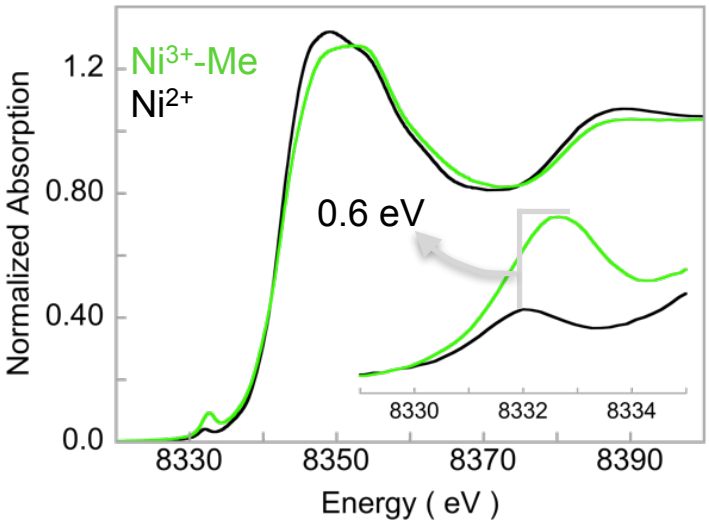
35% Iodide

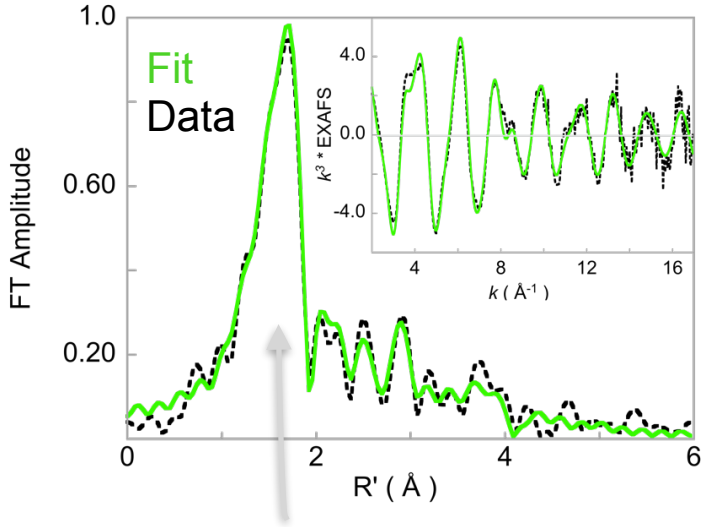
How can we be convinced of the formation of the Ni^{3+} species?

If present what is our confidence level of the Ni-Me distance?

Is there photoreduction/differences in the crystal that can lead to an erroneous Ni-Me distance?

Solution Structure of Organometallic Intermediate





first shell fit indicates presence of Me

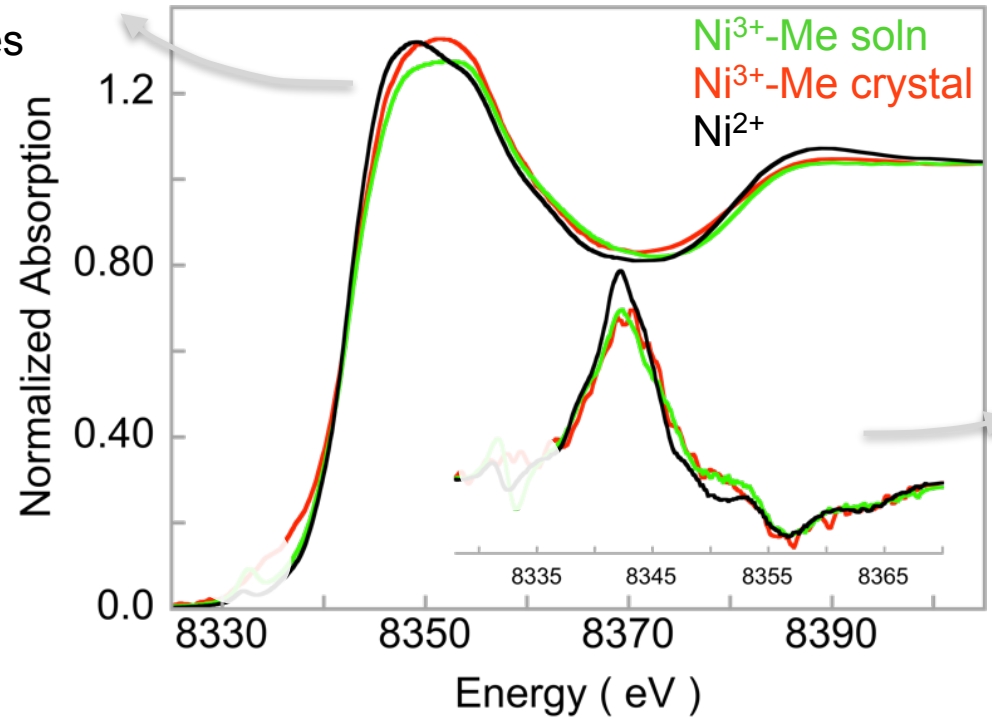
Ni K-edge data show formation of Ni^{3+} in solution

Ni EXAFS data show presence of Ni-Me bond

Combination of edge and EXAFS data helped develop geometric and electronic structure

Evidence for In-Crystallo Intermediate Formation

similar edge shape/energies



overlaying edge inflections

Ni K-edge data confirm the formation of the intermediate *in-crystallo*

The data preclude significant photoreduction in the crystal

Soln and crystal data indicate similar geometric structures → 2.08 Å used as benchmark

How is crystalline XAS Useful

- Biological EXAFS is a powerful technique that furnishes *atomic* resolution local structures of metalloprotein active site.
- XAS is a powerful technique to obtain valuable insights into the electronic structures of metalloproteins
- Solution and crystalline structures of metalloproteins may vary *intrinsically*.
- Crystal structures may be mixtures, or photoreduced - XAS/EXAFS bring additional info.
- Structures can be low resolution - Complementarity with EXAFS important for electronic structure interpretation.

Limitations of the EXAFS Method

Very accurate first shell distances (+/- 0.02 Å), but “sees” the average of all of a given photoabsorber.

Ability to identify types of ligands, but only if $\Delta Z > 1$ (for Z~6-17).

Not able to separate out contributions from different oxidations states of the same photoabsorber type (i.e. cannot separate Fe(III) EXAFS contribution from the Fe(IV) EXAFS contribution).

EXAFS range may be truncated due to the presence of Z+1 atoms (e.g Zn in Cu EXAFS and Fe in Mn EXAFS)

Take Home Messages

SLAC

What to look for in an EXAFS Paper ?

- A good, high quality data set. Are the EXAFS data shown? How far out in k-space have the data been obtained? What is the fit range?
- Explanation of data processing and analysis packages used.
- Assessment of how the “best-fit” was arrived at.
- Potential discussion of correlated parameters and resolution of the data.
- Statistics
- Be suspicious of...filtered data, large changes in E_0, too many parameters.

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National Institutes of Health.