# Applications of XAS in the Biological Sciences

**or**

# Some Interesting Examples & Pitfalls of Biological XAFS

**Erik Farquhar**

**Center for Synchrotron Biosciences**

**Case Western Reserve University**

**NSLS Beamline X3B (until 9/30/2014)**

**NSLS-II ISS & SM3**

# Biology was an Early Adopter of Synchrotron XAS

**X-Ray Absorption Spectroscopy Using Synchrotron Radiation for Structural Investigation of Organometallic Molecules of Biological Interest**

**(metalloporphyrins/methemoglobin)**

BRIAN M. KINCAID‡, P. EISENBERGER†, KEITH O. HODGSON*, AND S. DONIACH§

Stanford Synchrotron Radiation Project, Stanford University, Stanford, California 94305

*Proc. Nat. Acad. Sci. USA*
Vol. 72, No. 6, pp. 2340–2342, June 1975

**ABSTRACT** **The technique of x-ray absorption spectroscopy using tuneable, very intense x-rays from a high energy electron storage ring has been applied to study of the extended x-ray absorption fine structure for Cu and Ni tetraphenylporphyrin and methemoglobin. Preliminary**

structure of the same material. Such a comparison would be extremely useful in studying solution compared with solid state conformation of metalloproteins where crystal packing forces and other unknowns often lend uncertainty to conclusions drawn from x-ray diffraction data. Furthermore, the technique is element selective (as determined by the energy used) and is insensitive to nonmetallic impurities that do not affect the metal coordination site. We believe that this technique will provide a powerful method for studying a number of metalloenzyme and protein systems where subtle structural changes may be involved in the mechanism of activity.

**Protein crystallography was much harder in those days . . .**

**XAS was often the only way to get structural data on metalloprotein active site structure. . .**

# Biologically/Environmentally Relevant Metals

**By no means a complete assignment of elements!**

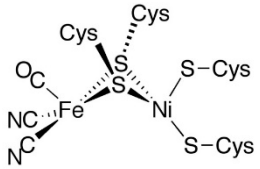
| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 H 1.00794 | | | | | | | | | | | | | | | | 1 H 1.00794 | 2 He 4.002602 |
| 3 Li 6.941 | 4 Be 9.012182 | | | | | | | | | | | 5 B 10.811 | 6 C 12.0107 | 7 N 14.00674 | 8 O 15.9994 | 9 F 18.9984032 | 10 Ne 20.1797 |
| 11 Na 22.989770 | 12 Mg 24.3050 | | | | | | | | | | | 13 Al 26.981538 | 14 Si 28.0855 | 15 P 30.973761 | 16 S 32.066 | 17 Cl 35.4527 | 18 Ar 39.948 |
| 19 K 39.0983 | 20 Ca 40.078 | 21 Sc 44.955910 | 22 Ti 47.867 | 23 V 50.9415 | 24 Cr 51.9961 | 25 Mn 54.938049 | 26 Fe 55.845 | 27 Co 58.933200 | 28 Ni 58.6934 | 29 Cu 63.546 | 30 Zn 65.39 | 31 Ga 69.723 | 32 Ge 72.61 | 33 As 74.92160 | 34 Se 78.96 | 35 Br 79.904 | 36 Kr 83.80 |
| 37 Rb 85.4678 | 38 Sr 87.62 | 39 Y 88.90585 | 40 Zr 91.224 | 41 Nb 92.90638 | 42 Mo 95.94 | 43 Tc (98) | 44 Ru 101.07 | 45 Rh 102.90550 | 46 Pd 106.42 | 47 Ag 107.8682 | 48 Cd 112.411 | 49 In 114.818 | 50 Sn 118.710 | 51 Sb 121.760 | 52 Te 127.60 | 53 I 126.90447 | 54 Xe 131.29 |
| 55 Cs 132.90545 | 56 Ba 137.327 | 57 La 138.9055 | 72 Hf 178.49 | 73 Ta 180.9479 | 74 W 183.84 | 75 Re 186.207 | 76 Os 190.23 | 77 Ir 192.217 | 78 Pt 195.078 | 79 Au 196.96655 | 80 Hg 200.59 | 81 Tl 204.3833 | 82 Pb 207.2 | 83 Bi 208.98038 | 84 Po (209) | 85 At (210) | 86 Rn (222) |
| 87 Fr (223) | 88 Ra (226) | 89 Ac (227) | 104 Rf (261) | 105 Db (262) | 106 Sg (263) | 107 Bh (262) | 108 Hs (265) | 109 Mt (266) | 110 (269) | 111 (272) | 112 (277) | | 114 (289) (287) | | 116 (289) | | 118 (293) |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 58 Ce 140.116 | 59 Pr 140.90765 | 60 Nd 144.24 | 61 Pm (145) | 62 Sm 150.36 | 63 Eu 151.964 | 64 Gd 157.25 | 65 Tb 158.92534 | 66 Dy 162.50 | 67 Ho 164.93032 | 68 Er 167.26 | 69 Tm 168.93421 | 70 Yb 173.04 | 71 Lu 174.967 |
| 90 Th 232.0381 | 91 Pa 231.03588 | 92 U 238.0289 | 93 Np (237) | 94 Pu (244) | 95 Am (243) | 96 Cm (247) | 97 Bk (247) | 98 Cf (251) | 99 Es (252) | 100 Fm (257) | 101 Md (258) | 102 No (259) | 103 Lr (262) |

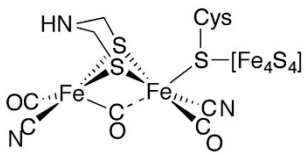
**~ 30-50% of all proteins are metalloproteins**

**Many elements are bio-relevant … even more in environmental contexts**

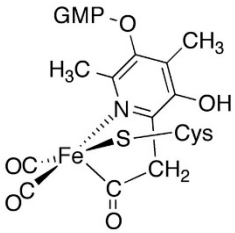
# Metalloproteins carry out many FUNDAMENTAL reactions




[NiFe]$H_2$ase

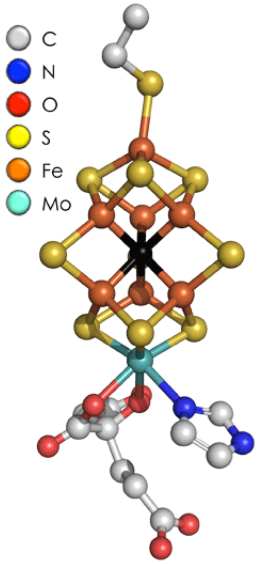



[FeFe]$H_2$ase




[Fe]$H_2$ase

**Hydrogenases:** $2H^+ + 2e^- \leftrightarrow H_2$



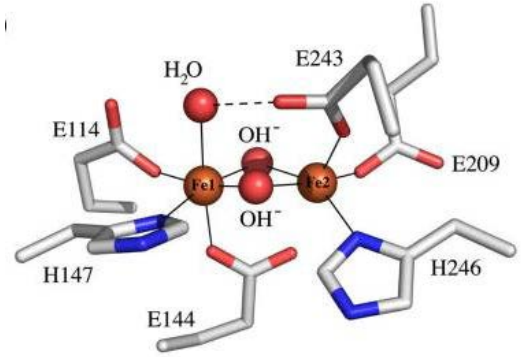

FeMoCo

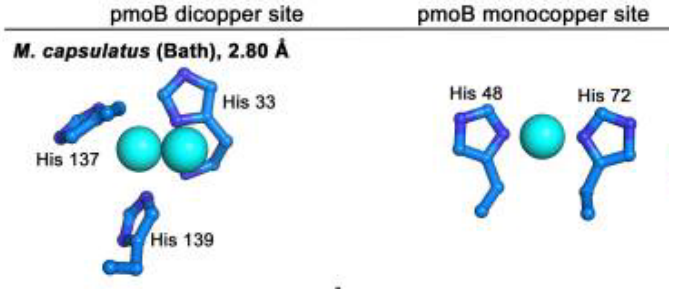
## Nitrogenases

Nitrogen fixation

$N_2 + 8e^- + 8H^+ \rightarrow$

$2NH_3 + H_2$



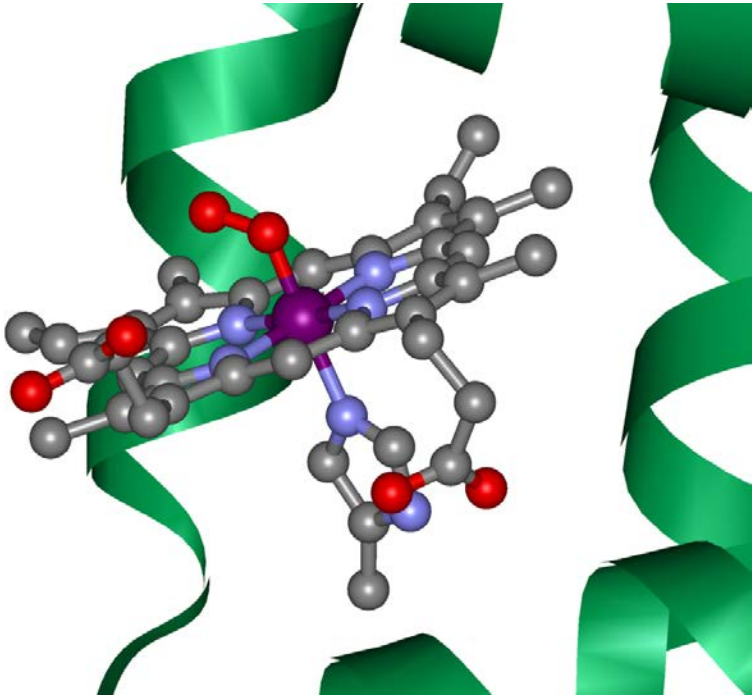

## Methane monooxygenases

$CH_4 + O_2 \rightarrow CH_3OH$

Both Fe and Cu forms are found in Nature

- **What is the local electronic/geometric structure around the metal?**
- **Does that structure change with conditions or during a reaction?**

# What Shall We Talk about Today . . .



**XAS in biology is an enormous field with 40 years of history . . .**

**. . . not so different from other subject areas.**

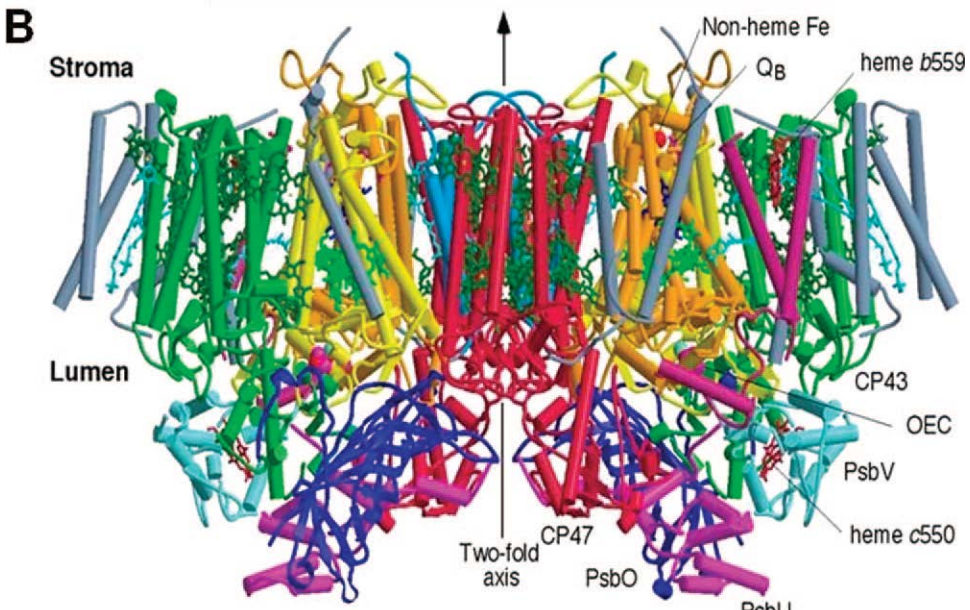
**Two themes:**

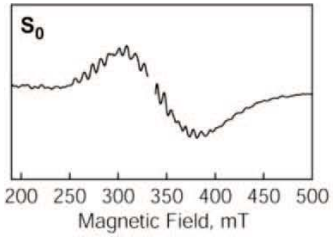
1. **Some examples of applying XAS to an biological problem.**
2. **Some examples of pitfalls in bioXAS**

**Examples of the application of XAS to biological problems:**

a. **Metalloprotein radiation damage – Photosystem II OEC**
b. **A typical recent bioXAS problem – RPE65**
c. **Probing intermediates by RFQ XAS – Methane Monooxygenase**

