EXAFS : Application to Biological Systems

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Outline

- Application of EXAFS to Metals in Biology
- Modern Approach to Biological XAS
- Single Crystal XAS
- Oxyhemoglobin
- DypB

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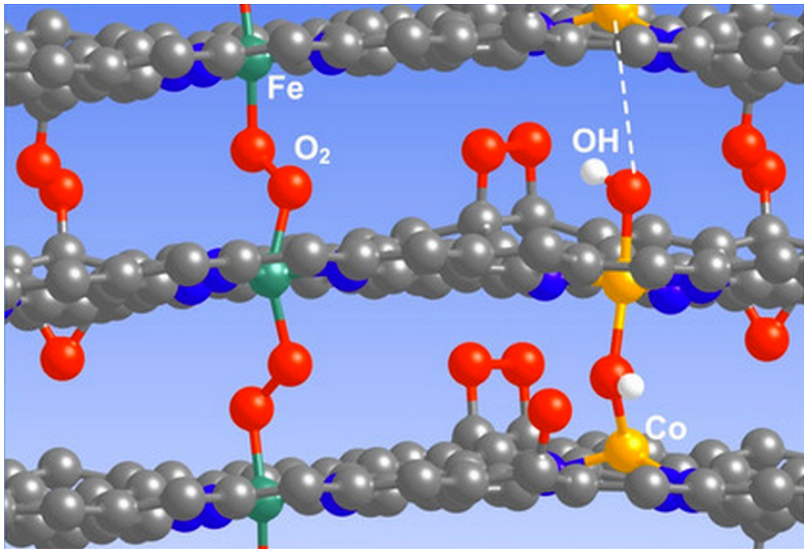
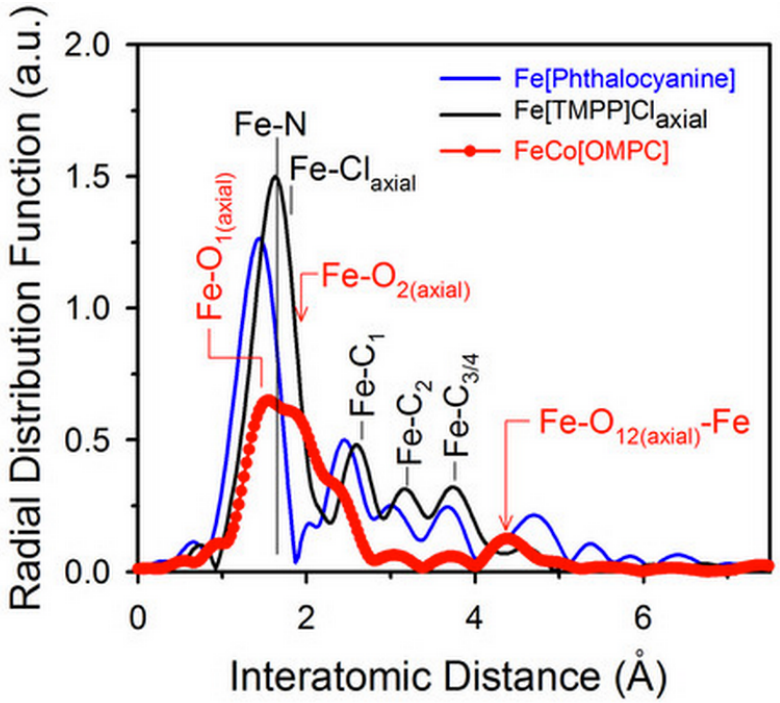
Ordered Systems





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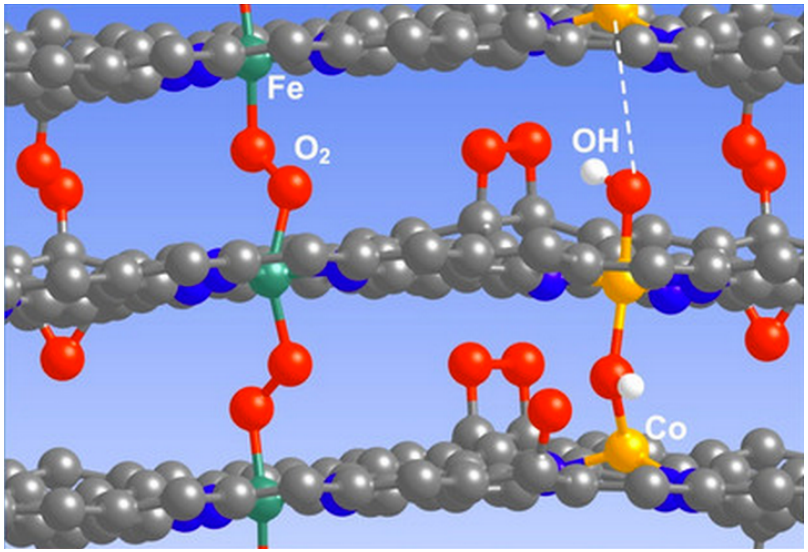
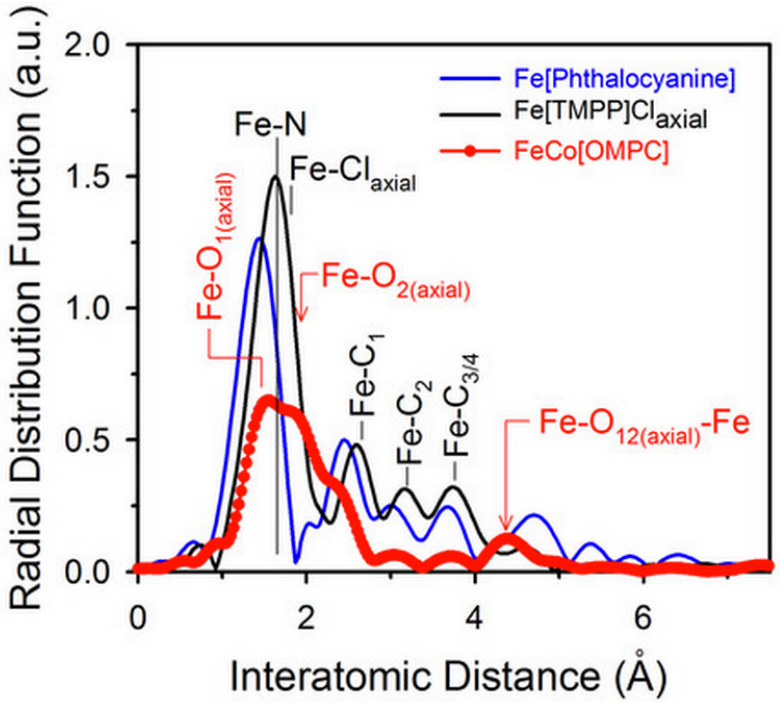
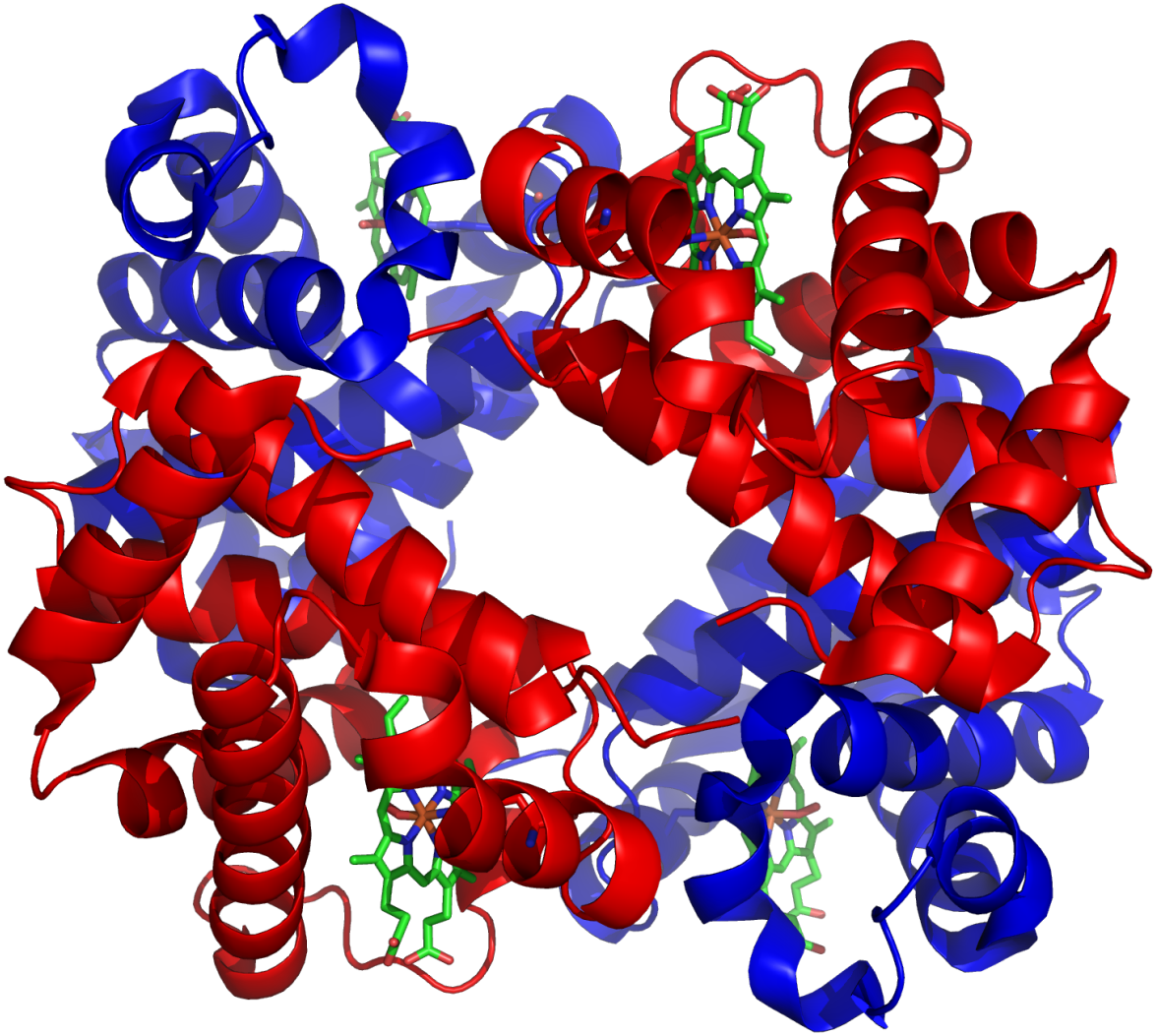




- EXAFS more ordered (data to high *k*)
- Shell by Shell analysis is popular and FT based fitting is helpful.
- Rigid structure - similarity between related systems.

Biological Systems



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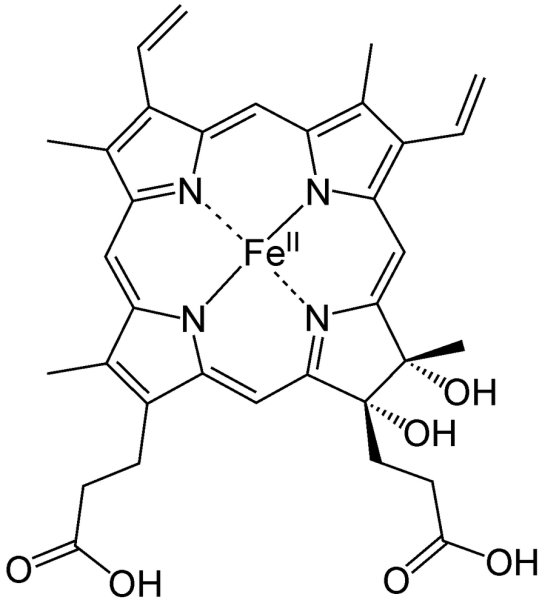

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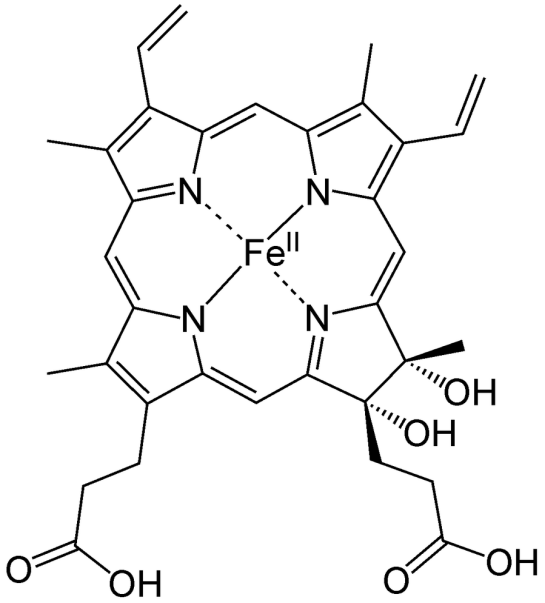