# Photosystem II and the OEC



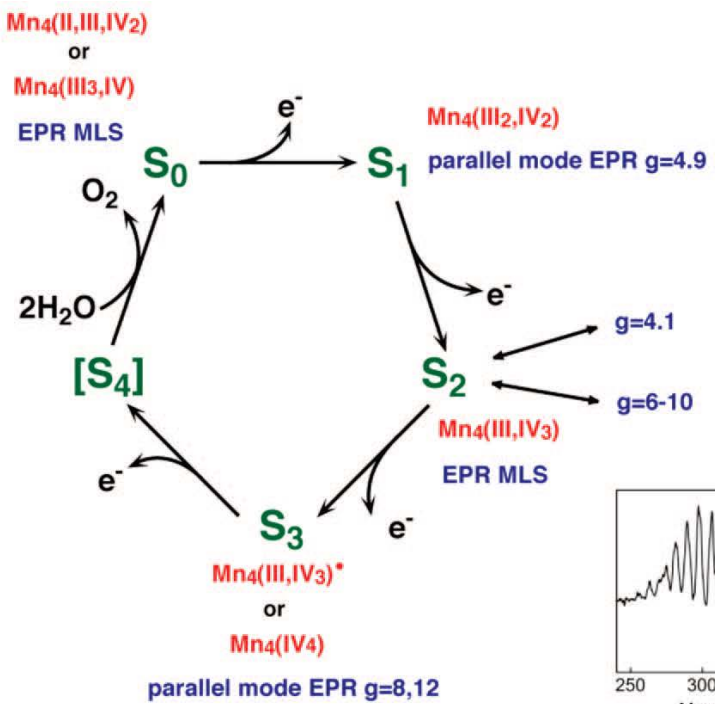



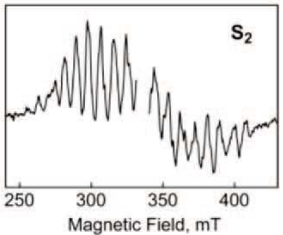

**Protein crystallography of Photosystem II: what you see is what it is?**

**Oxygen Evolving Complex**

**$2H_2O \rightarrow O_2$**

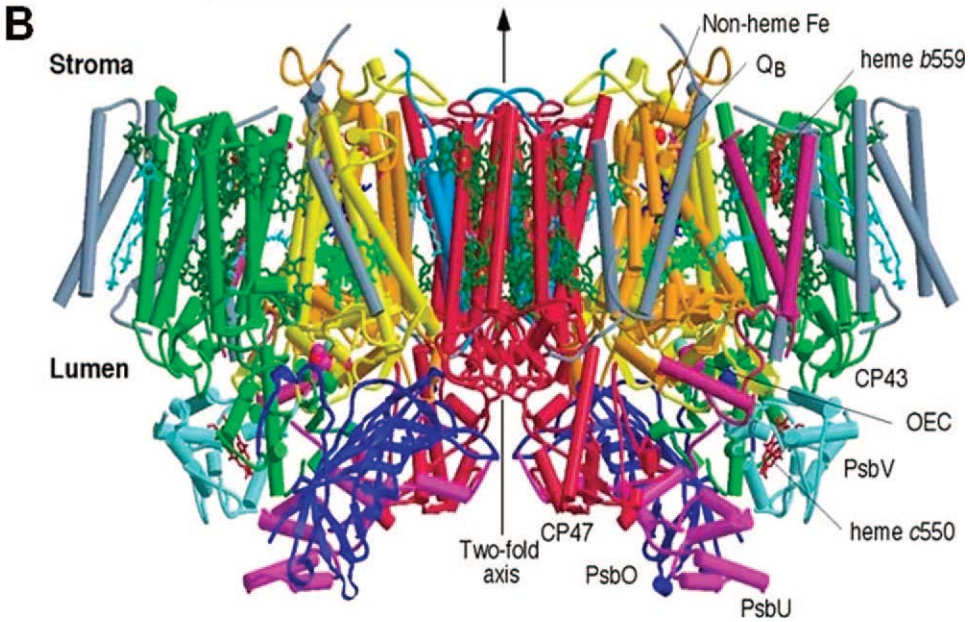
**Via a $Mn_4Ca$ cluster**




*Inorg. Chem.* **2008,** *47*, 1711-1726

# Photosystem II and the OEC: MX HELPFUL or NO?

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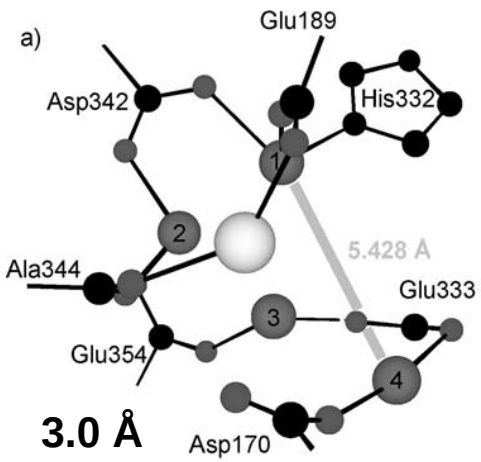
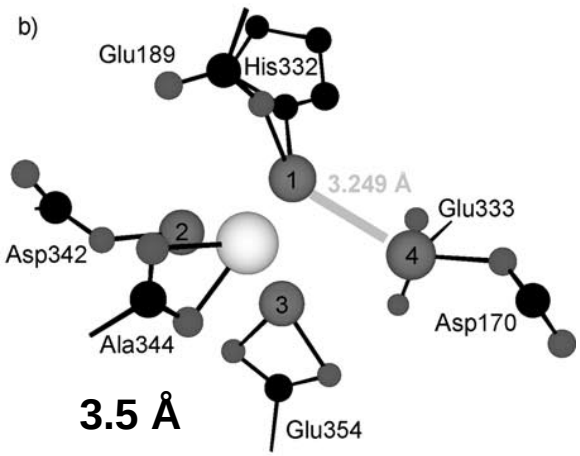
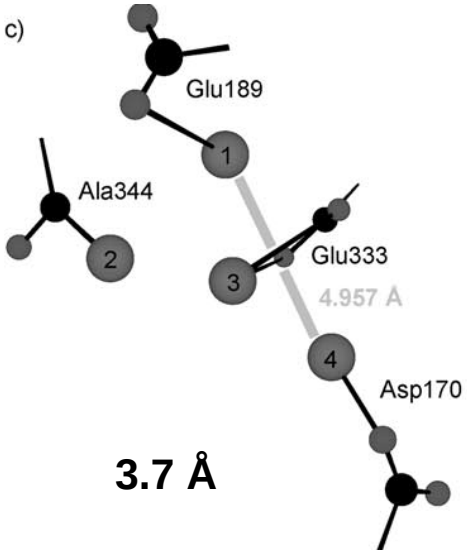



**3.0 Å**




**3.5 Å**



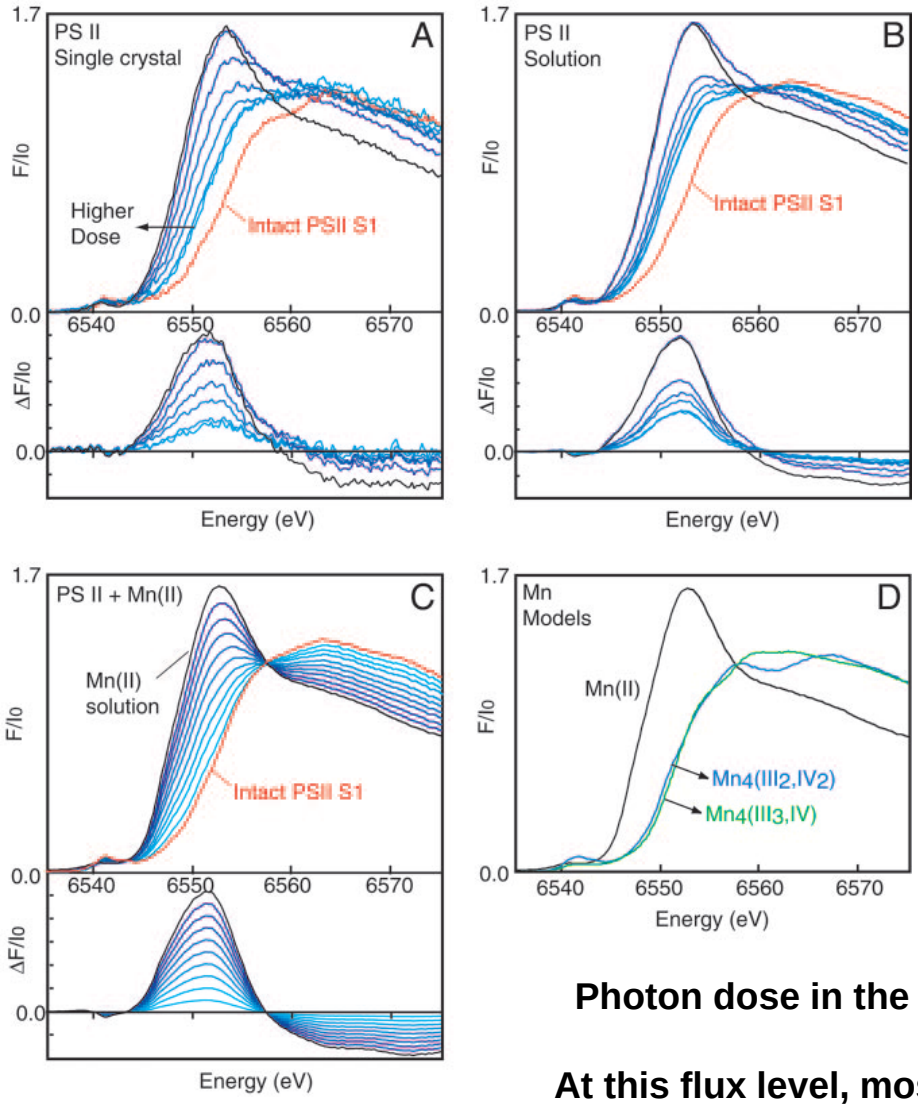

**3.7 Å**

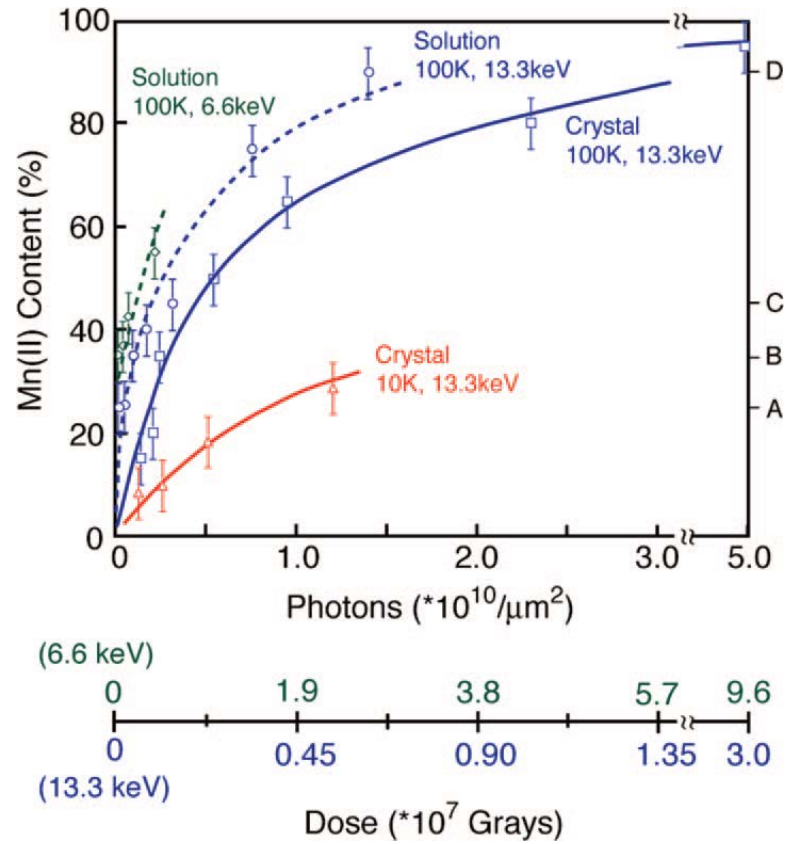
**Note the dramatically different positions of $Mn_4$, metal ligands from structure to structure**

*Chem. Eur. J.* **2007**, *13*, 5082 – 5089

**"X-ray damage to the $Mn_4Ca$ complex in single crystals of photosystem II: A case study for metalloprotein crystallography"**

Junko Yano, Jan Kern, Klaus-Dieter Irrgang, Matthew J. Latimer, Uwe Bergmann, Pieter Glatzel, Yulia Pushkar, Jacek Biesiadka, Bernhard Loll, Kenneth Sauer, Johannes Messinger, Athina Zouni, and Vittal K. Yachandra