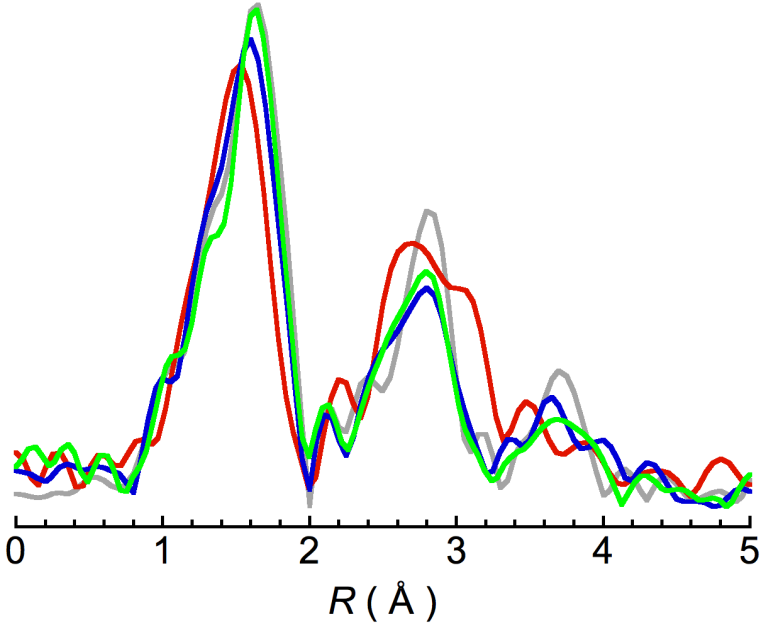

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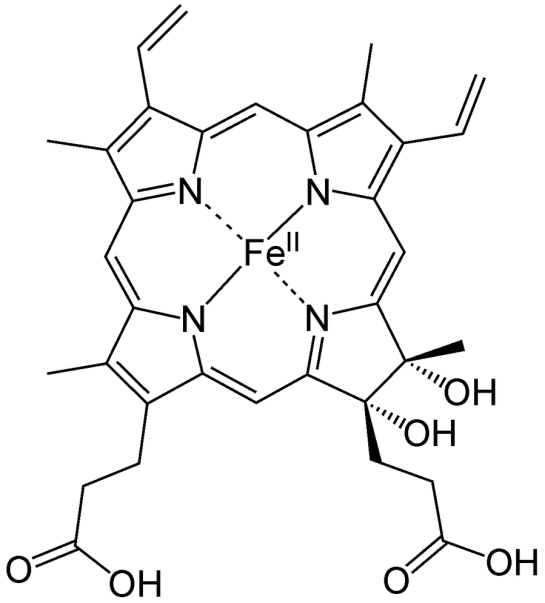

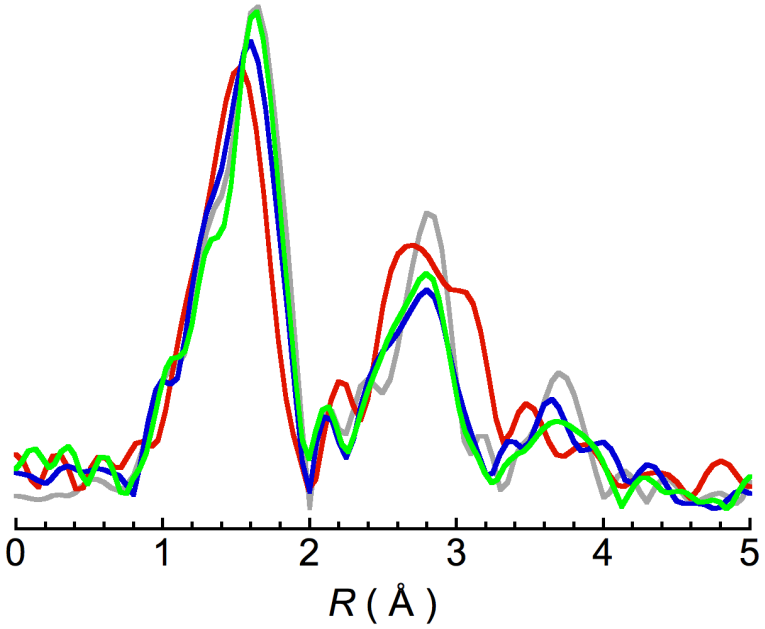

- EXAFS are more disordered (data to k=11-15 Å).
- Complete EXAFS analysis necessary for meaningful interpretation.
- Confidence mostly in first shell & second shell metal coordination.

Experimental Considerations

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

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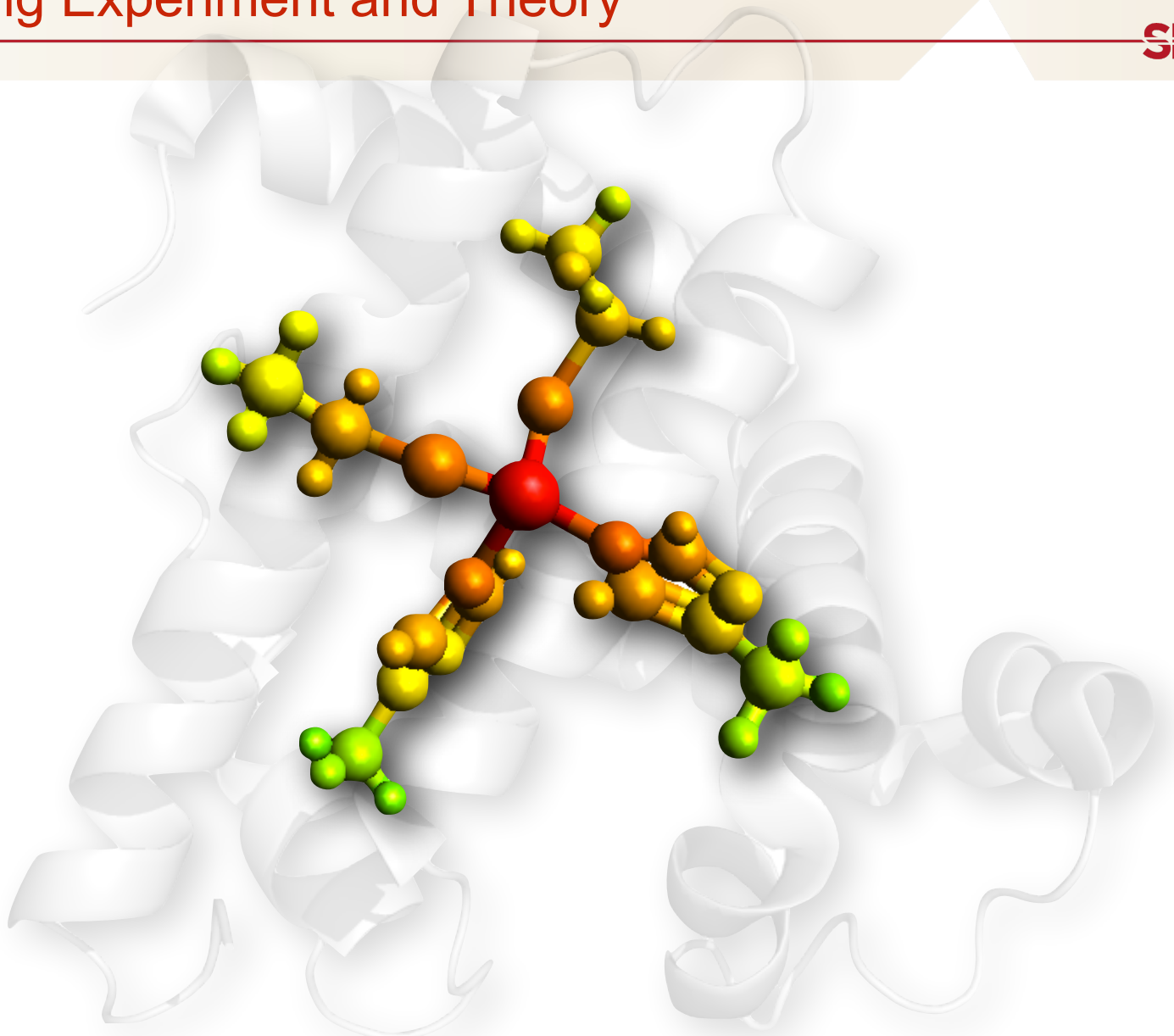
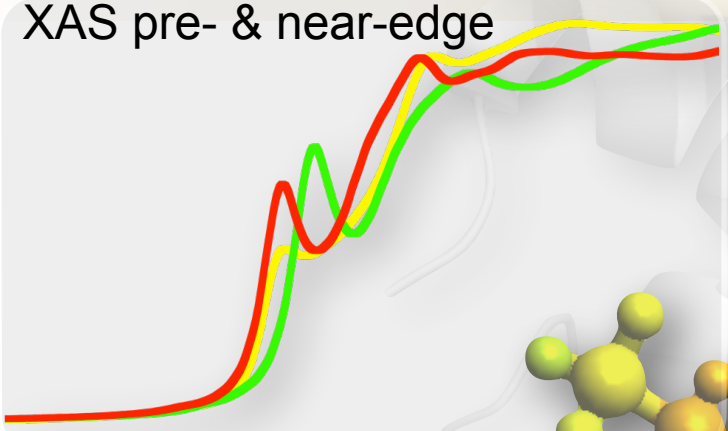
Combining Experiment and Theory

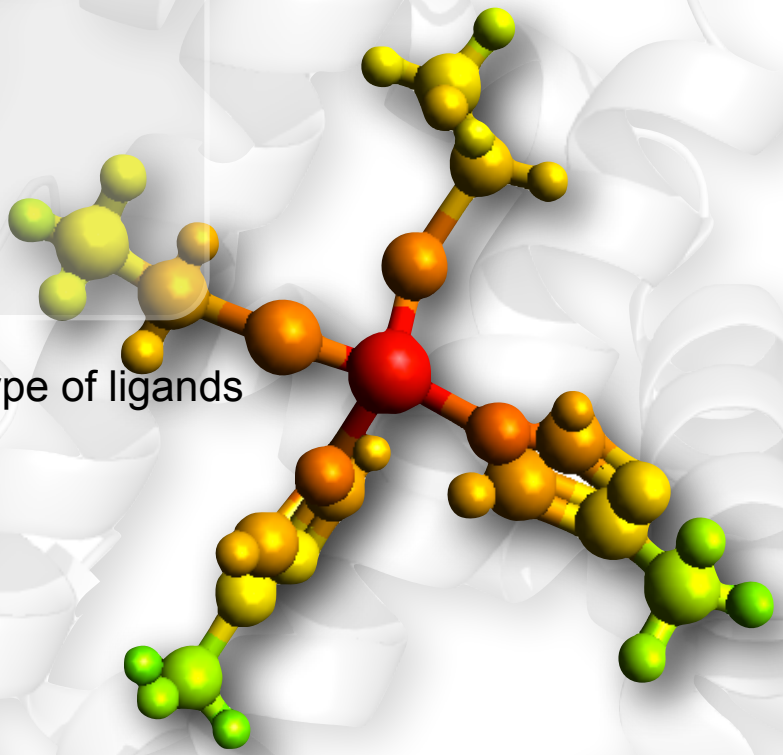


Combining Experiment and Theory

XAS pre- & near-edge



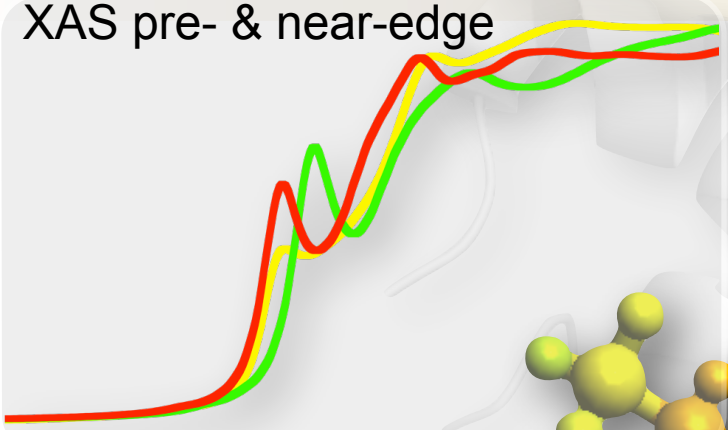
Electronic information:
covalency, bond strength, type of ligands



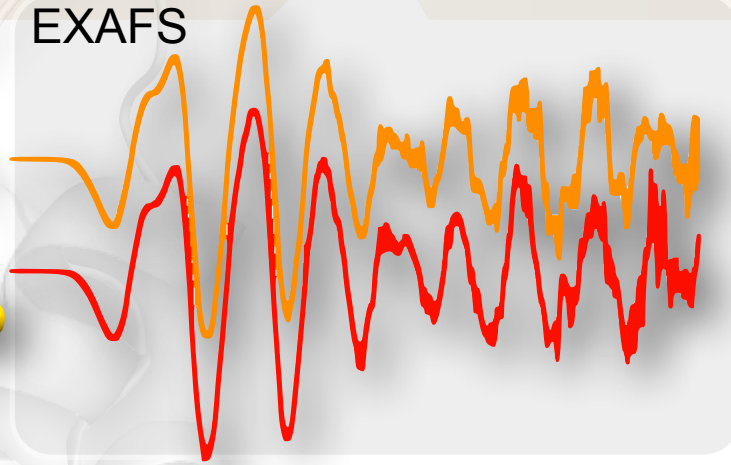
Combining Experiment and Theory

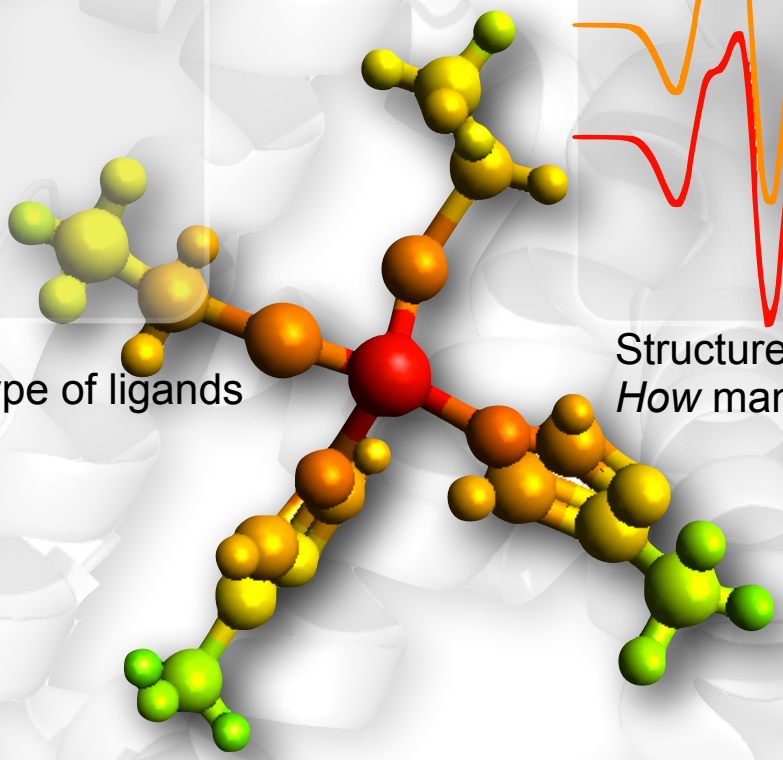
SLAC

XAS pre- & near-edge



EXAFS





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

Combining Experiment and Theory

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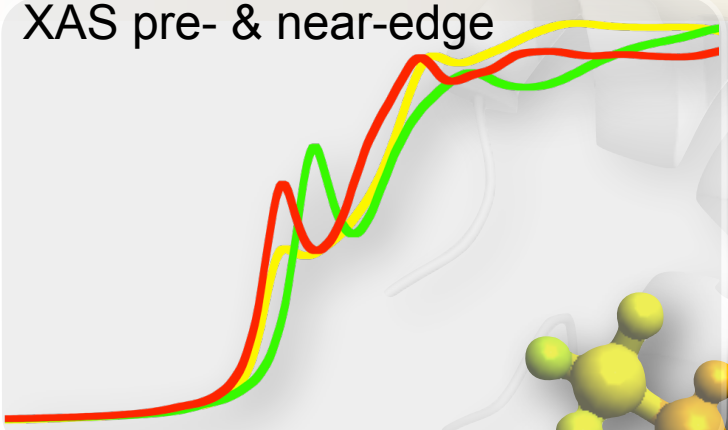

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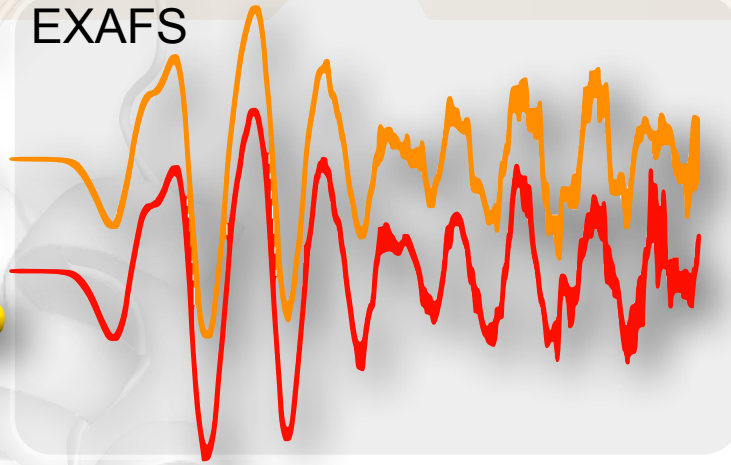

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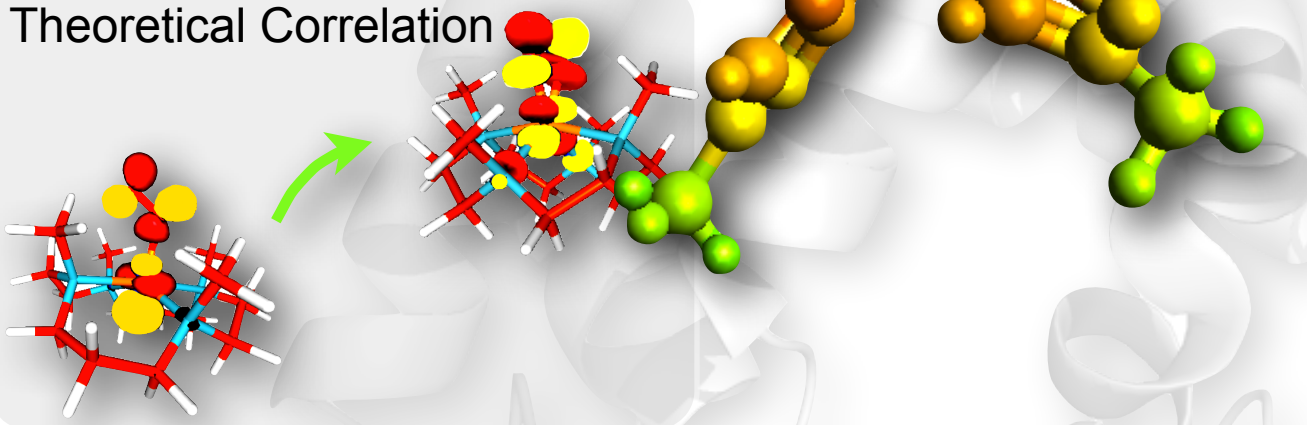
Theoretical Correlation

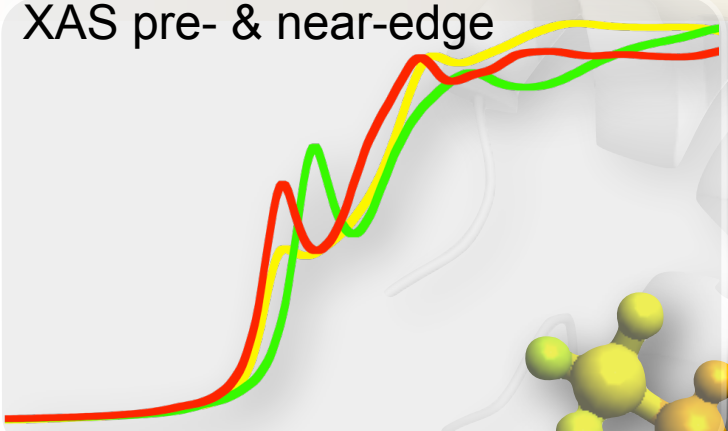


Detailed Electronic Information

Combining Experiment and Theory

SLAC

XAS pre- & near-edge



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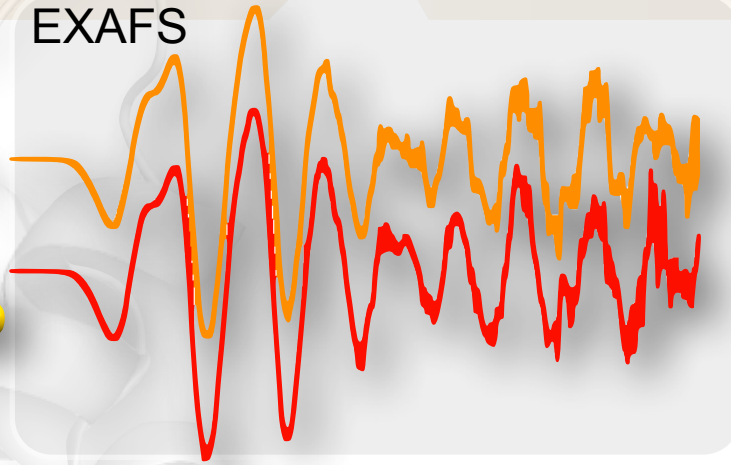

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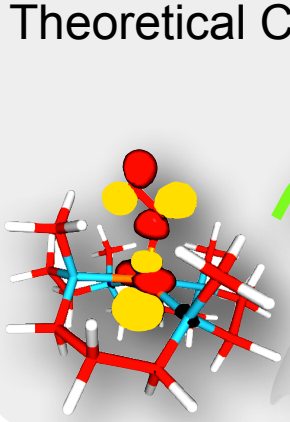
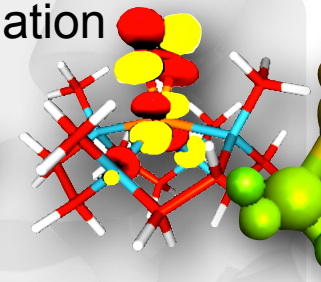
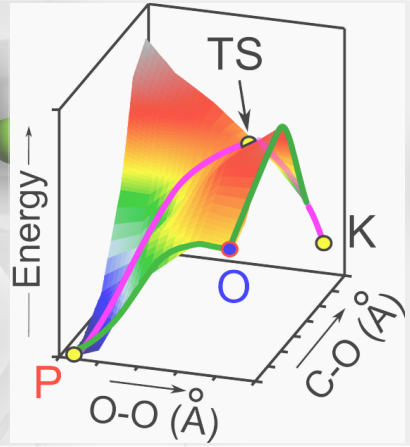




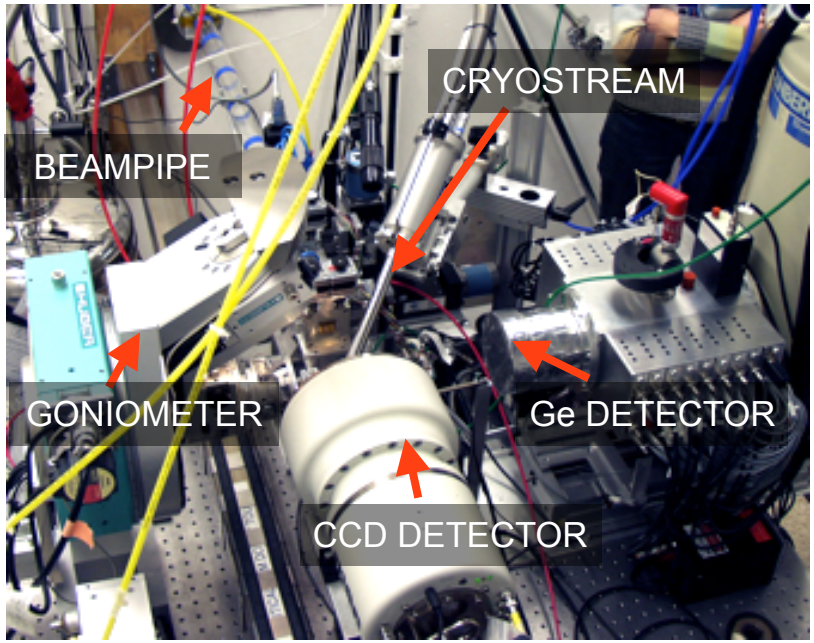


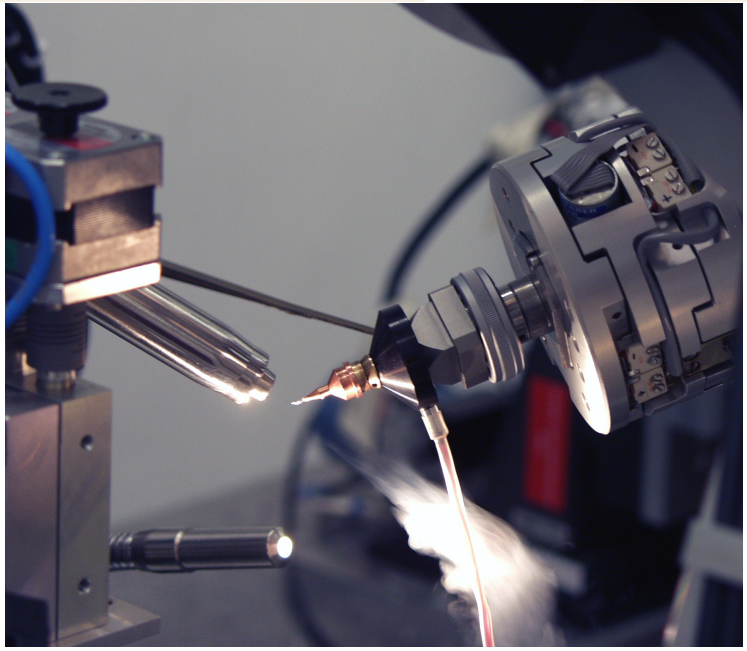
Detailed Electronic Information

Structure Function Correlation

- Application of EXAFS to Metals in Biology
- Modern Approach to Biological XAS
- Single Crystal XAS
- Oxyhemoglobin
- DypB