**Photon dose in the MX studies ranged from 1 – 6 x $10^{10}$ photons/$\mu m^2$.**

**At this flux level, most of the Mn has been photoreduced back to Mn(II).**

**The safe dose is several orders of magnitude lower ($10^7$-$10^8$)!!!**

*PNAS* **2005**, *102*, 12047–12052

*Inorg. Chem.* **2008,** *47,* 1711-1726

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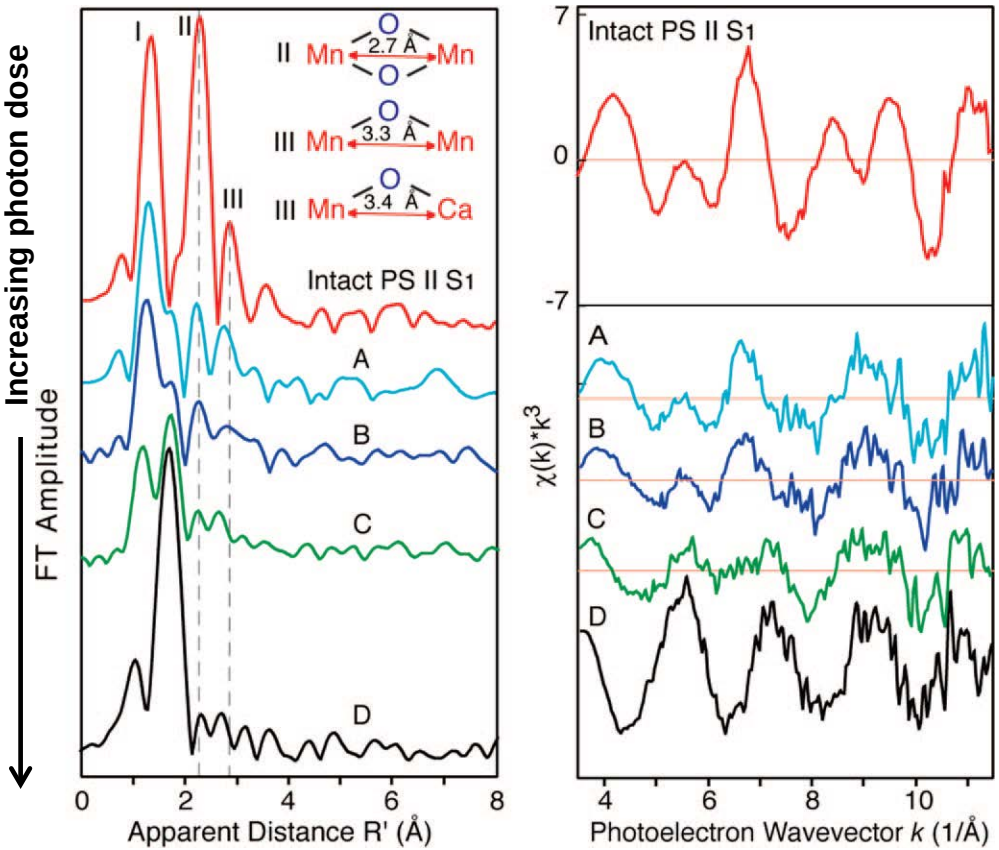
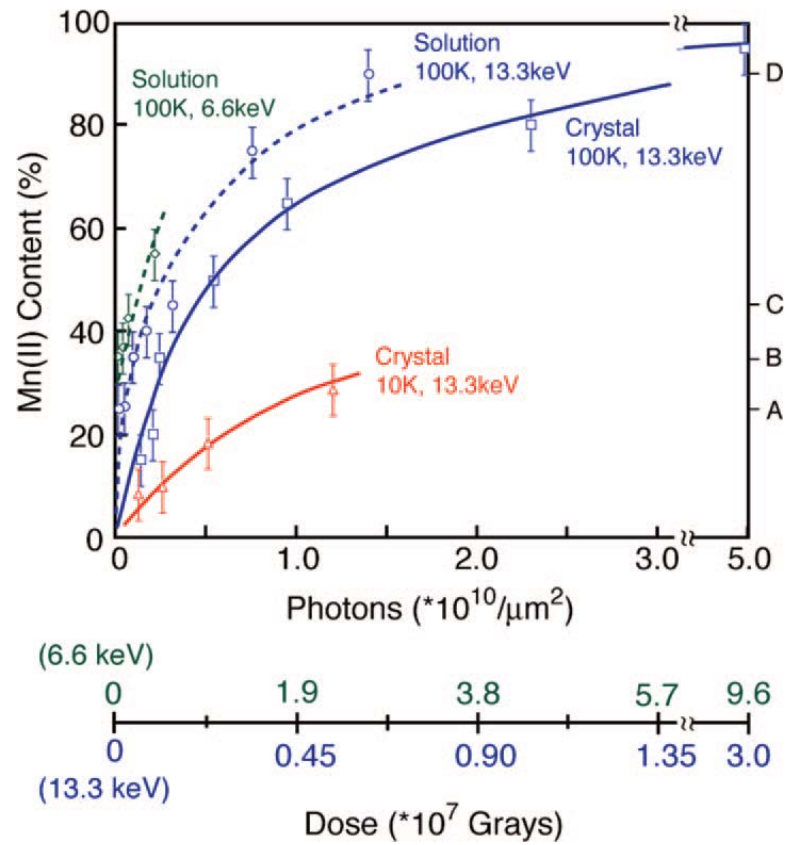



**Spectral changes of PS II Mn EXAFS due to radiation damage.** (left) The FT of the EXAFS spectrum from an intact PS II solution sample is on top (red). The FTs from PS II solution samples exposed to 0.05, 0.1, 0.3, and 1.4 × $10^{10}$ photons/μm$^2$ at 13.3 keV and 100 K and containing 25 (blue), 35 (dark blue), 45 (green), and 90% (black) photoreduced Mn(II) are labeled A-D, respectively. An average dose of ~3.5 × $10^{10}$ photons/μm$^2$ was used for XRD studies. (right) Corresponding k$^3$-space EXAFS spectra of PS II




**Progressive photoreduction leads to loss of Mn–Mn vectors in EXAFS and apparent conversion to $[Mn(H_2O)_6]^{2+}$**

*PNAS* **2005**, *102*, 12047–12052

*Inorg. Chem.* **2008,** *47,* 1711-1726

# PS II OEC: XAS provides new info for crystallography . . .

**Correlated single crystal XAS gave useful insights into photosensitivity of the $Mn_4Ca$ cluster of the OEC.**

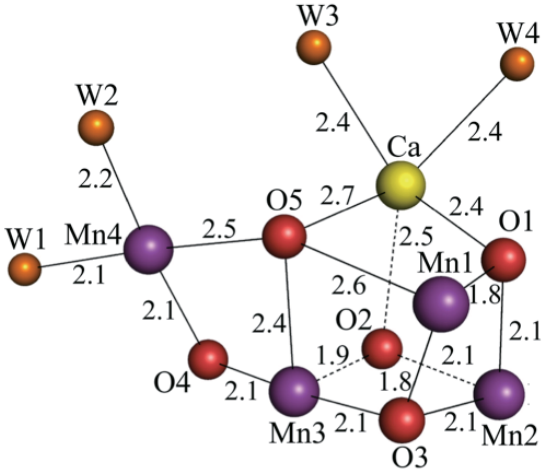
**Helped account for discrepancies in the MX data.**

**Useful lesson for further X-ray diffraction studies, which was taken to heart.**

## Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å

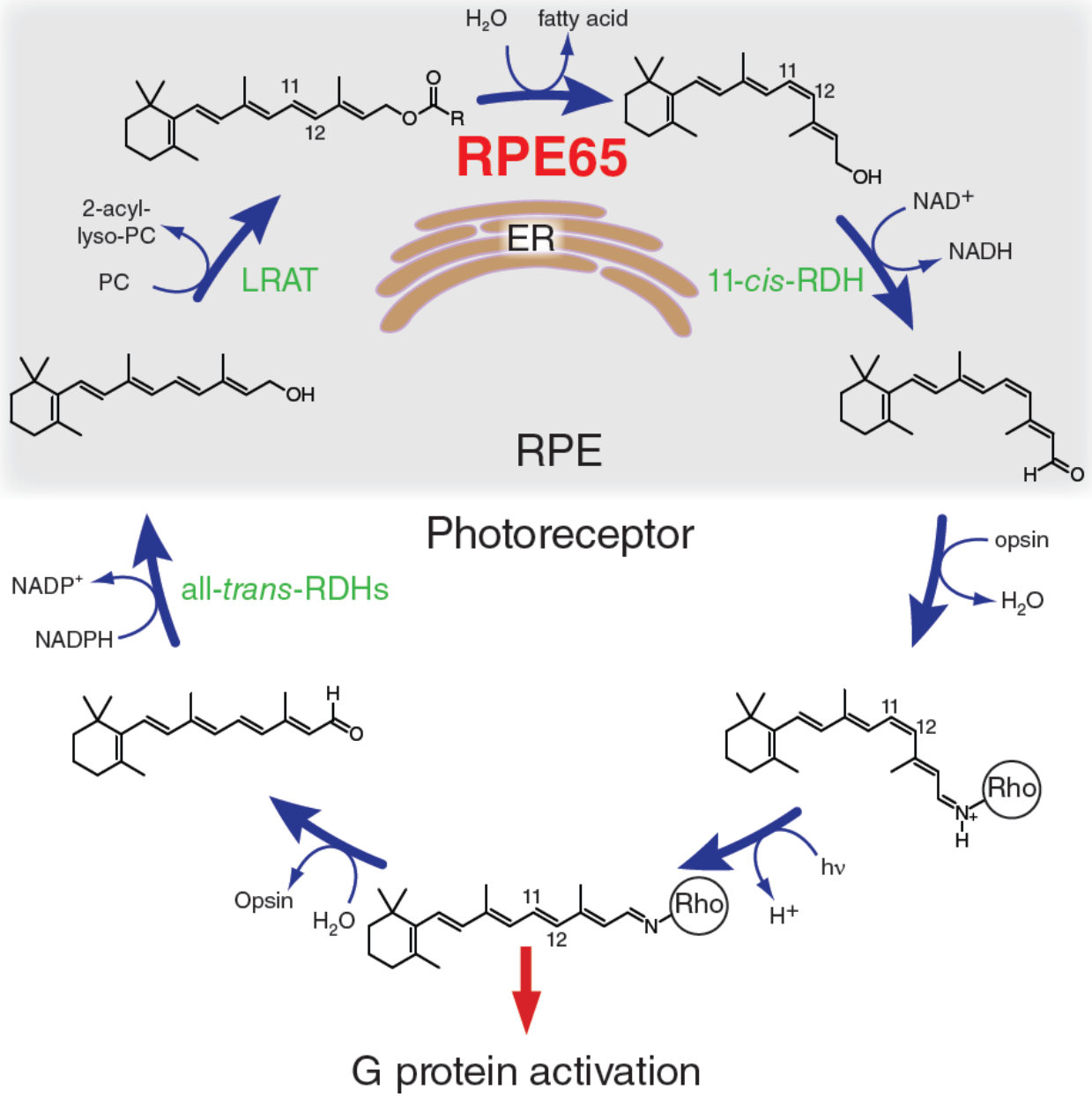
Yasufumi Umena[1]†*, Keisuke Kawakami[2]†*, Jian-Ren Shen[2] & Nobuo Kamiya[1]†

"Because the whole data set was divided into nine spots on the crystal, each spot received a total dose of 0.28 x $10^{10}$ photons/$um^2$ …
This dose is much lower than that used previously[3–6], and is also at a low level of the dose range reported to induce possible radiation damage in the $Mn_4CaO_5$ cluster[33]."




*Nature* **2011**, *473*, 55-60

# Mammalian Vision Requires Chromophore Regeneration



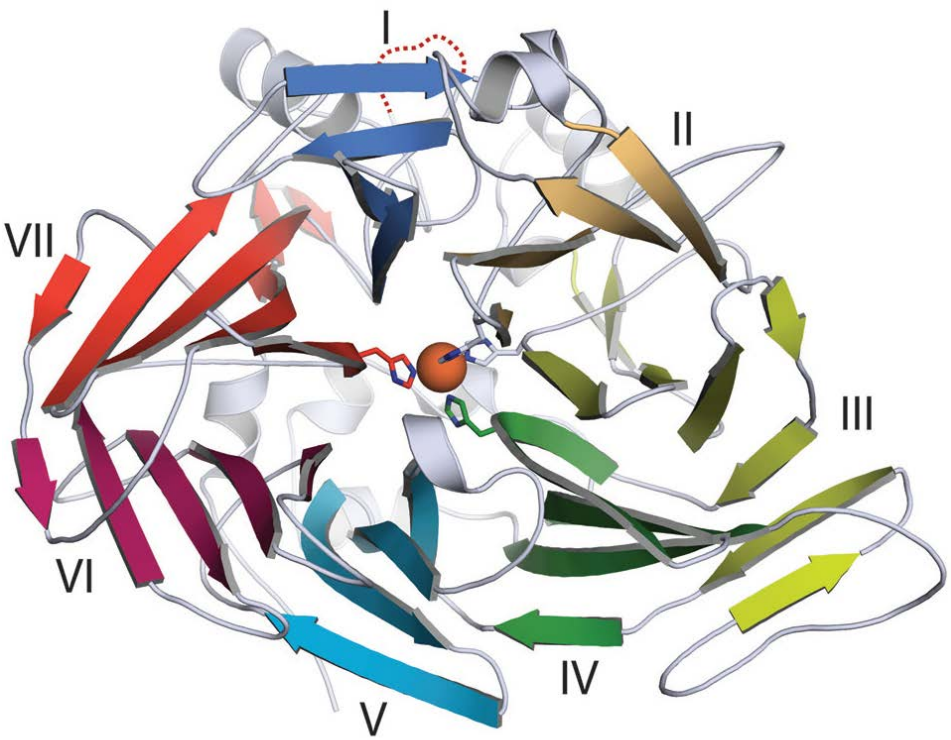

Scheme courtesy of Philip Kiser

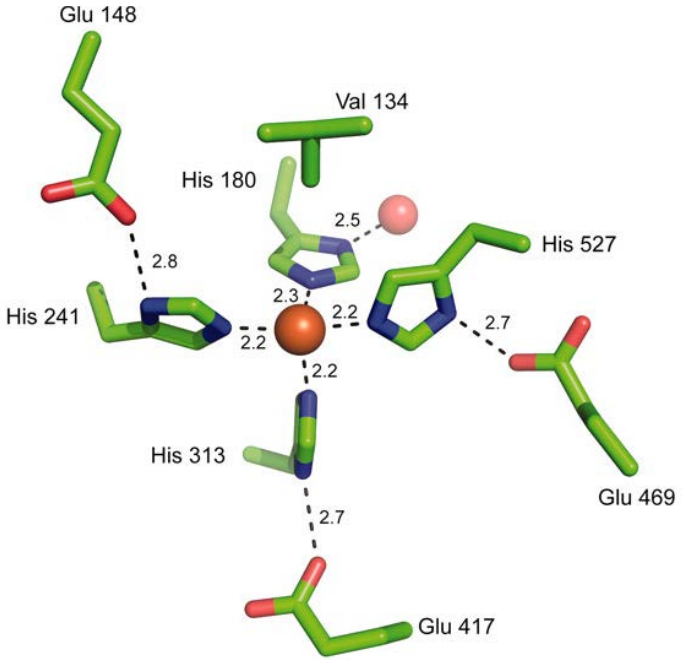
# Structure of the Delipidated Form of RPE65

RPE65: 65 kDa membrane protein associated with photoreceptor layer in the eye

Exhibits sequence homology with apo-carotenoid oxygenases, which require Fe(II) / $O_2$. RPE65 also requires iron for activity. Redox role more ambiguous.