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

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

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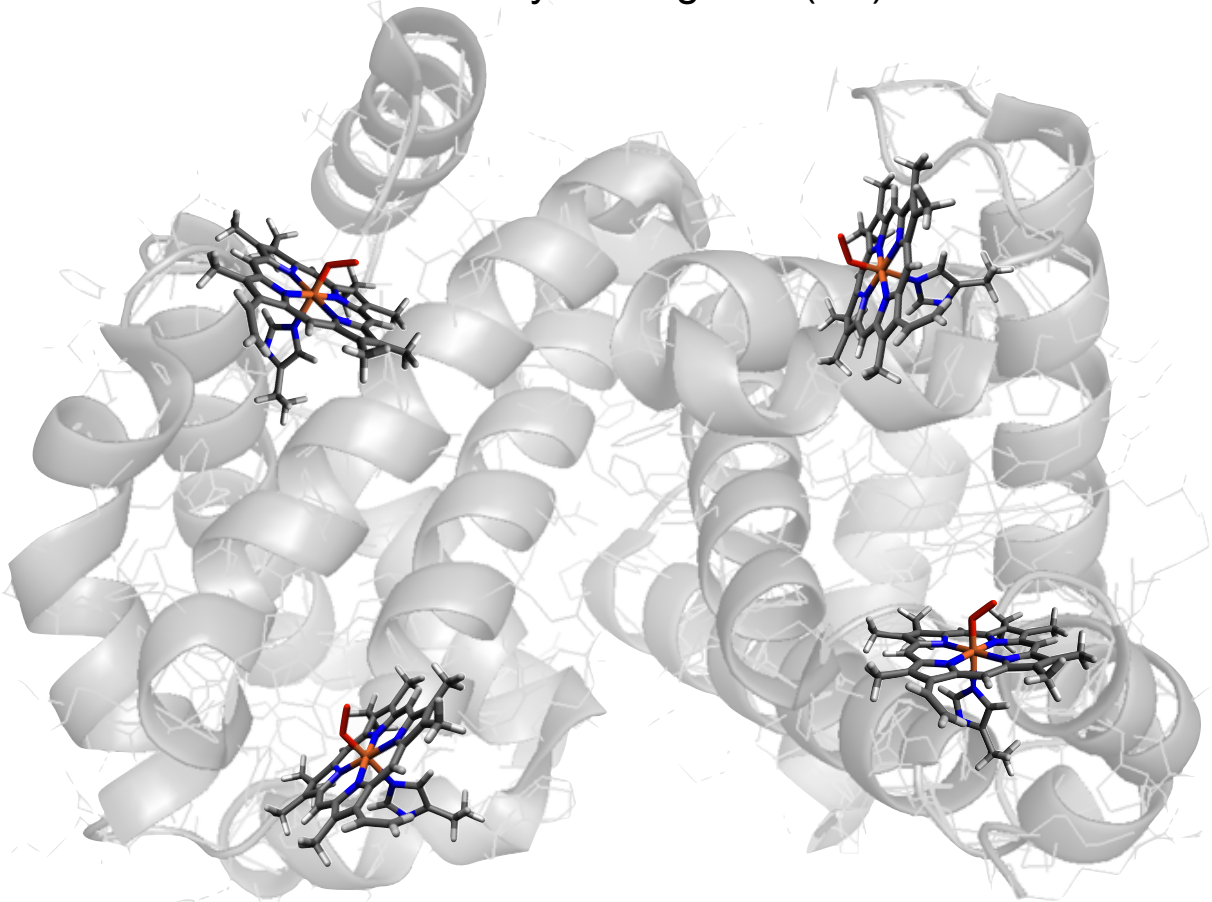
Direct Comparison to Crystallography

- 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

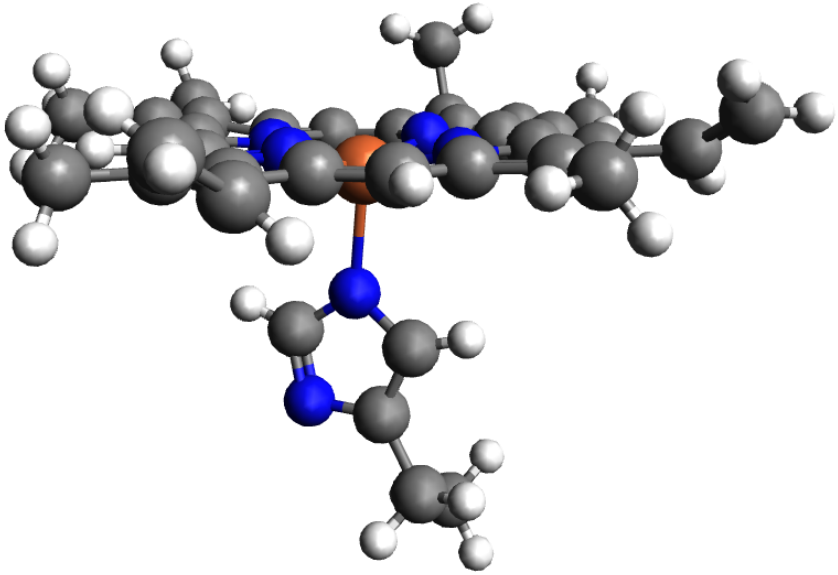
Human interaction with O_2 Mediated by Hemoglobin (Hb)



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

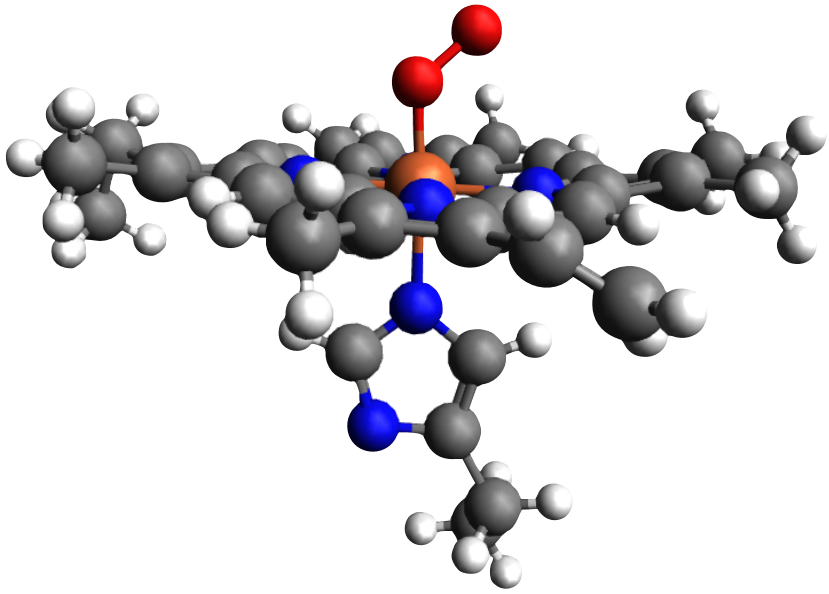
Oxyhemoglobin

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

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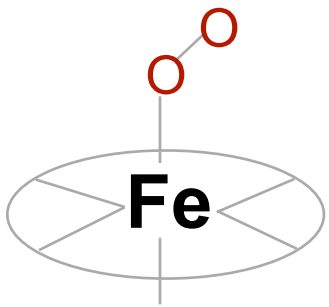
Ferrous

Fe^{2+} O_2

S=0, S=0

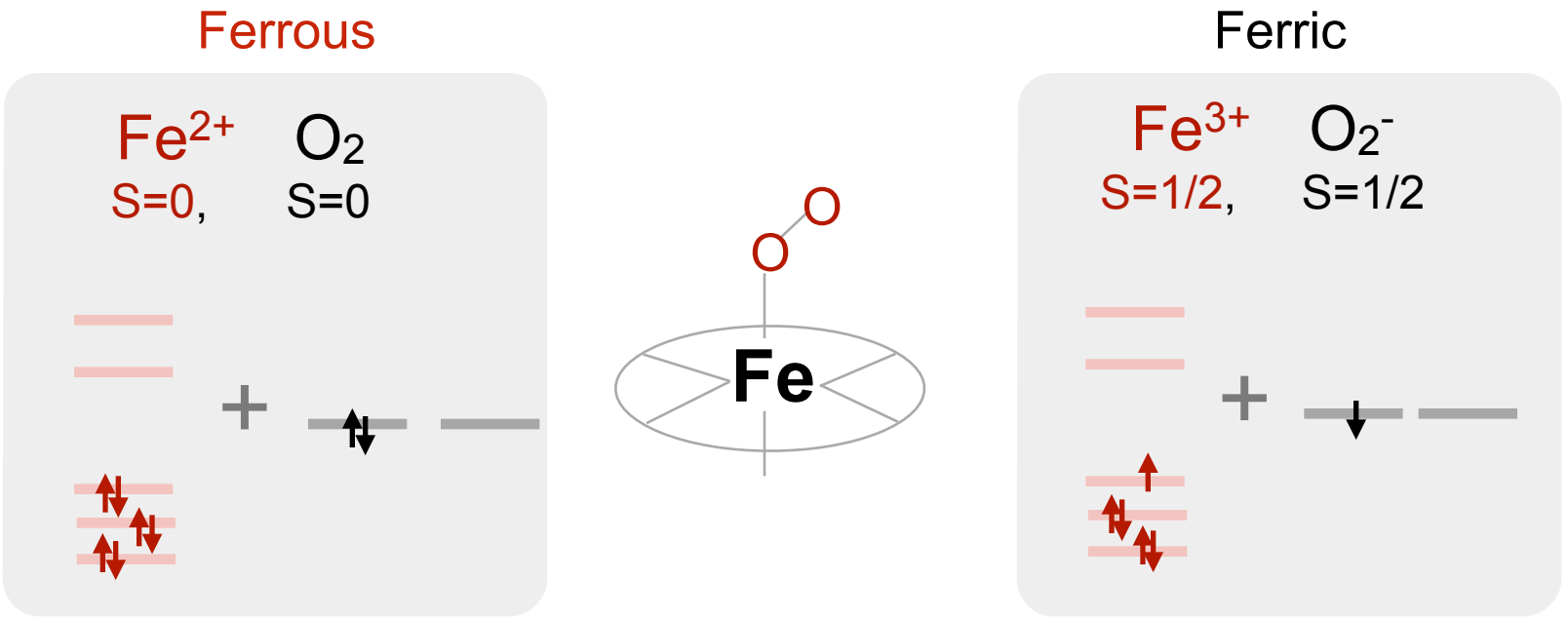


Ferric

Fe^{3+} O_2^-

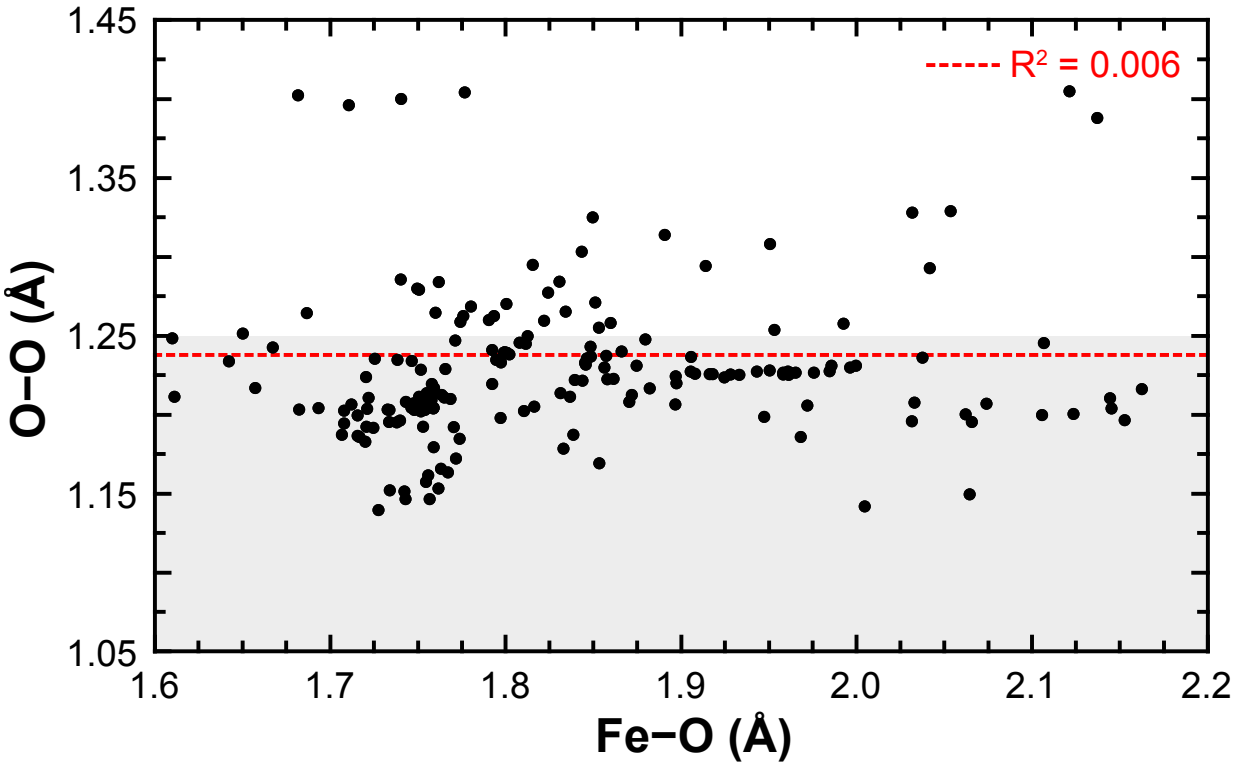
S=1/2, S=1/2

Electronic Structure



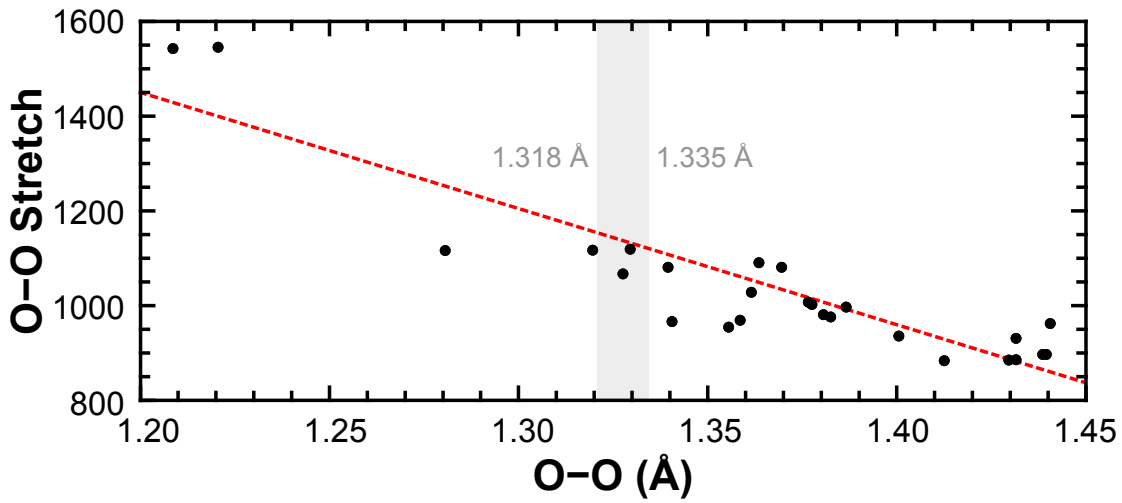
Is the metal center Reduced (Ferrous) or Oxidized (Ferric)?