2.14 Å structure of delipidated, solubilized form reported in 2009.



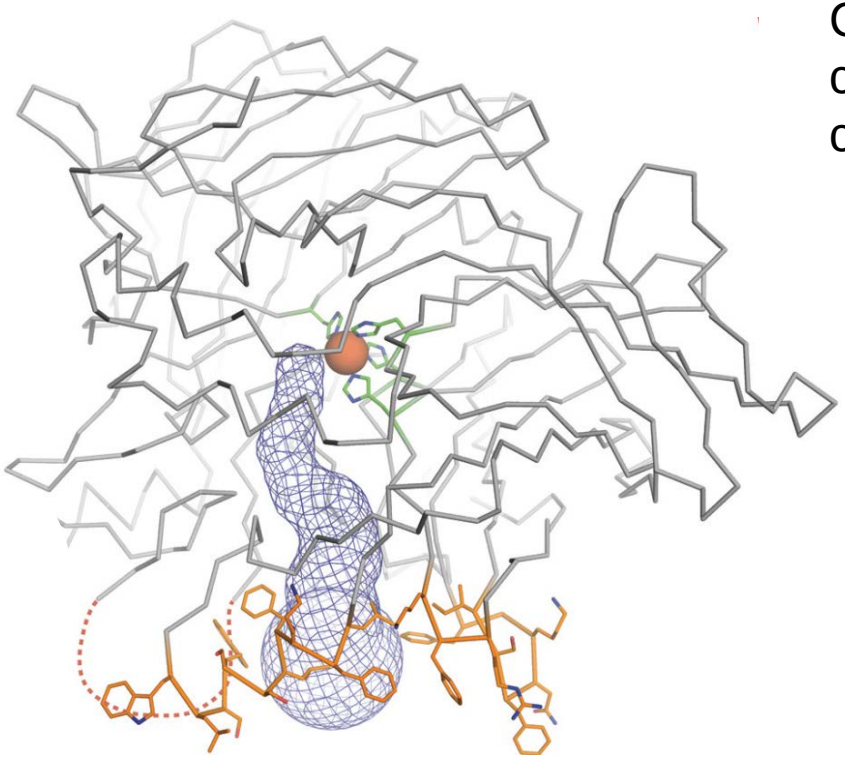




**This form is not active towards isomerizing all-*trans*-retinal**

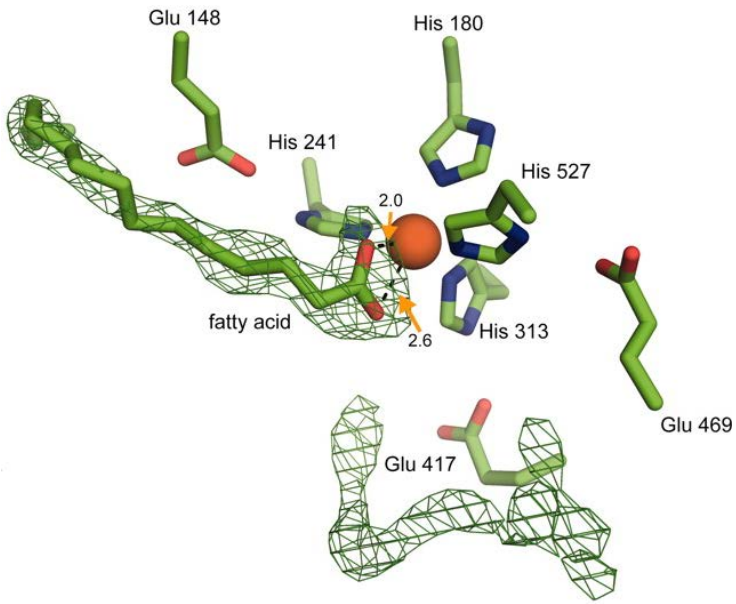
Kiser, *et al.*, *PNAS U.S.A.*, **2009**, *106*, 17325-17330

# Structure Analysis Reveals Intriguing Features

Evidence for substrate channel from the membrane-binding side of the protein.



Channel is partially occupied by a long-chain molecule, perhaps a fatty acid, with carboxylate moiety binding iron.




## Questions:

**MX:** Why is the delipidated solubilized form inactive? Structural differences in the membrane-bound form?

**XAS:** What else can we learn about the iron center?

Kiser, *et al.*, *PNAS U.S.A.*, **2009**, *106*, 17325-17330

# XAS Demonstrates that RPE65 Contains an Iron(II) Center

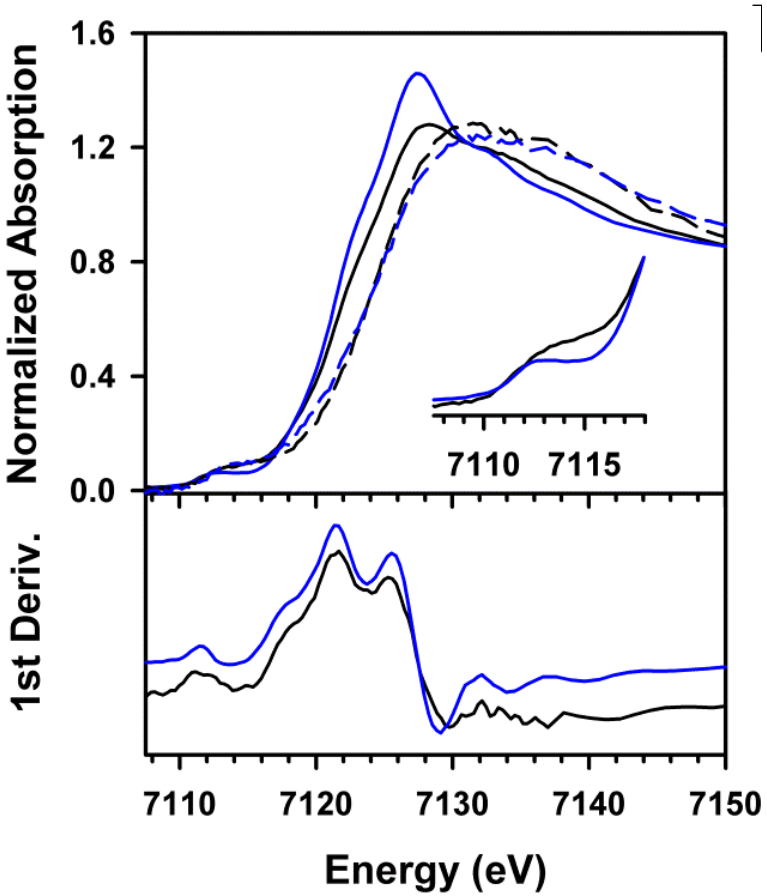
**Protein isolated from actual cow eyes:**

- **limited quantities, difficult to obtain**
- **EPR or Mössbauer not ready options**
- **XAS was an accesible way to probe iron valence**

- **As-isolated RPE65 contains a 5/6C iron(II) center (edge/pre-edge)**

- **$H_2O_2$ treatment blue-shifts the edge, consistent with Fe oxidation.**

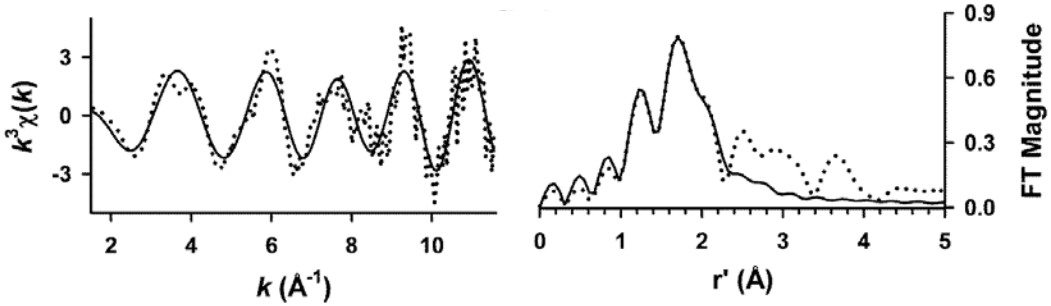
- **XANES analysis of 4His-ligated Fe(II)-ACO, a soluble carotenoid oxygenase, is identical.**



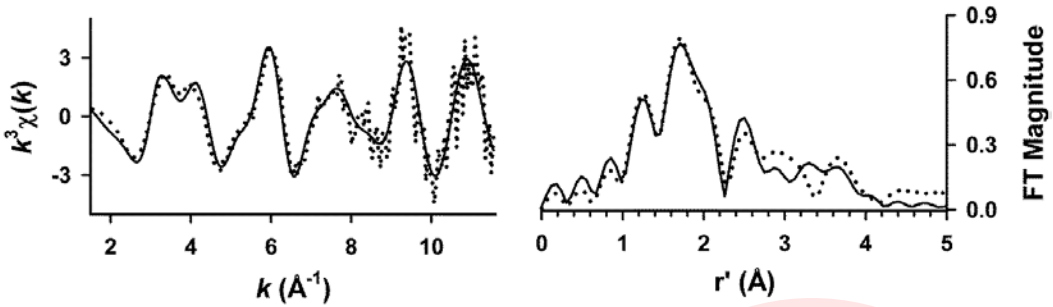

**RPE65 in black, ACO in blue.**
**$H_2O_2$ treated forms in dashes**

Kiser, *et al.*, *PNAS*, **2012**

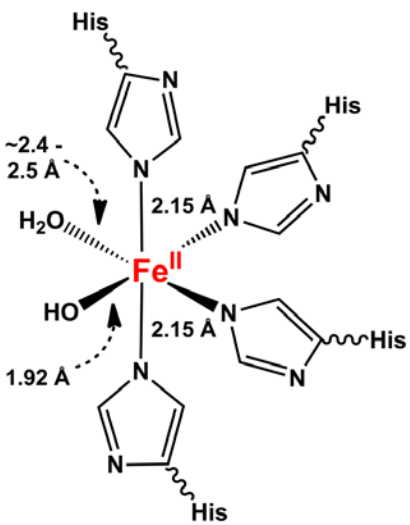
# EXAFS Analysis Suggests Carboxylate Binding

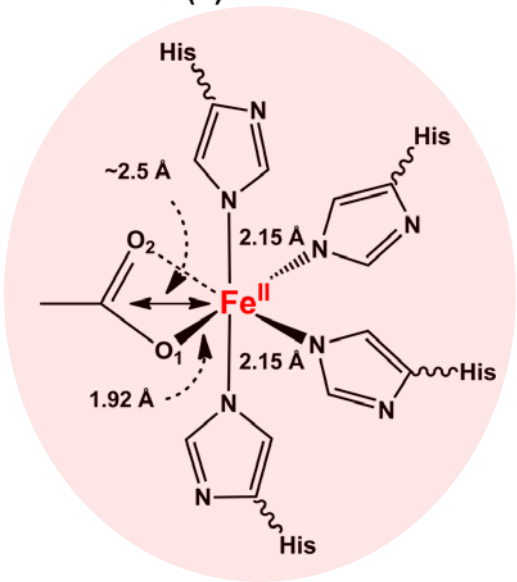



4 Fe–N/O @ 2.15 Å ($\sigma^2$ = 0.003)
1 Fe–O/N @ 1.93 Å ($\sigma^2$ = 0.003)
1 Fe–C/N/O @ ~2.50 Å ($\sigma^2$ = 0.0005)




Outer shell features simulated nicely with multiple-scattering from 3-4 His ligands (principal Fe–$N_{His}$ @ 2.15 Å)




Favor carboxylate due to consistency with crystal structure. Limited(?) evidence for very ordered solvent ligands in first coordination sphere.

Theoretical XANES simulations might be interesting . . .

Kiser, *et al.*, *PNAS*, **2012**

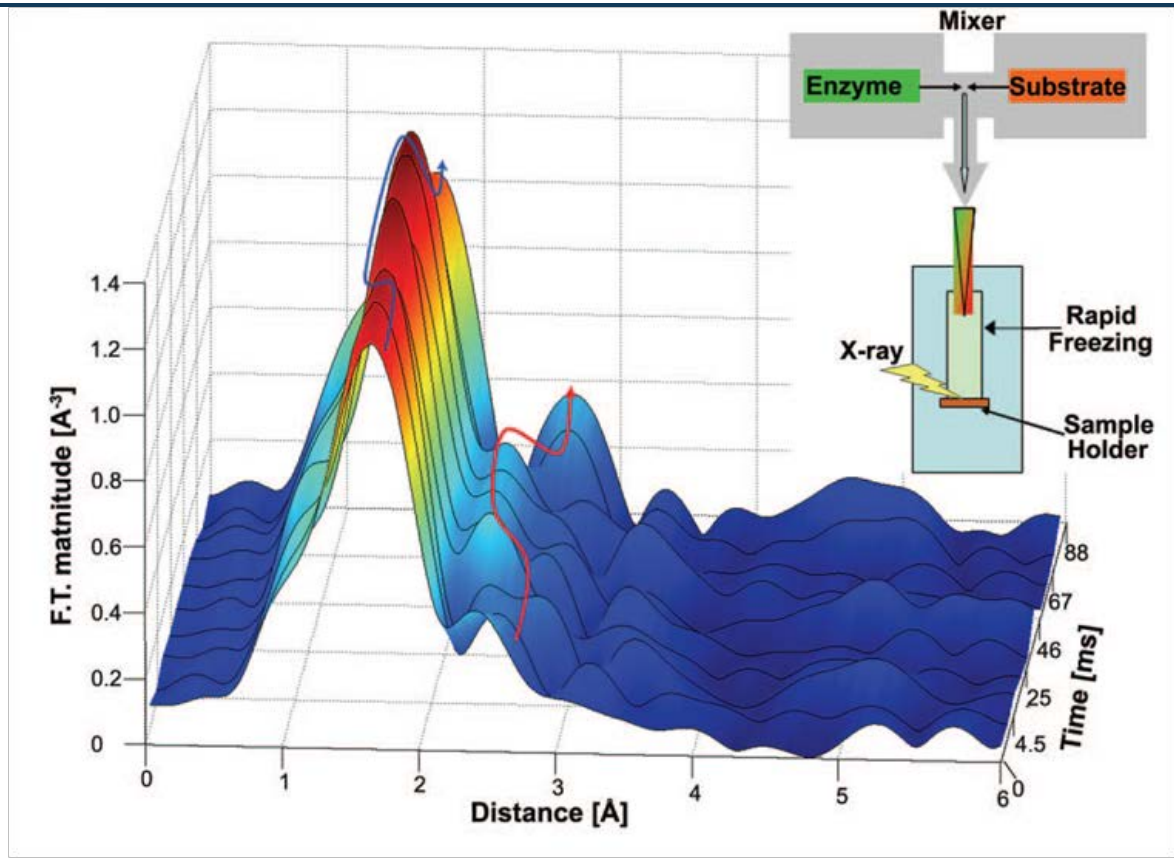
# RFQ XAS: Intermediates

Reaction intermediates tend not be amenable to crystallization, by their very nature. Structural information via XAS enables a better chemical understanding.

## Problems:

1. Limited material quantities
2. Low – very low concentrations (sub-mM absorber)
3. Rxns are v. fast (tens to hundreds of milliseconds)

The usual transmission-based Quick-EXAFS experiments not really feasible




Time-resolved XAS measurements of TNF-alpha converting enzyme during enzymatic catalysis using RFQ [Solomon et al., *PNAS* **104**, 4931 (2007)].

## Solution:

**Rapid Freeze Quench – quench reaction at specific timepoints by freezing.**

Collect XAS data on frozen samples and look for changes relative to initial starting material and final product.

Ideally: have data from other techniques that tells you something about rxn timescale

# A Classic RFQ-XAS Example – Methane Monooxygenase

**An $Fe_2^{IV}O_2$ Diamond Core Structure for the Key Intermediate Q of Methane Monooxygenase**

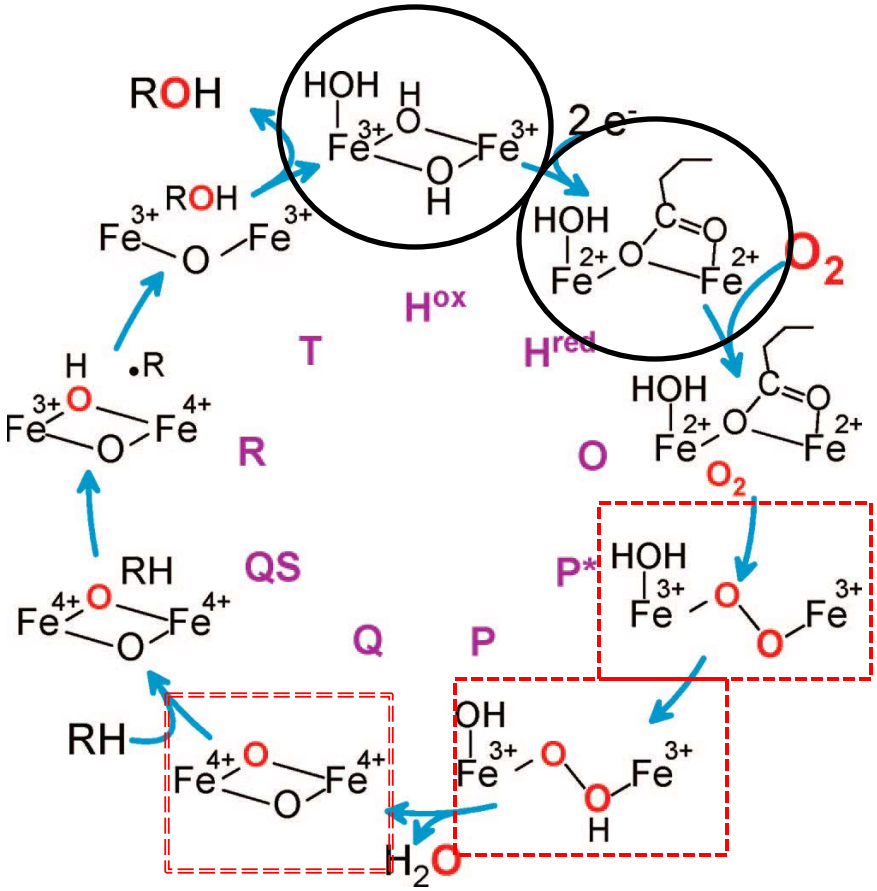
Lijin Shu, Jeremy C. Nesheim, Karl Kauffmann, Eckard Münck, John D. Lipscomb, Lawrence Que Jr.

$$CH_4 + O_2 \rightarrow CH_3OH$$

**Some pretty clever people did many experiments to work out how MMO works.**

**What is the geometric structure of the critical intermediate in this enzyme?**

**Use RFQ XAS to find the answer**

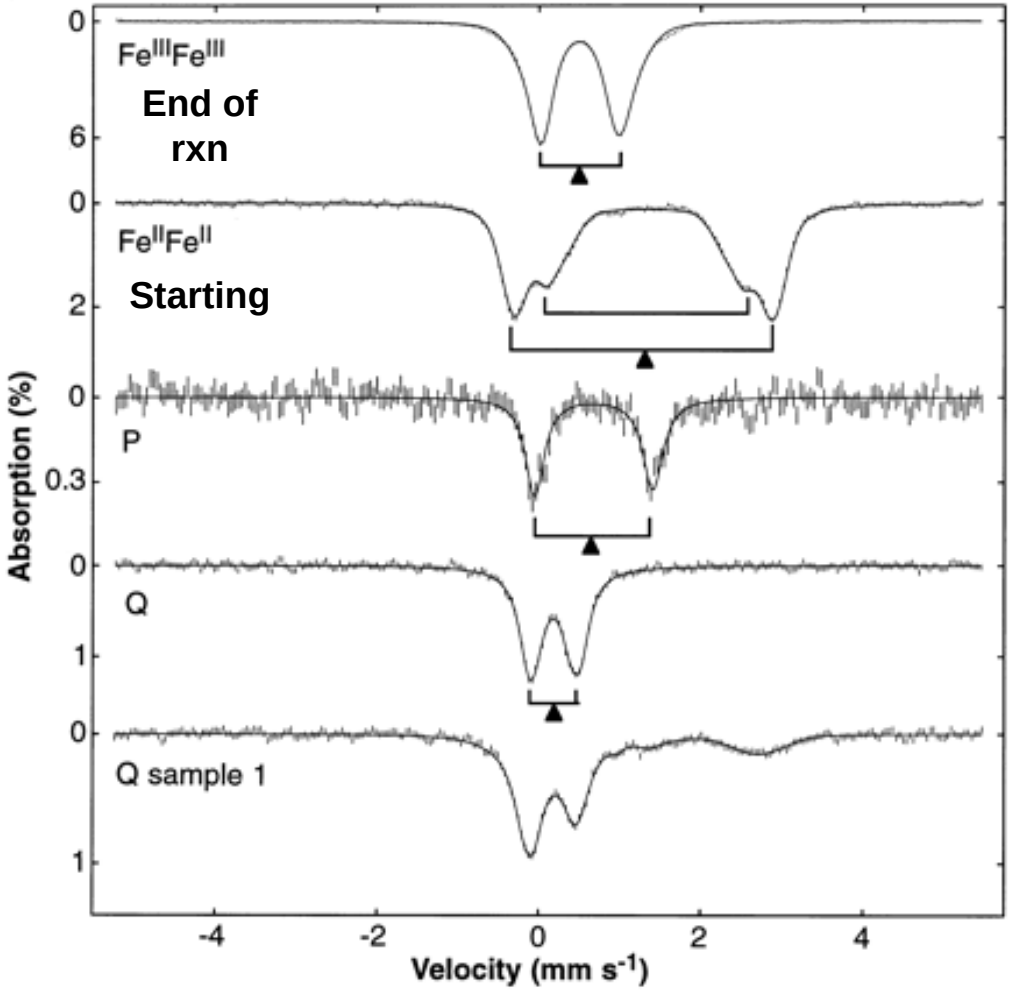


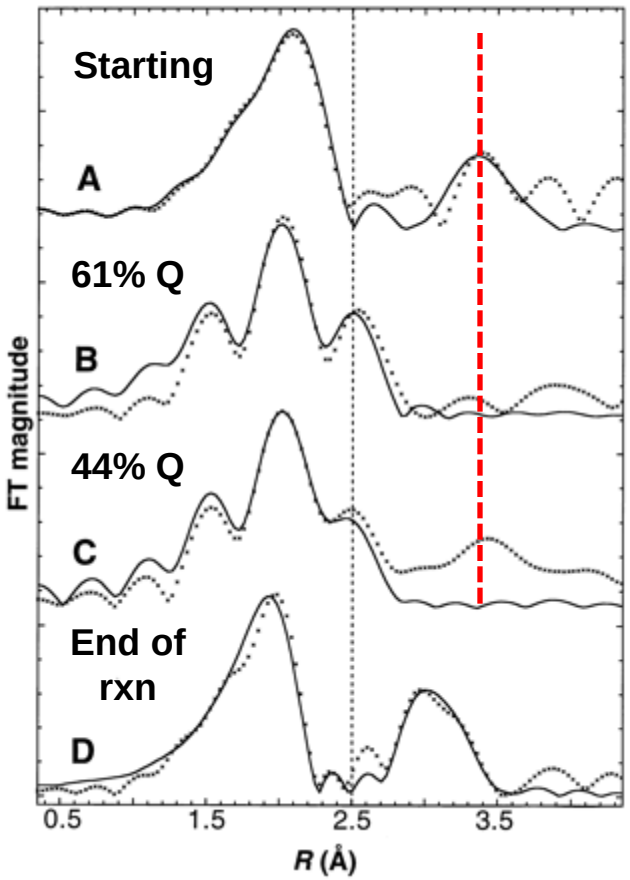

*Science*, **1997**, *275*, 515-518
*Acc. Chem. Res.* **2007**, *40*, 475–483

# Correlated Spectroscopy Helps . . .

**They are not shooting in the dark!**

tandem Mössbauer to verify rxn time scale, intermediates; data on oxidation state, site symmetry