Crystallography



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

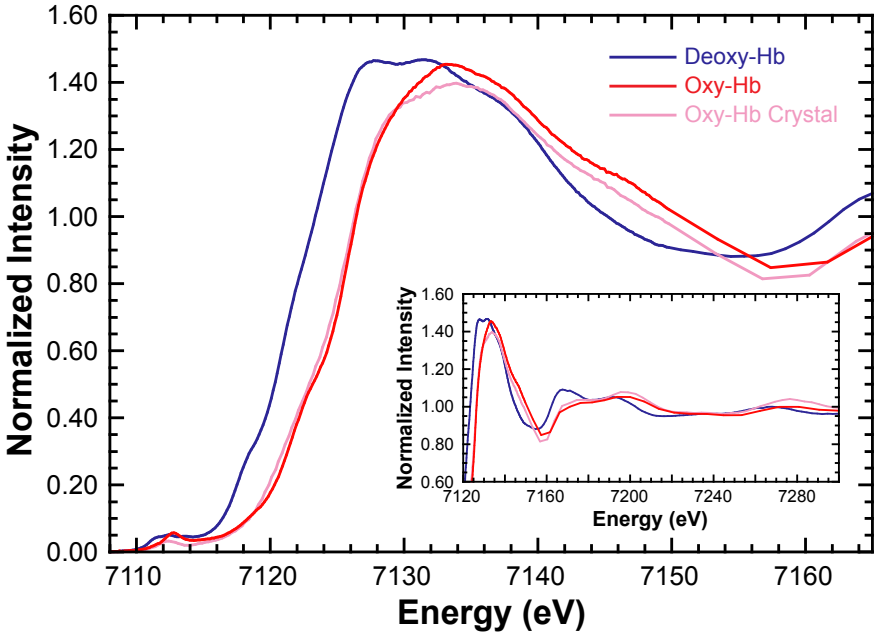
Solution Spectroscopy

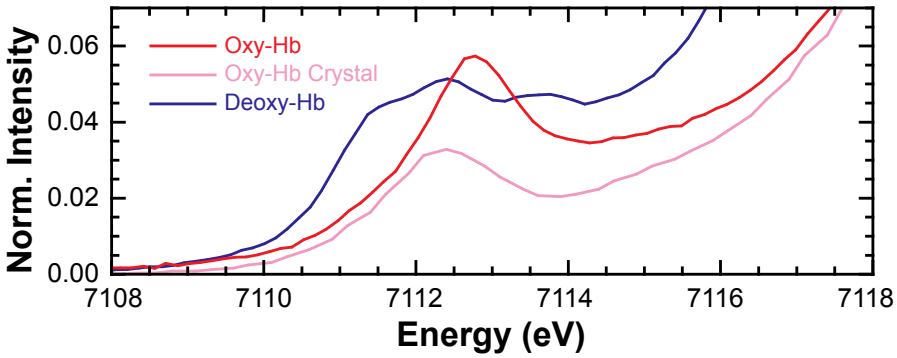


- O-O Bond Distance derived from spectroscopy (rRaman) indicates Oxidized (Ferric).

Discrepancy between solution spectroscopy and x-ray crystallography??

Solution & Crystal XAS

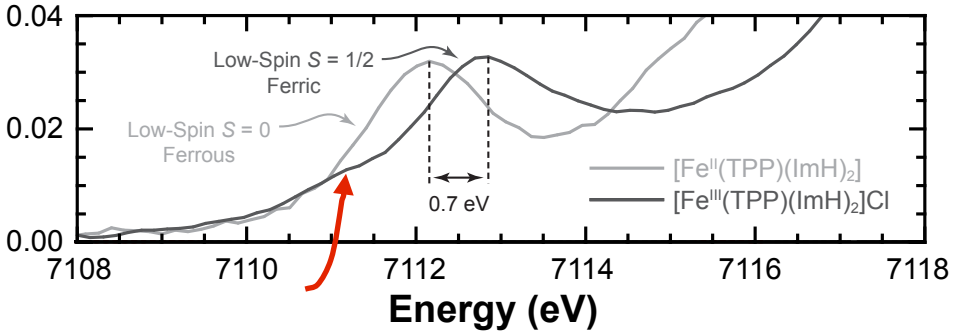




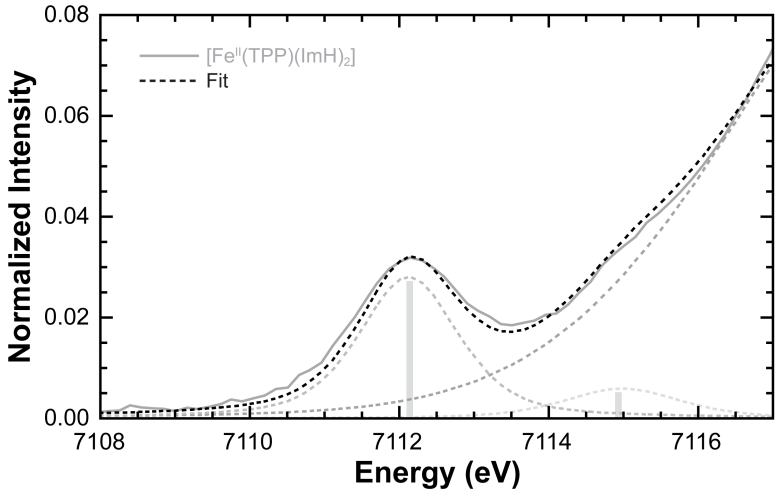
- Crystal near-edge similar to solution.
- Structure analysis shows very similar O_2 bound geometry.
- Fe K-edge and pre-edge distinctly different from starting material - deoxyHb
- *Curiously* - Fe K-pre-edge for oxyHb in solution and crystalline forms are different.
- Since geometric structure is similar,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

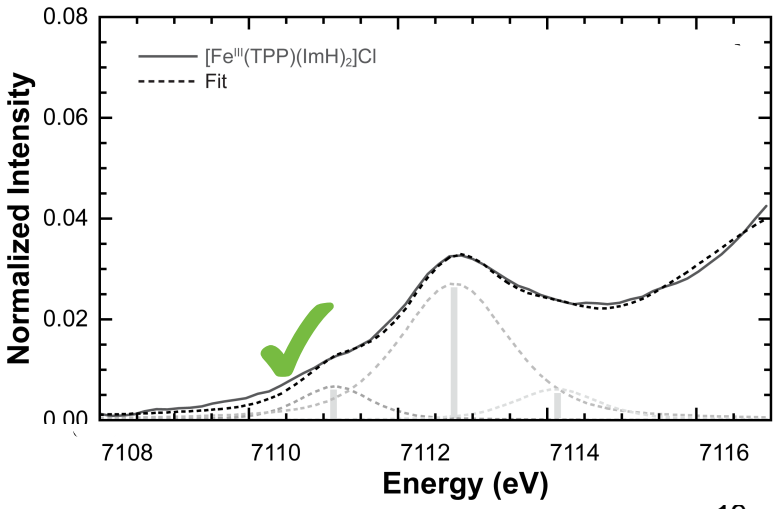
Model Comparison

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

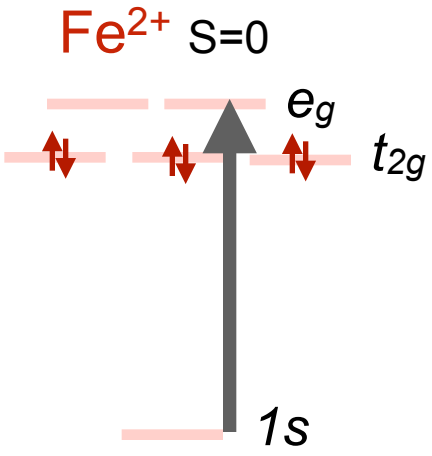


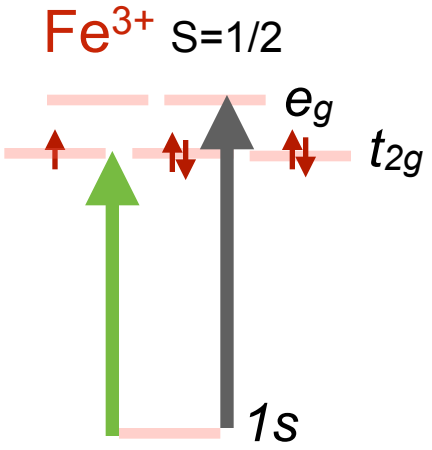
pre-pre-edge feature ??

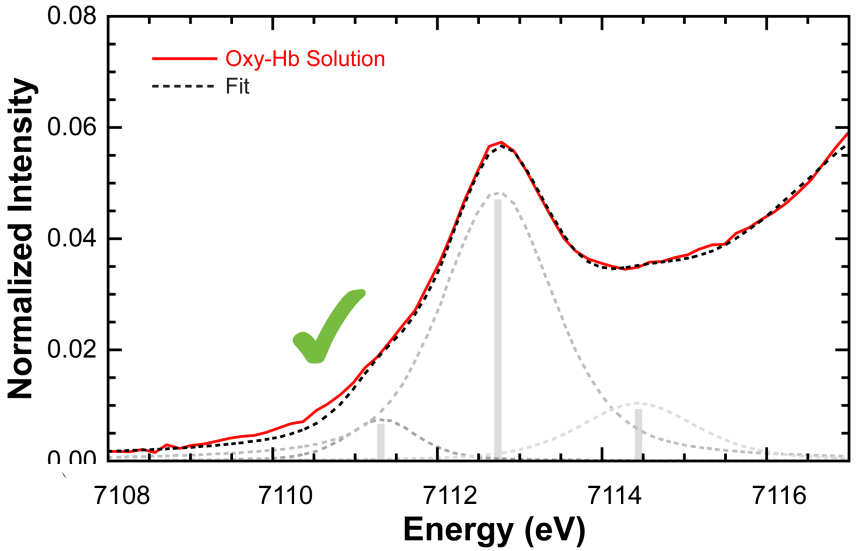


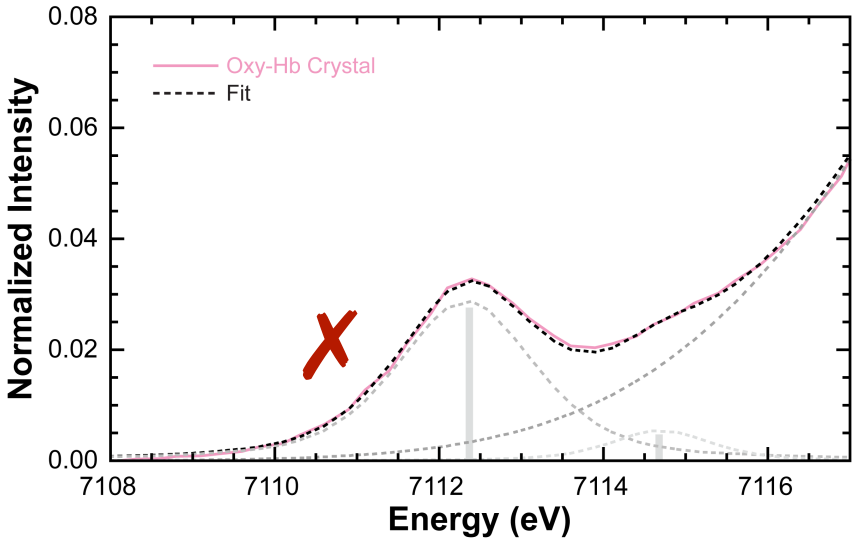


Solution & Crystal Pre-edge

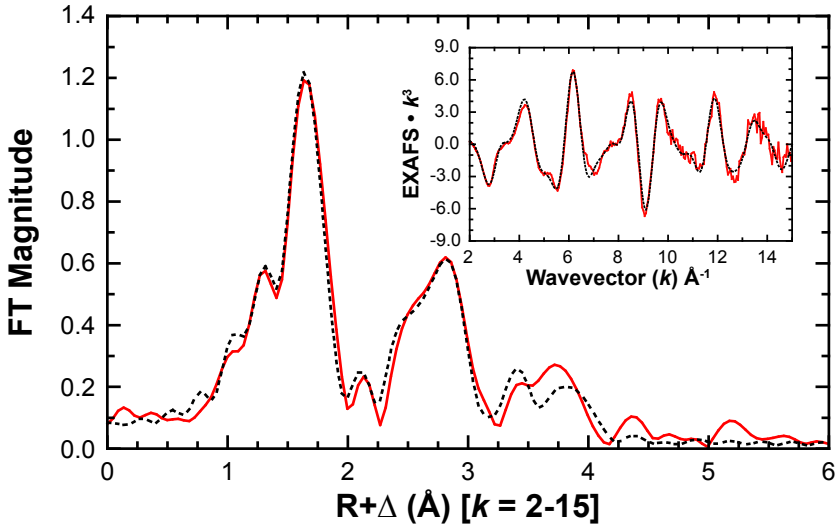


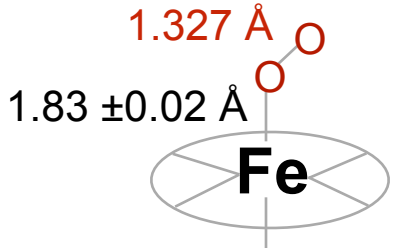


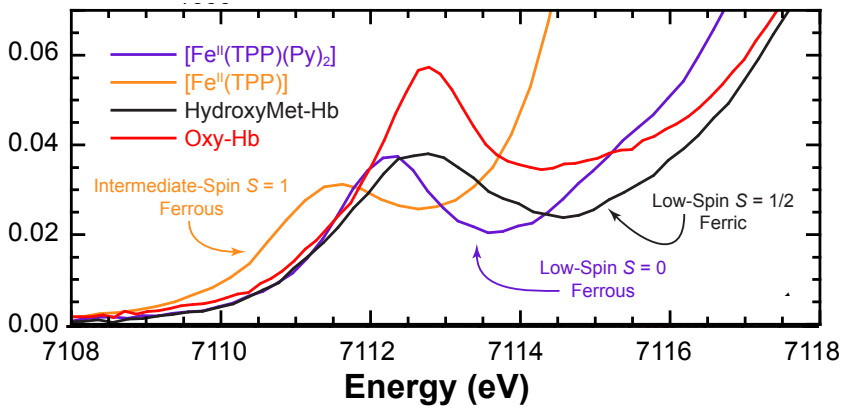


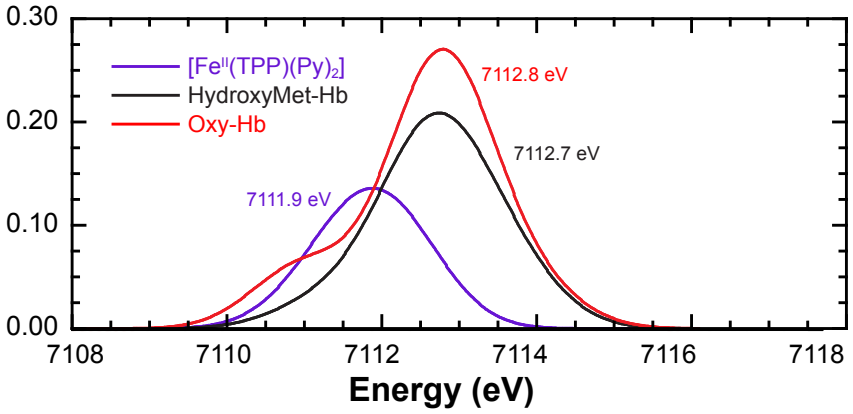


Solution EXAFS and DFT



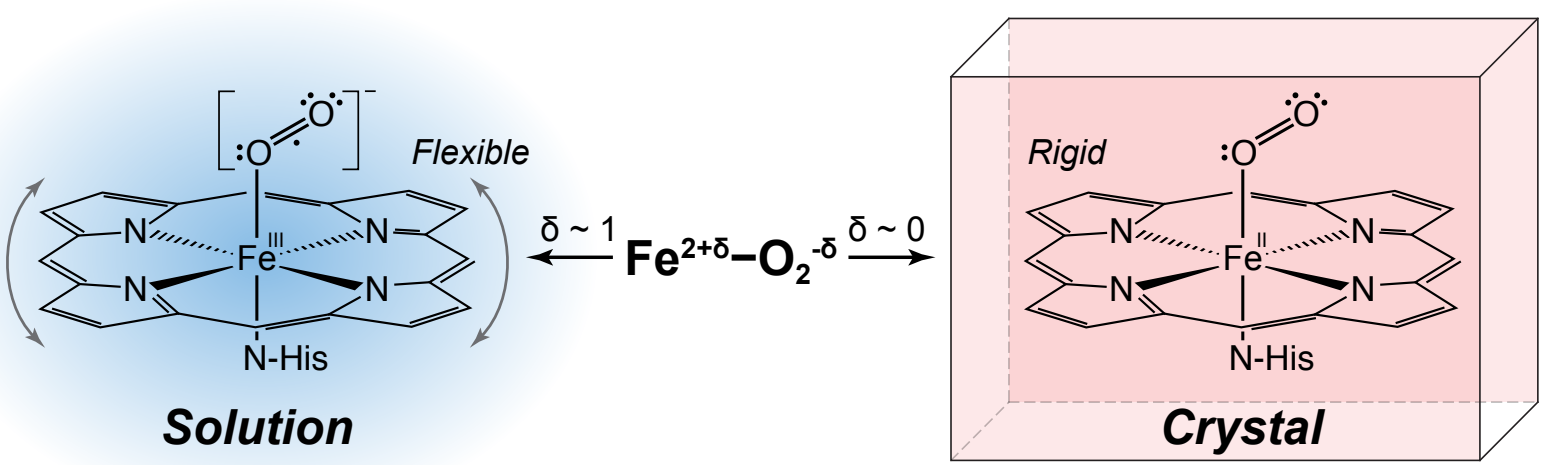






- DFT reproduces EXAFS distances and the differences in pre-edge.

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.

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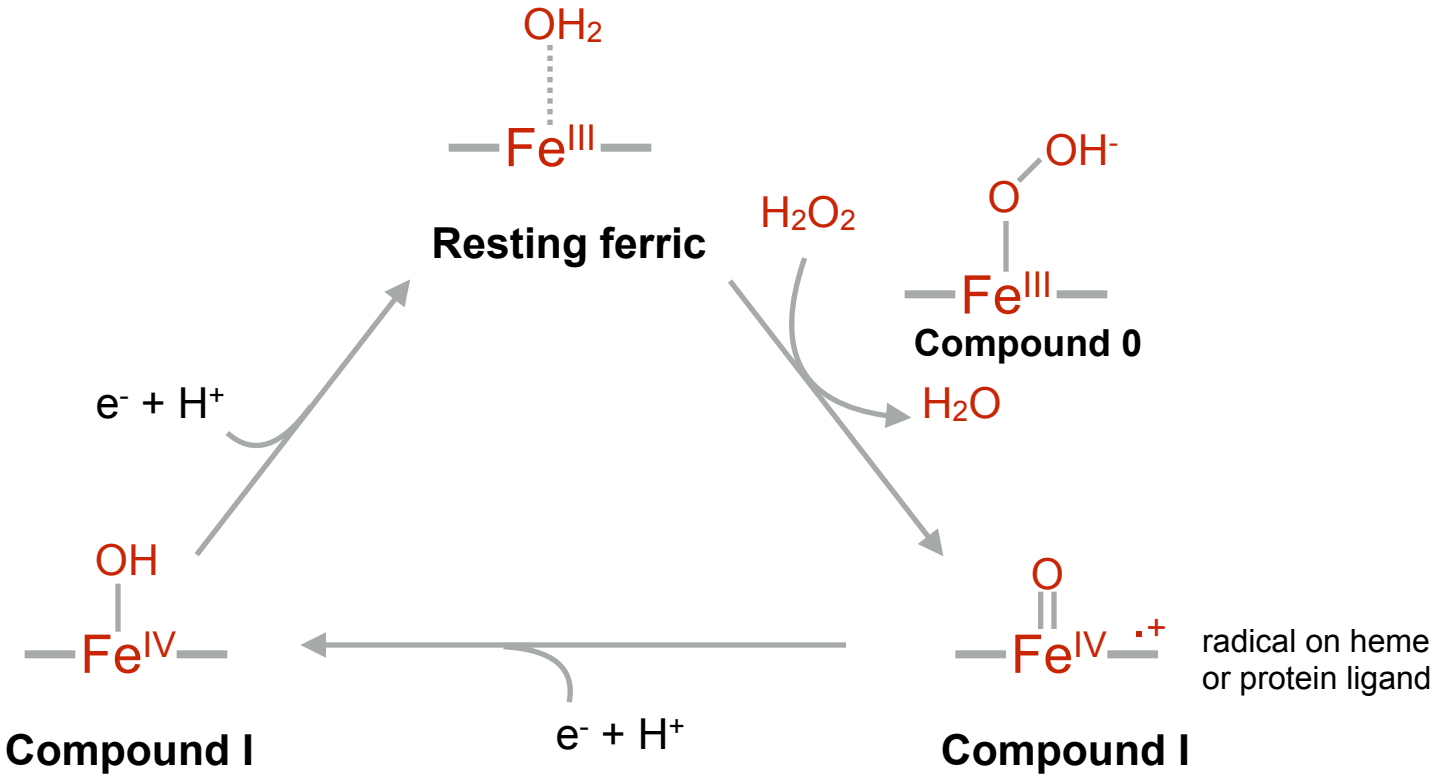
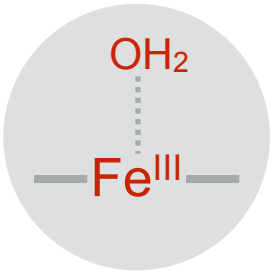
Dye-decolorizing Peroxidase B (DypB)

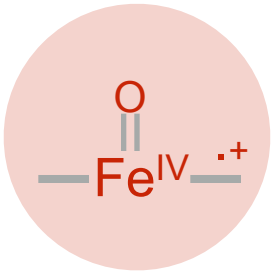
- Recently discovered heme protein with peroxidase-like activity.
- Capable of oxidizing anthraquinone dyes, lignin and even Mn^{2+}.
- Remarkable specificity for a wide range of reductive substrates.