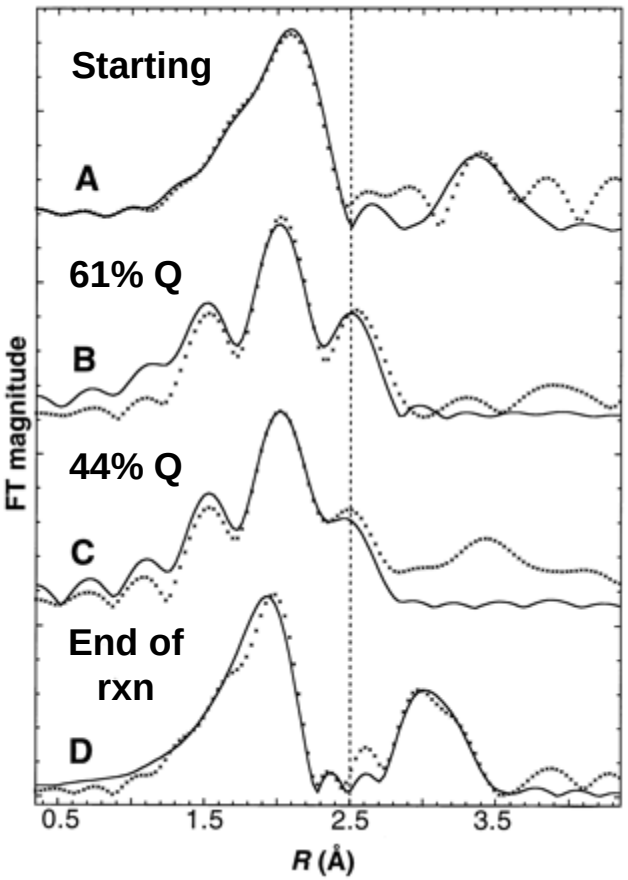

**Clear differences between starting point, intermediate, and end product!**

*Science*, **1997**, *275*, 515-518

# A Classic RFQ-XAS Example – Methane Monooxygenase

| Sample | Fit of EXAFS region | Pre-edge area (units) |
|---|---|---|
| $Fe^{II}Fe^{II}$ MMOH | 4 O/N at 2.20 Å (0.0067)<br>0.5 O/N at 2.02 Å (0.0039)<br>1 Fe at 3.43 Å (0.0062) | 10 ± 1 |
| Sample 1 (61% Q) | 4 O/N at 2.05 Å (0.0115)<br>0.6 O/N at 1.77 Å (−0.0003)<br>0.6 Fe at 2.46 Å (0.0086)* | 22 ± 1 |
| Sample 2 (44% Q)† | 4 O/N at 2.03 Å (0.0063)<br>0.5 O/N at 1.78 Å (−0.0039)<br>0.5 Fe at 2.47 Å (0.0058)* | 18 ± 1 |
| Decayed Q | 5 O/N at 2.01 Å (0.0059)<br>0.6 Fe at 3.03 Å (0.0015)<br>0.4 Fe at 3.32 Å (0.0061) | 14 ± 1 |

*Attempts to fit this 2.46 Å shell with low atomic number atoms gave rise to unacceptable fits. †The 3.4 Å feature in the spectrum (Fig. 2C) of this sample was not included in the window used for the fit. When the window was extended to include this feature, it represented 0.5 Fe at 3.42 Å, which we attribute to contributions from the $Fe^{II}Fe^{II}$ and $Fe^{III}Fe^{III}$ forms of MMOH.

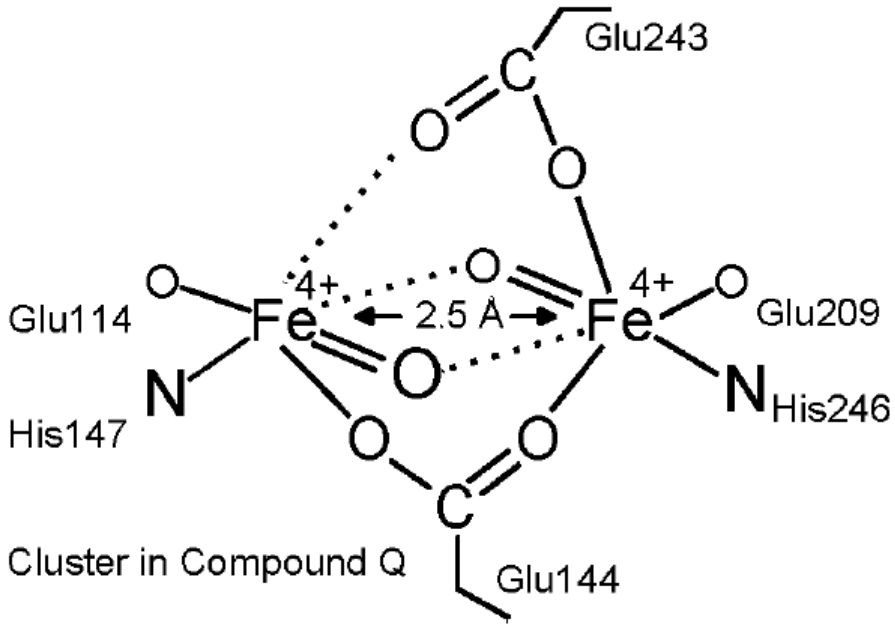



**XAS fitting analysis indicates a substantial shortening of the Fe···Fe vector to 2.5 Å, appearance of a short 1.77 Å Fe–O bond**

***n* for both shells is equal to % of intermediate**

*Science*, **1997**, *275*, 515-518

# A Structural Model from the XAFS Data




Recall that best fit consisted of
4-5 Fe–O/N @ 2.05 Å
Fe⋯Fe @ ~2.5 Å,
short Fe–O @ 1.77 Å

Structural model nicely accounts for EXAFS parameters.

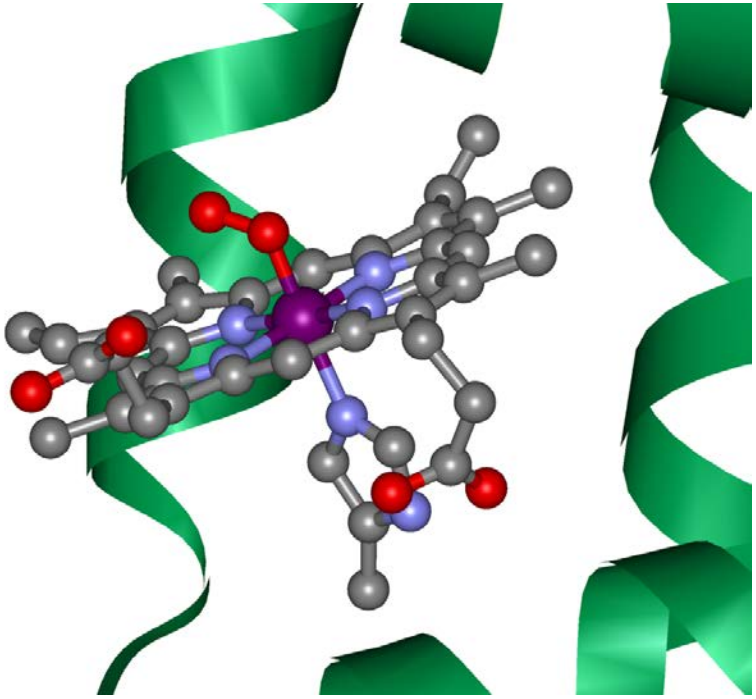
**Basis for further investigation by other methods (computations, development of new catalysts, etc).**

## Lessons / Things to Think About

1. Watching for simple changes even with ***imperfect samples*** can provide a lot of insight into structure.
2. XAS was one of several tools in this experimental program and was used to answer a specific question. *Incredible fits to data are just pretty pictures unless you have context.*

*Science*, **1997**, *275*, 515-518

# What Shall We Talk about Today . . .



**XAS in biology is an enormous field with 40 years of history . . .**

**. . . not so different from other subject areas.**

**Two themes:**

1. **Some examples of applying XAS to a problem.**
2. **Some examples of pitfalls in bioXAS**

**Examples of some pitfalls in applying XAS to biological problems:**

a. **Counting N and S ligands at Zn sites**

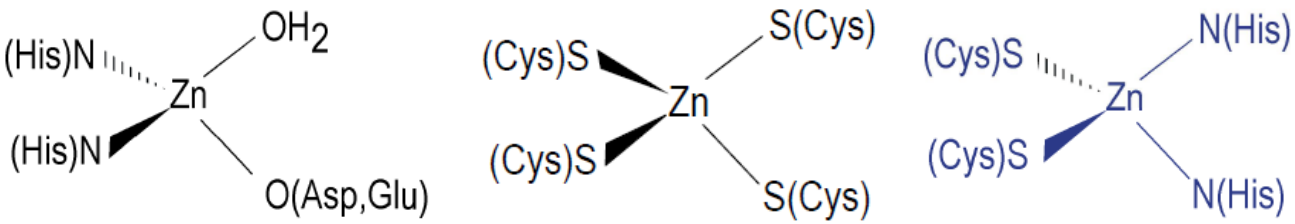
b. **Detecting metal-metal scattering**

# Zinc XAS of N/S sites – Not as Easy as it Seems

**The Limitations of X-ray Absorption Spectroscopy for Determining the Structure of Zinc Sites in Proteins. When Is a Tetrathiolate Not a Tetrathiolate?**

Penner-Hahn, et al . . .

**Zinc(II) is the second most abundant transition metal in humans**
**Variety of structural and catalytic roles**
**Frequently found in a 4C $T_d$ geometry, with $N_{His}$ and/or $S_{Cys}$ ligands**



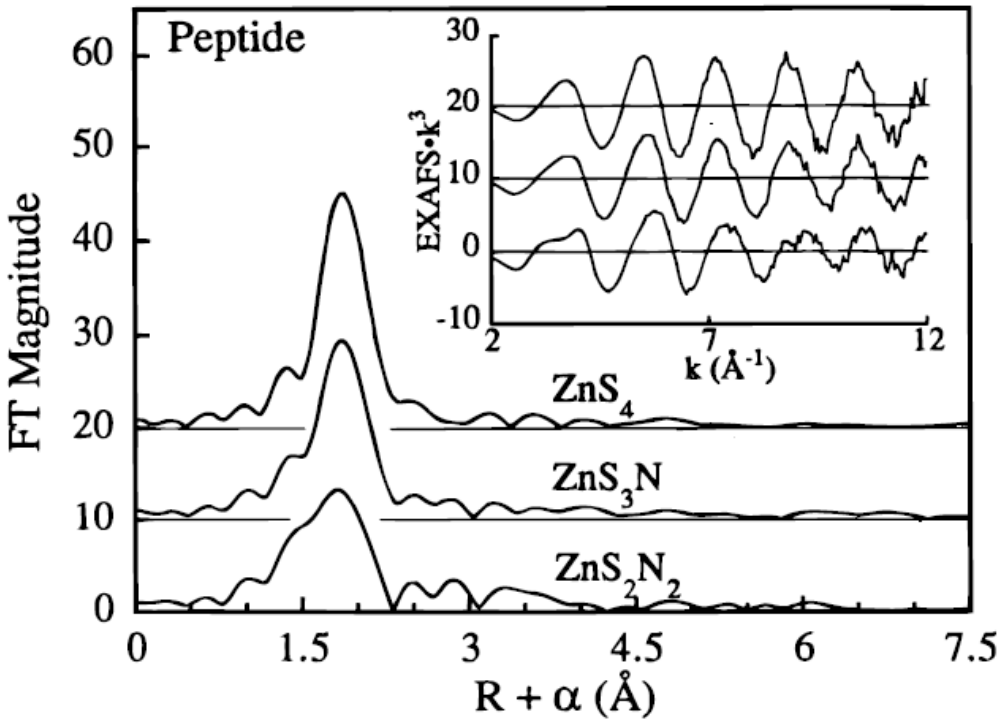

Typically,
$Zn–N_{His}$ ~ 2.05-2.1 Å,
$Zn–S_{Cys}$ ~ 2.3 Å

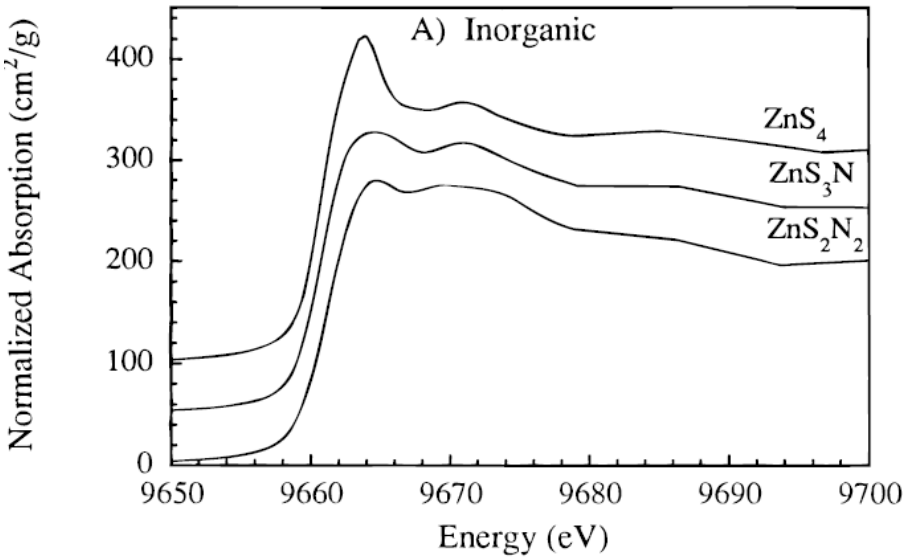
**Zn(II) is spectroscopically quiet, thus XAS is one of the very few physical methods available for characterization of Zn(II) sites in proteins.**