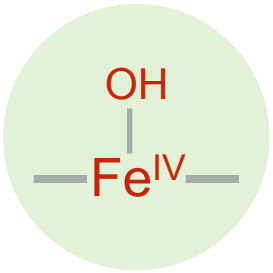
Peroxidase Catalytic Cycle



Characterizing Compound I & II







Characterizing Compound I & II

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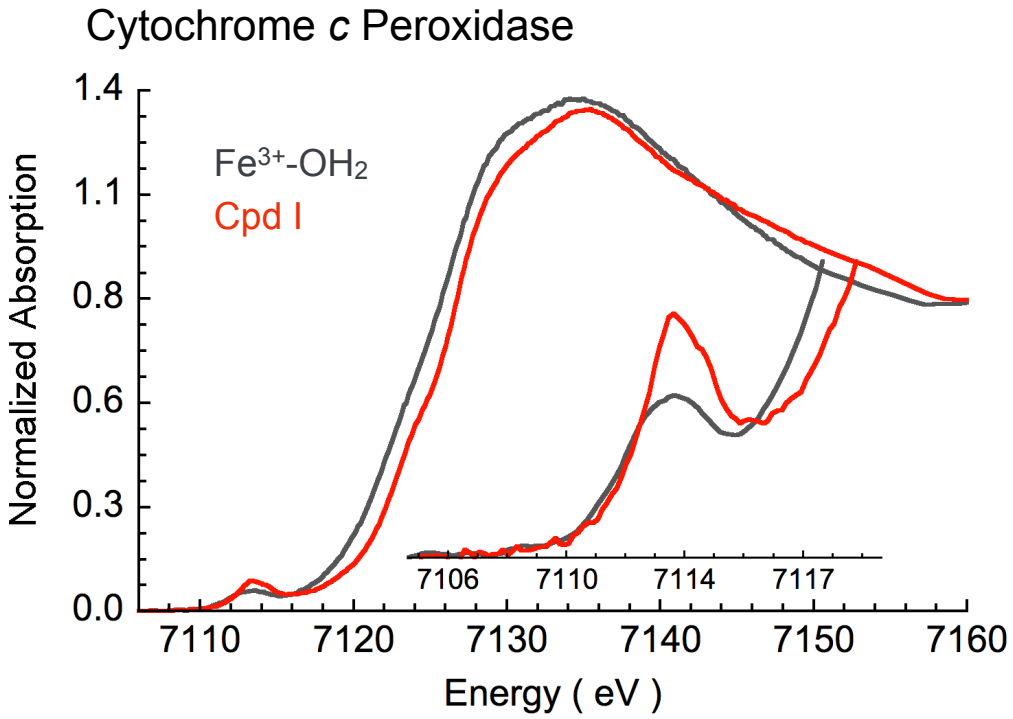
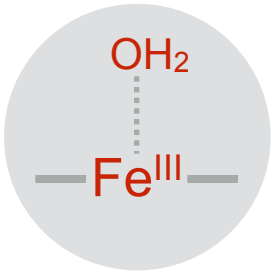
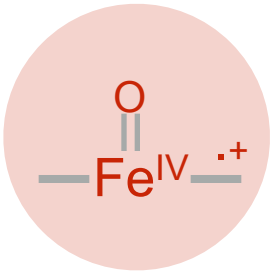
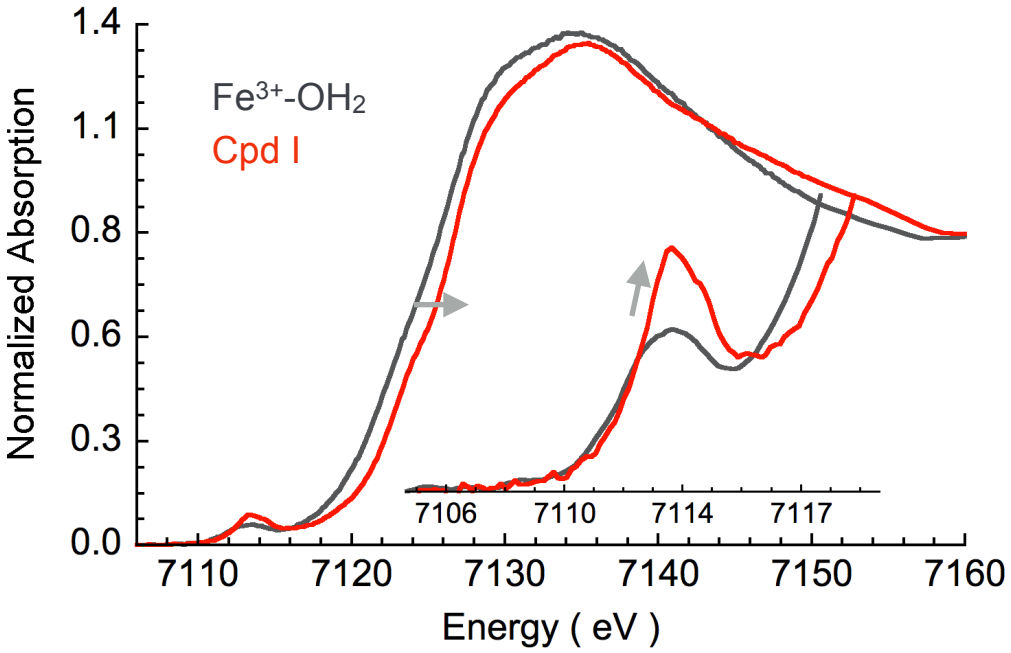


Characterizing Compound I & II





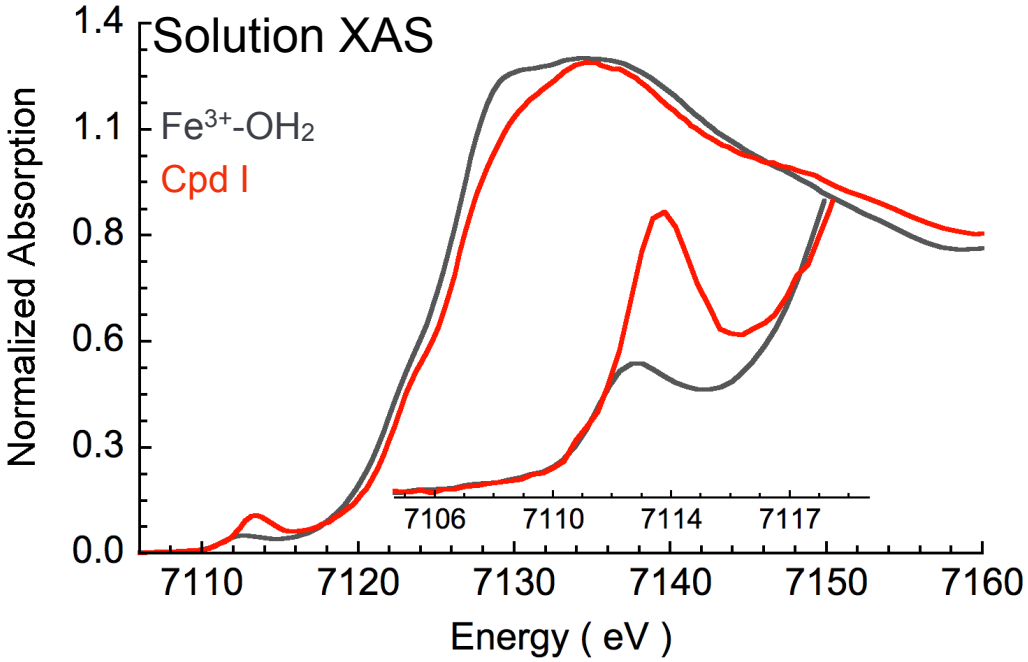
Cytochrome *c* Peroxidase



Expected Spectral Change

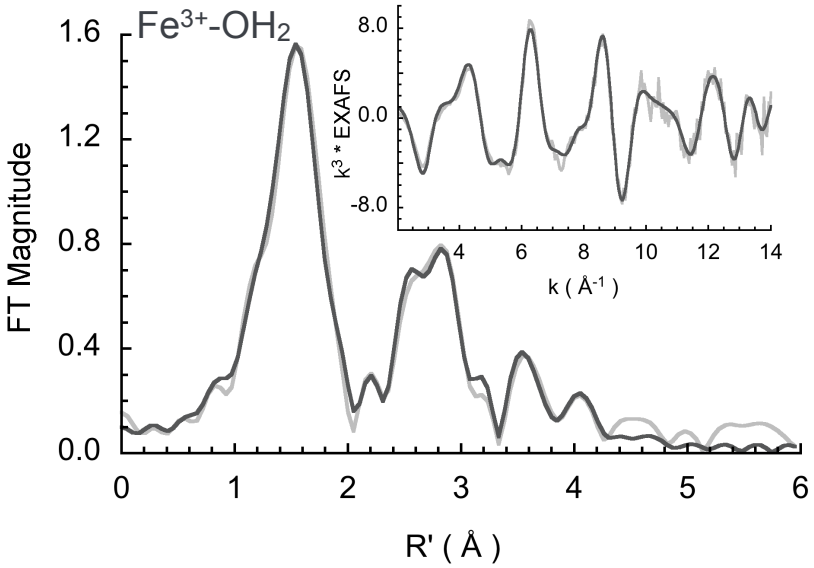
- > 1 eV shift in rising edge
- > intensity & energy of pre-edge

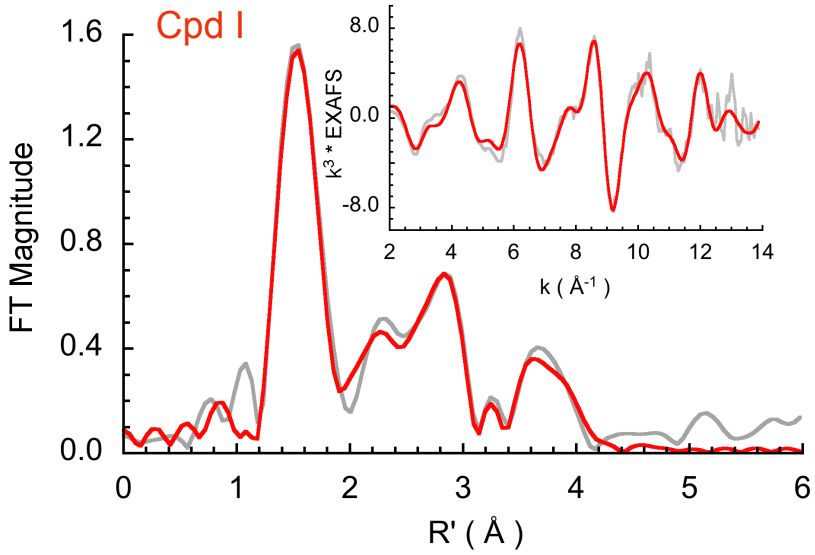
Solution XAS & EXAFS on DypB



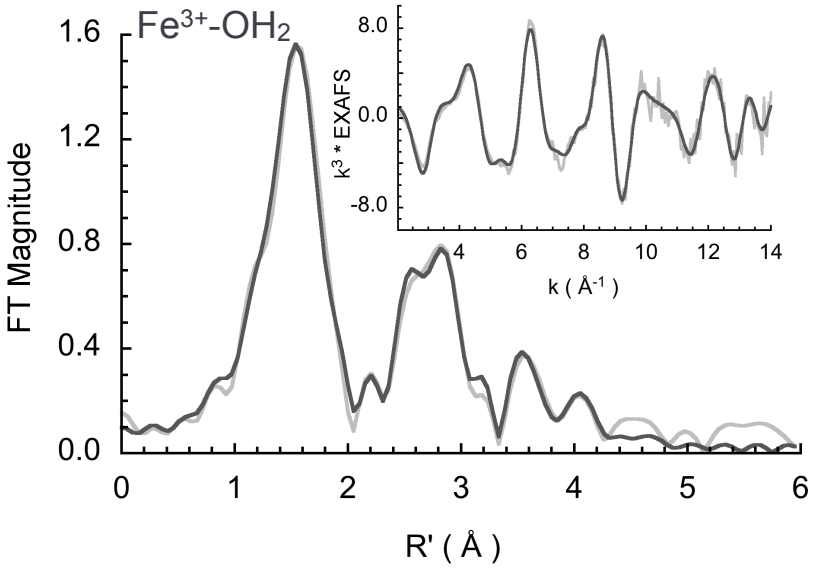
- ✓ > 1 eV shift in rising edge
- ✓ > intensity & energy of pre-edge