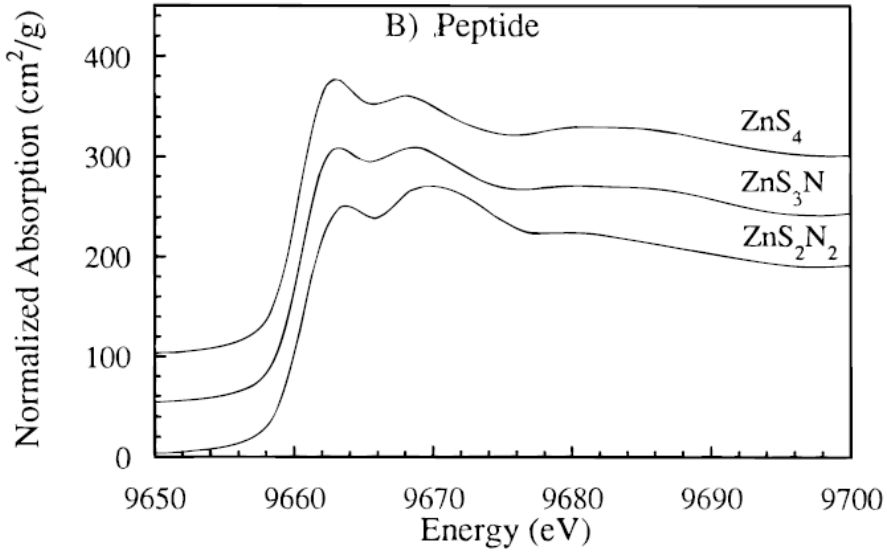
"…several examples of incorrect discrimination between S and N ligands. In GAL4, the tetrathiolate Zn site[11] was incorrectly modeled as having $ZnS_3O$ ligation.[12] The opposite error, overestimation of the fraction of sulfur ligands, occurred for the Zn site in aminolevulinate dehydratase, where the $ZnCys_3(O)$ site[13] was modeled as having $ZnS_4$ ligation,[14] and in the Rieske [2Fe-2S] cluster, where the $FeS_3N_1$ average site[15] was modeled as having $FeS_{3.5}N_{0.5}$ ligation.[16]"

*J. Am. Chem. Soc.* **1998,** *120*, 8401-8409

# No Clear Fingerprint to Count N and S



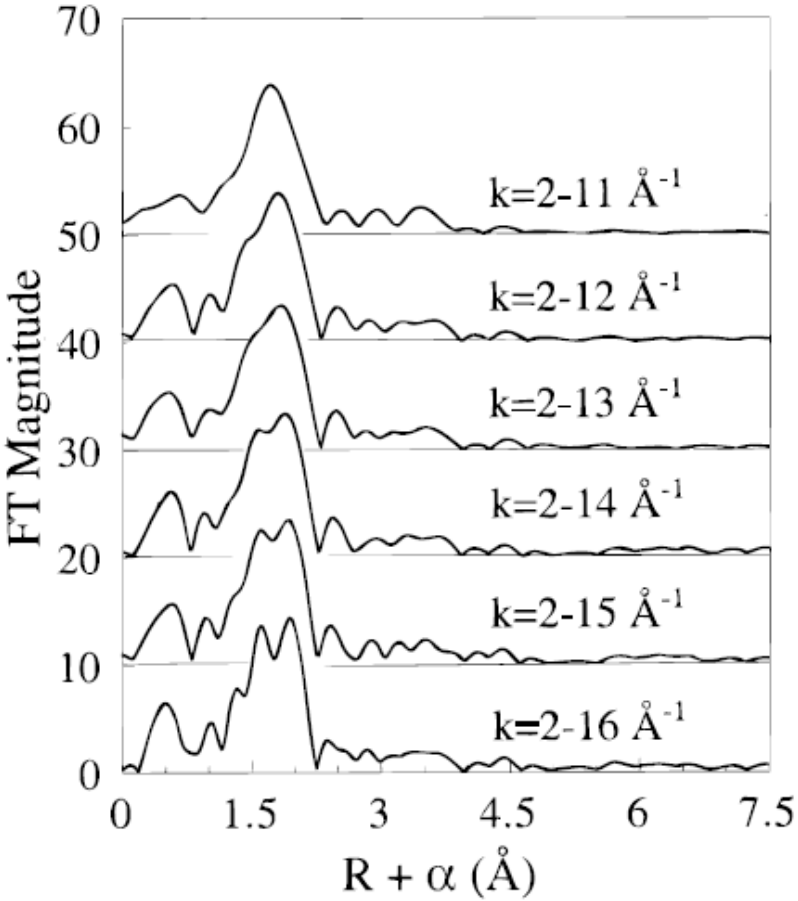

***k*³-weighted Zn XAFS is not that different over a typical biological XAS *k*-range of *k*=2-12 Å⁻¹.**

**XANES shows some differences, but no clearly useful fingerprint.**

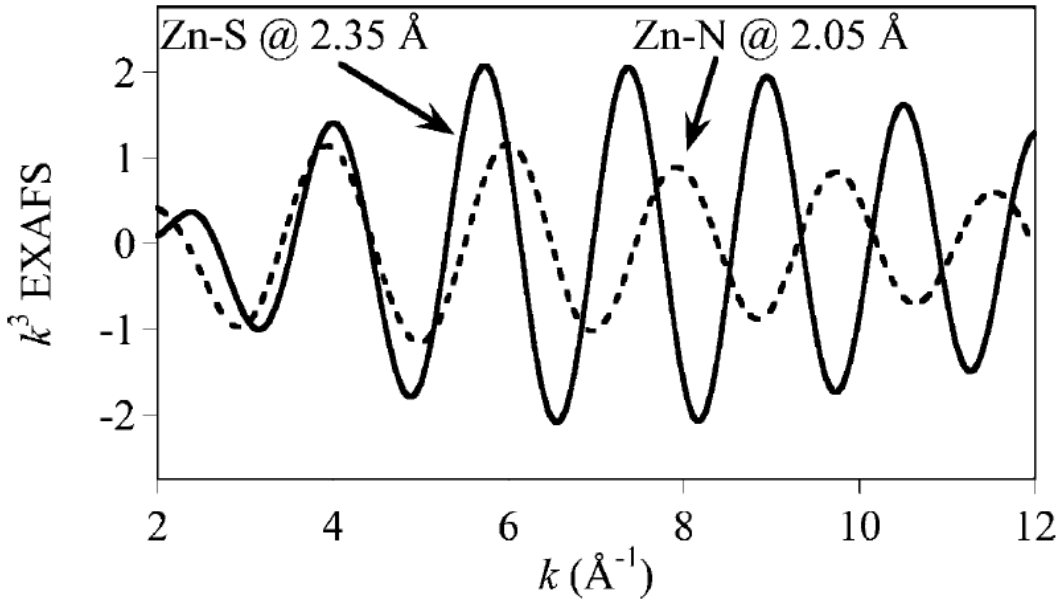
*J. Am. Chem. Soc.* **1998,** *120*, 8401-8409

# Zinc XAS of N/S sites – Not as Easy as it Seems

**Longer k-ranges provide increasing resolution, w/ two distinct shells in the FT (verified by fitting)**




**Can't count on getting high quality data!**




**Out of phase Zn–S and Zn–N – destructive interference**

**The authors of this study described an analysis protocol with parameter constraints and fit statistics to reliably analyze mixed N/S Zn(II) sites.**

**Simultaneous multiple *k*-weight refinements might be beneficial for this problem . . . has yet to be systematically explored.**

*J. Am. Chem. Soc.* **1998,** *120*, 8401-8409

# The Special Case of Histidines

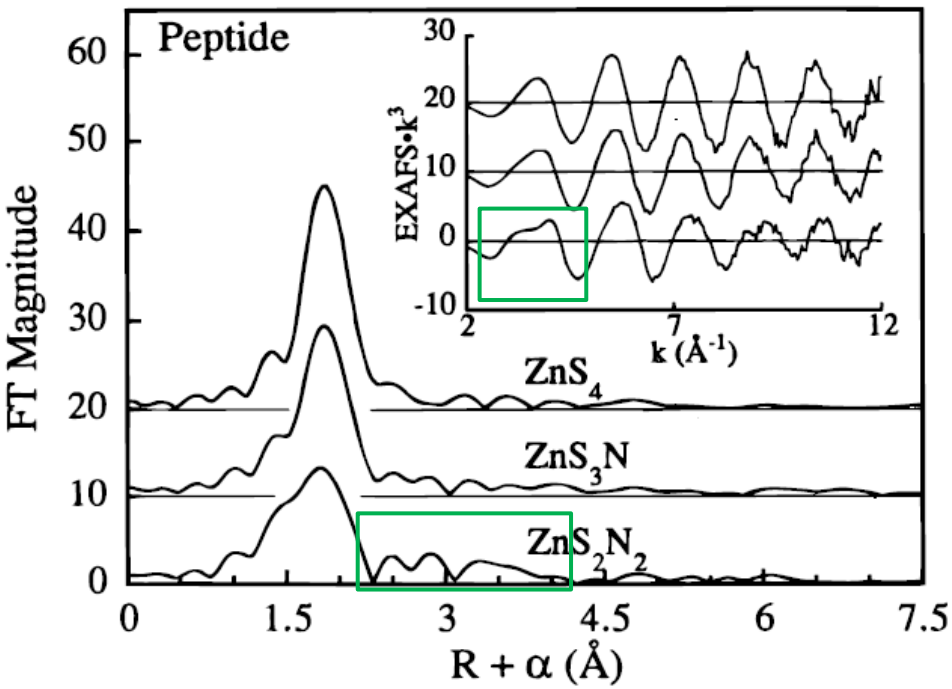
**Histidines exhibit unique EXAFS features that can be easily identified**

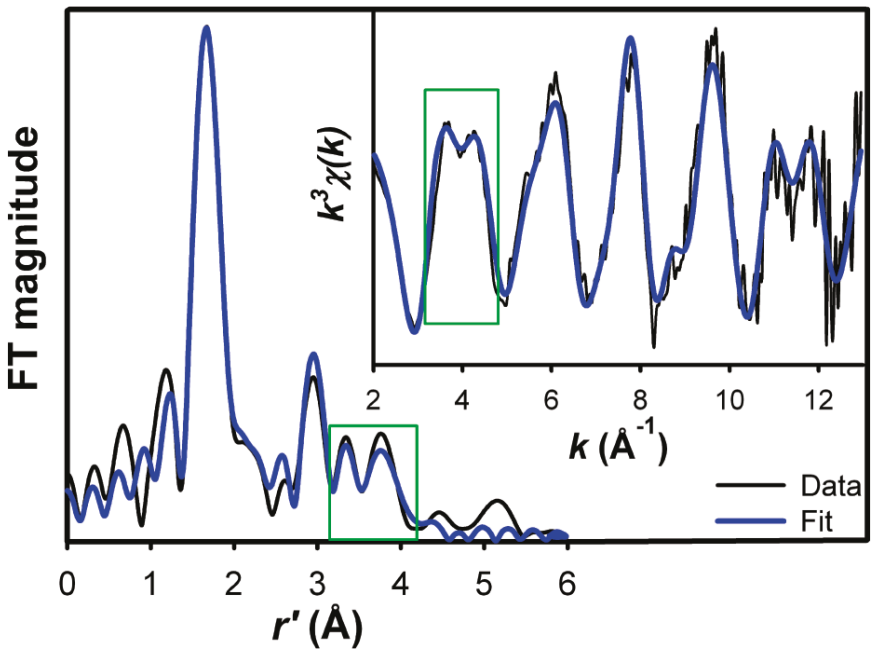
Typified by weak features in FT at r' = 3-4 Å, and a double hump in the EXAFS at k ~ 4-5 $Å^{-1}$.

**NOTE: Presence of His not always clearcut (e.g. disorder)**

In proteins with mixed Zn-$S_{Cys}$/$N_{His}$ ligation, detecting/fitting for bound His could provide another means to precisely quantify N/S mix (and distinguish N from O).