Solution XAS & EXAFS on DypB

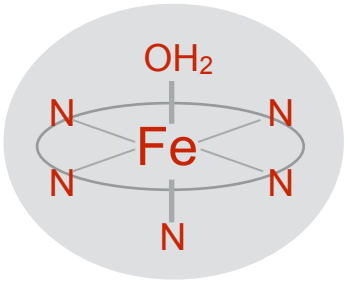


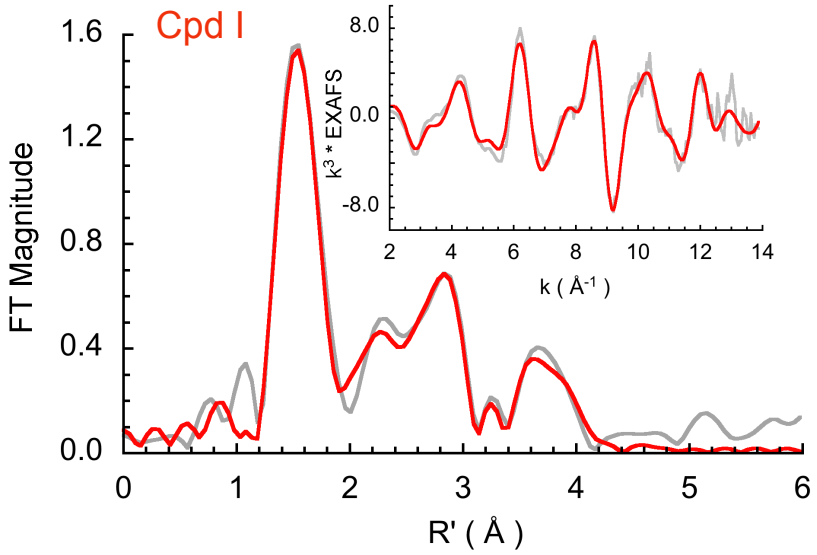


Solution XAS & EXAFS on DypB

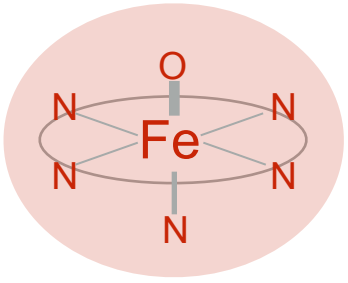


2 Fe-N 1.92 Å, 4 Fe-N 2.02 Å

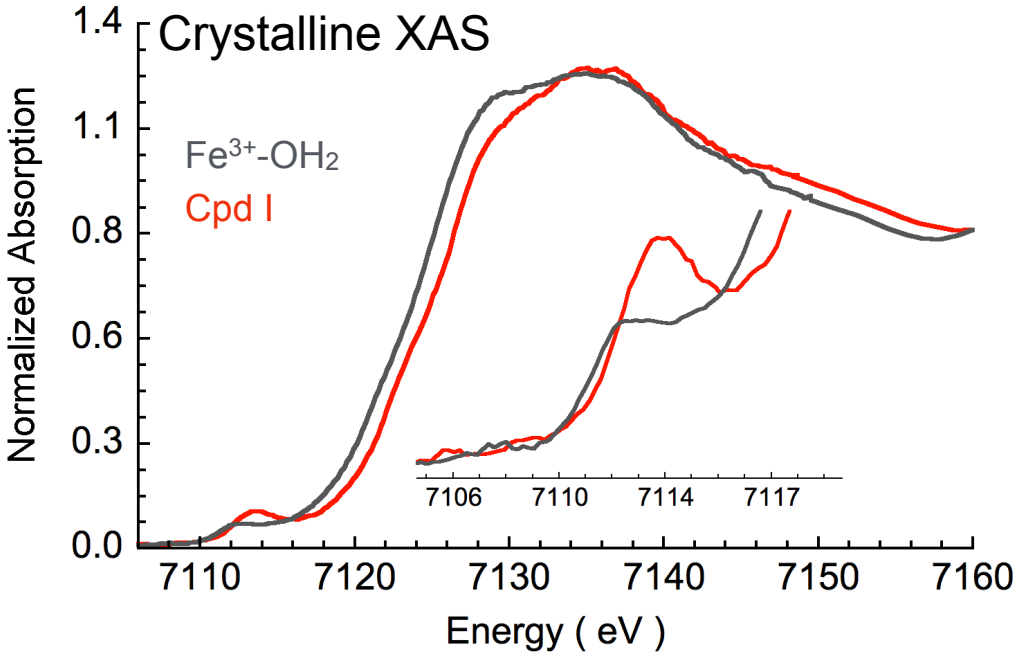




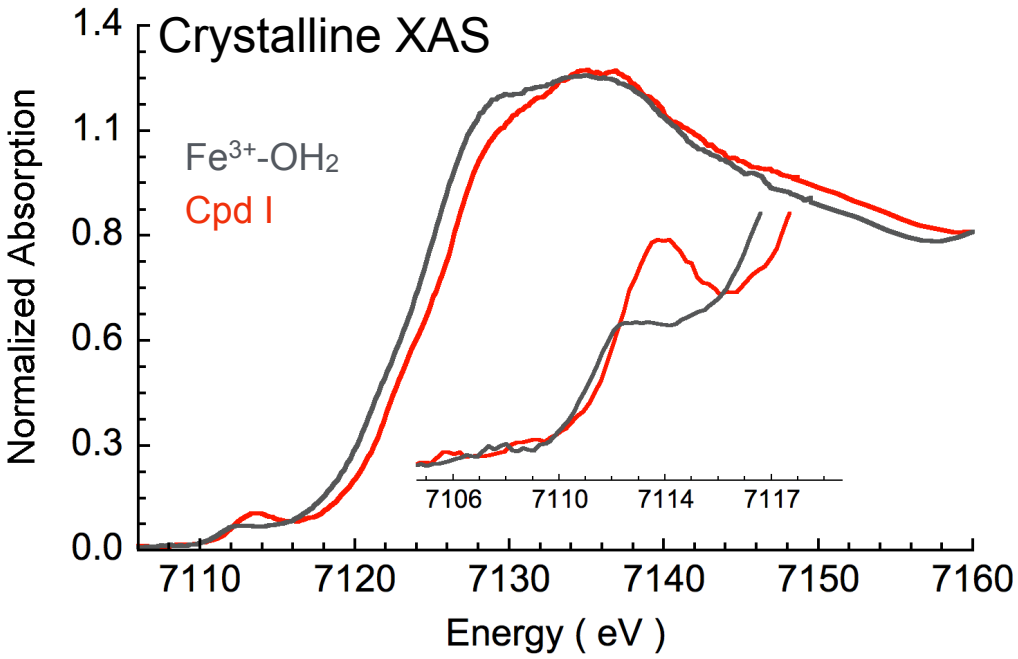
1 Fe-O 1.67 Å, 5 Fe-N 2.00 Å



Optimizing Crystallization Conditions



Optimizing Crystallization Conditions

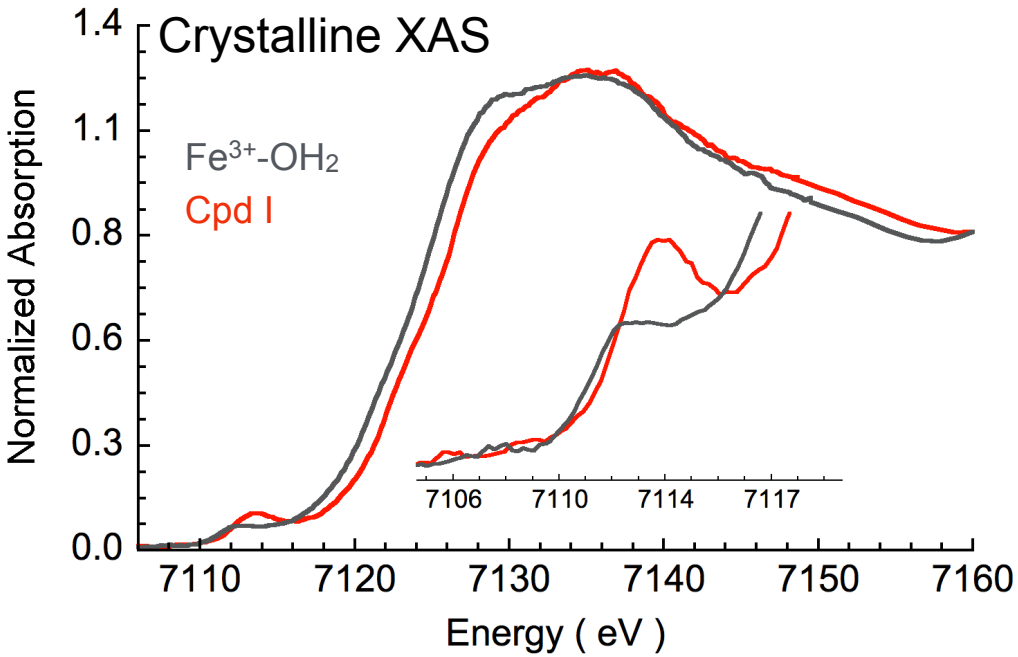


✓ > 1 eV shift in rising edge

✓ > intensity & energy of pre-edge

? *Some differences in solution & xtal*

Optimizing Crystallization Conditions



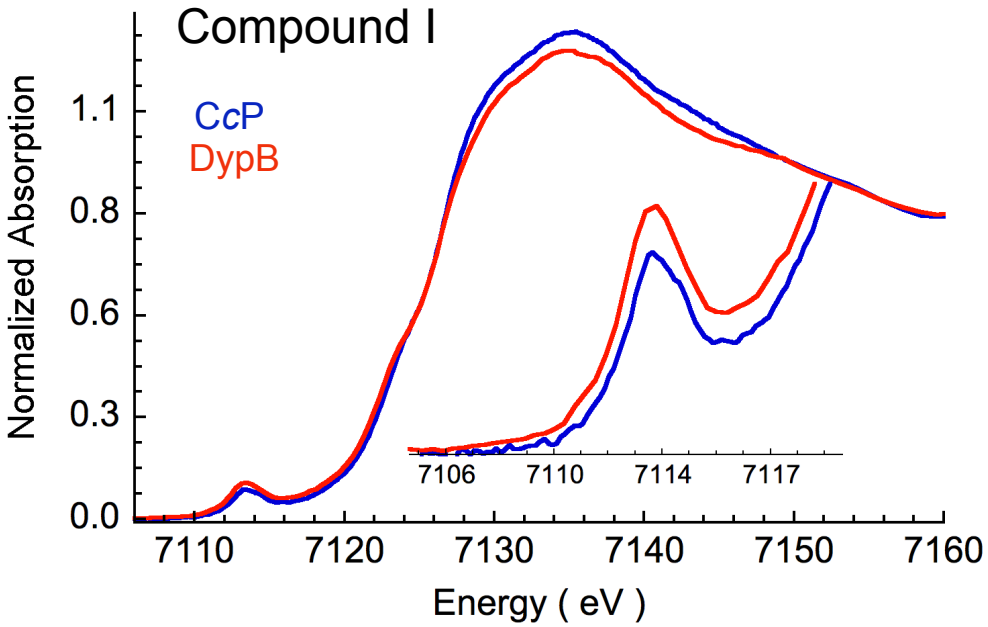
✓ > 1 eV shift in rising edge

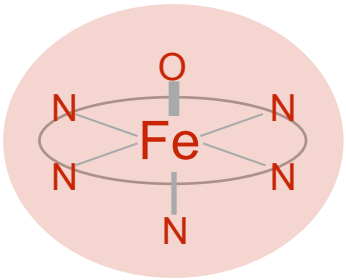
✓ > intensity & energy of pre-edge

? *Some differences in solution & xtal*

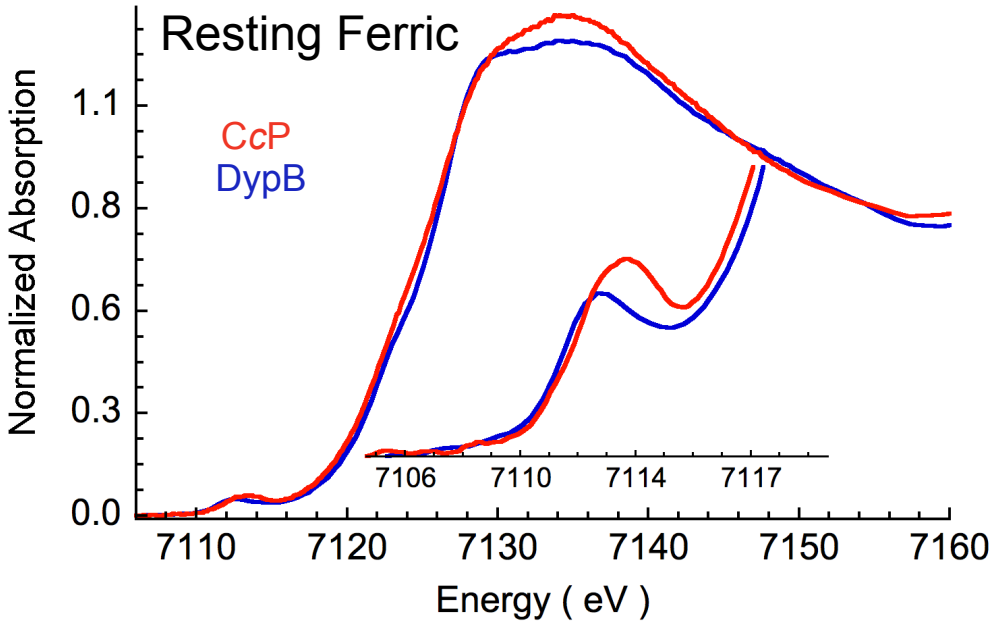
Electronic structure validation for crystallography.

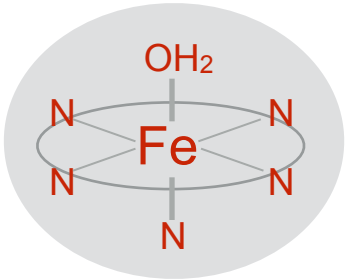
Ongoing Work: Electronic Structure of DypB



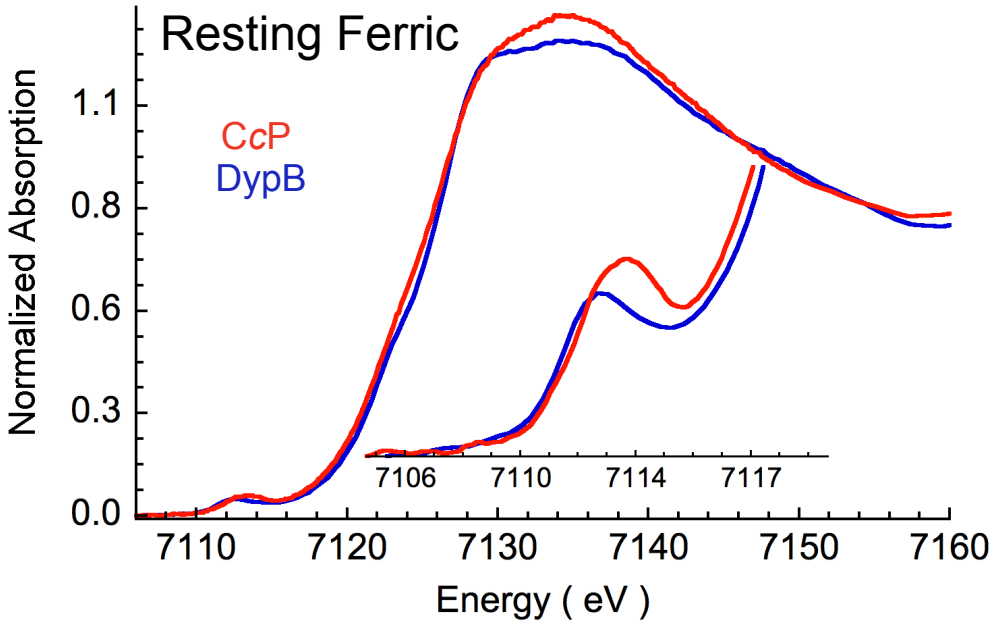


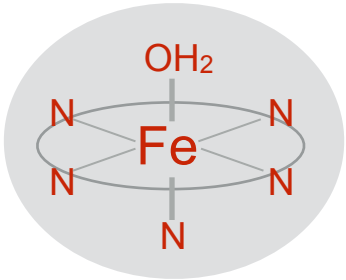
Ongoing Work: Electronic Structure of DypB





Ongoing Work: Electronic Structure of DypB





- Why are the ferric forms of CcP and DypB different?
- What are the differences in ligand field that lead to differences in the pre-edges?
- Is it a first sphere or second sphere effect?

Take Home Messages

- 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*.
- Researchers should feel encouraged to combine XAS and Crystallography, *routinely.*

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Department of Energy.
National Institutes of Health.

Thank You For Your Attention

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