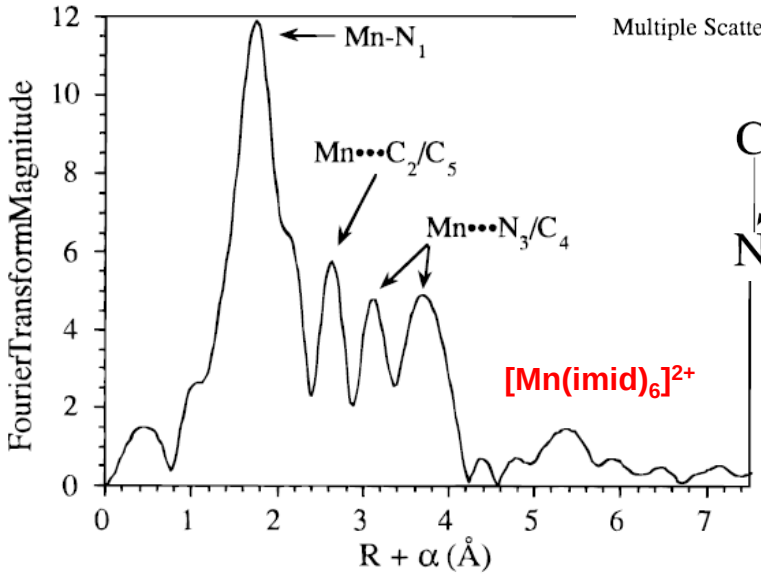
**Not necessarily a trivial exercise . . .**

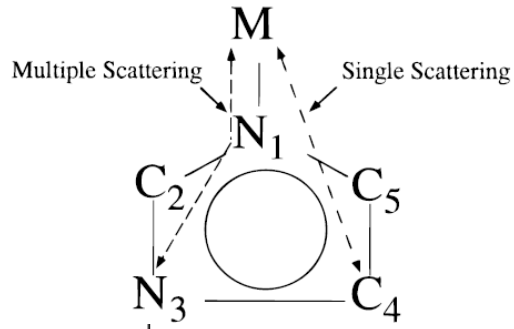


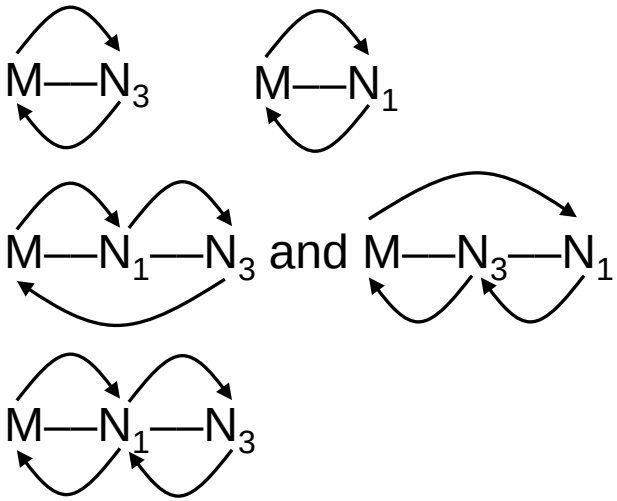




*Biochemistry* **1997**, *36*, 9847-9858
*J. Am. Chem. Soc.* **1998,** *120***,** 8401-8409
*J. Am. Chem. Soc.* **2011**, *133*, 6938–6941

# XAS of Histidines – Multiple Scattering!




Large set of two-body (single scattering), three/four body (multiple scattering) paths for metal-bound His.

Scattering intensity greatly affected by $\theta$ and $\phi$ angles.

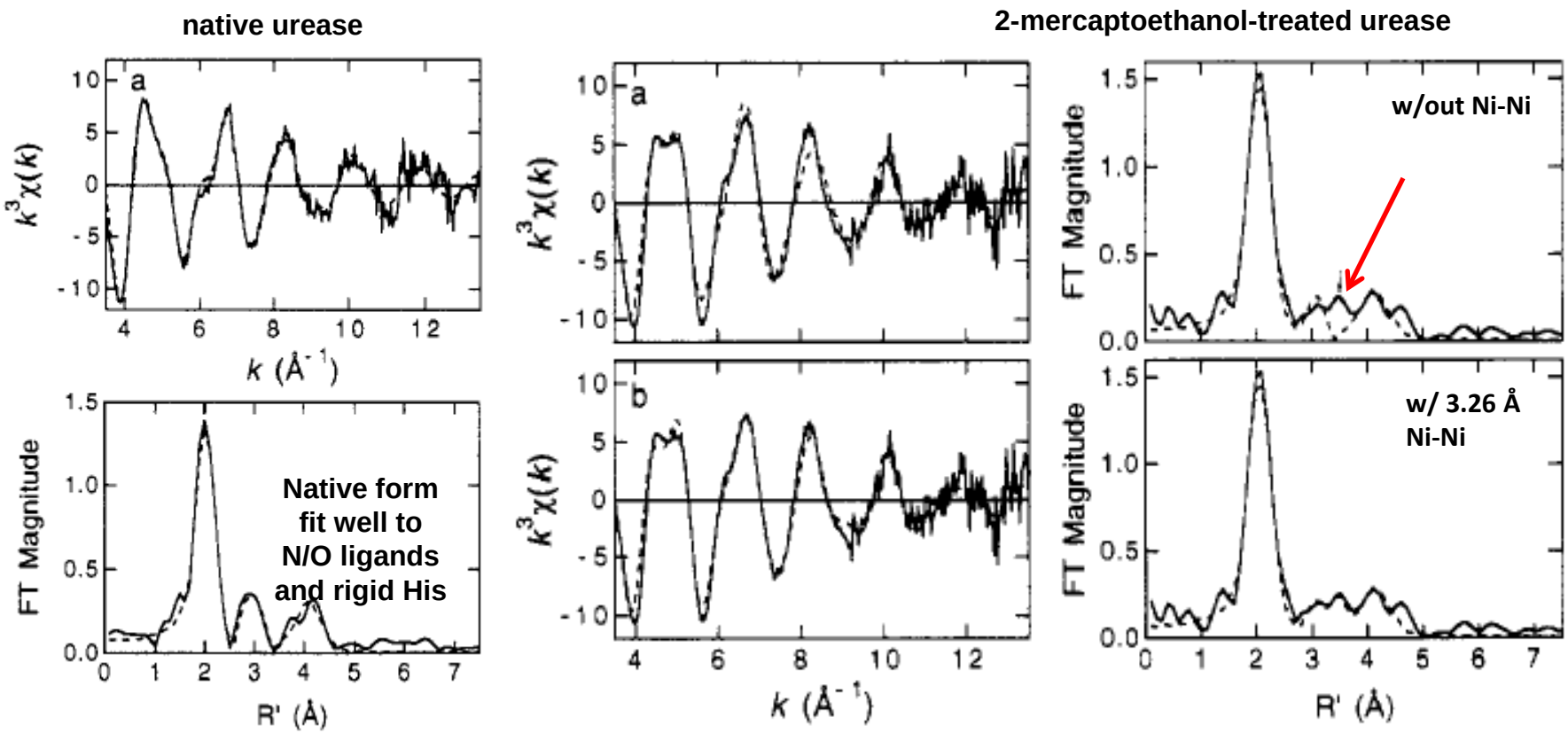
**Parameter constraints become extremely important when dealing with His MS – many paths/variables**

Most refinement schemes treat the imidazole as a rigid body: *r'* for 2/3/4 body paths constrained to constant difference, various correlation schemes for $\sigma^2$, etc.

*Biochemistry* **1997**, *36*, 9847-9858
*J. Am. Chem. Soc.* **2008**, *130*, 17826–17835

# Detecting Metal-Metal Scattering in Biology

Urease contains a dinuclear Ni(II) site … no MX data, so they used XAS.




"We cannot distinguish between two possible explanations for the absence of Ni-Ni interaction in the EXAFS of the native urease samples: (a) The Ni-Ni separation is too long to be observed or (b) the Ni-Ni separation is short, **but without a bridging ligand, the uncorrelated motion** of the two nickels produces a large Debye-Waller factor, damping its EXAFS contribution."

**Crystal structure of native form later showed a 3.5 Å Ni–Ni separation**

*Inorg. Chem.* **1994**, *33*, 1589-1593

# Detecting Metal-Metal Scattering in Biology

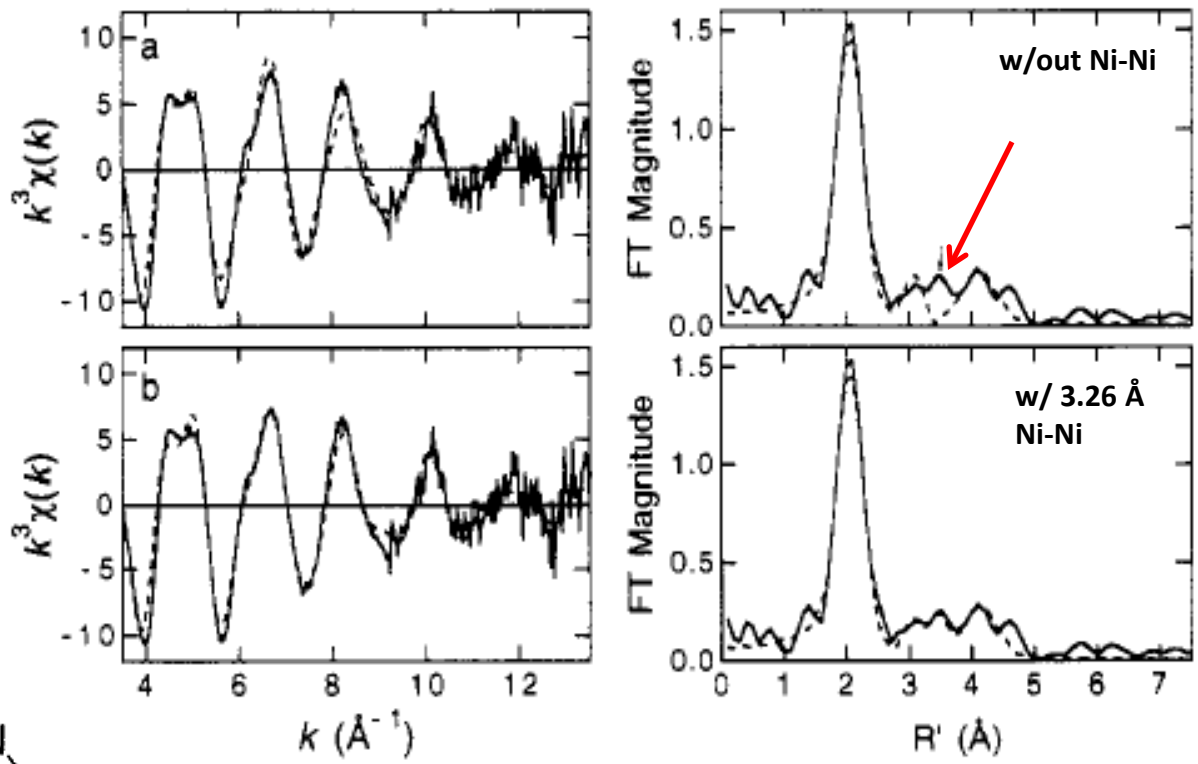
## Why the difference in detectability of the Ni–Ni interaction?

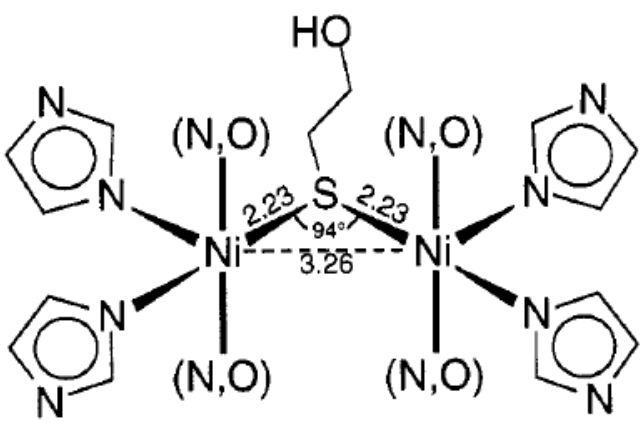
**Again, having data from other techniques is important!**

Magnetic studies showed only a weak interaction between both Ni(II) ions in the native form, increased when they added 2-mercaptoethanol . . .

2-ME bridges the Ni(II) site, and there are no bridging ligands otherwise . . .

**2-mercaptoethanol-treated urease**

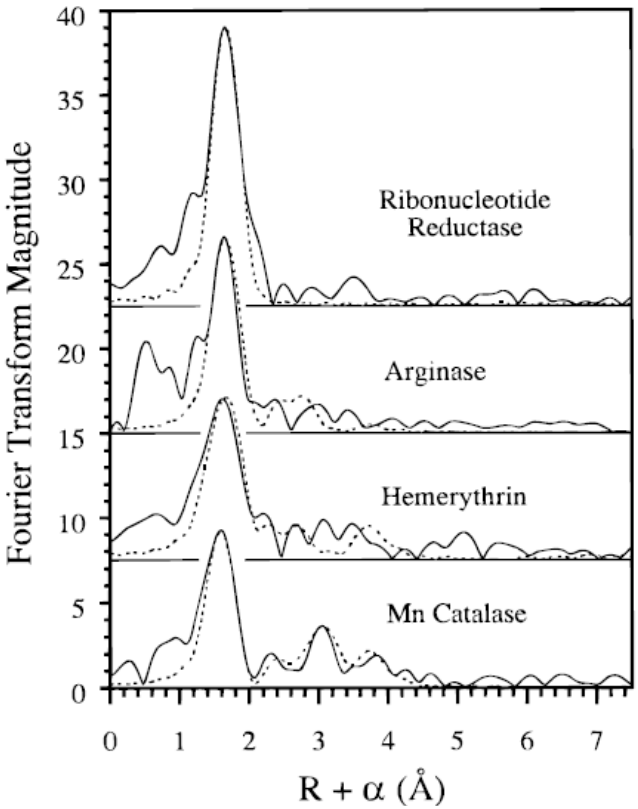


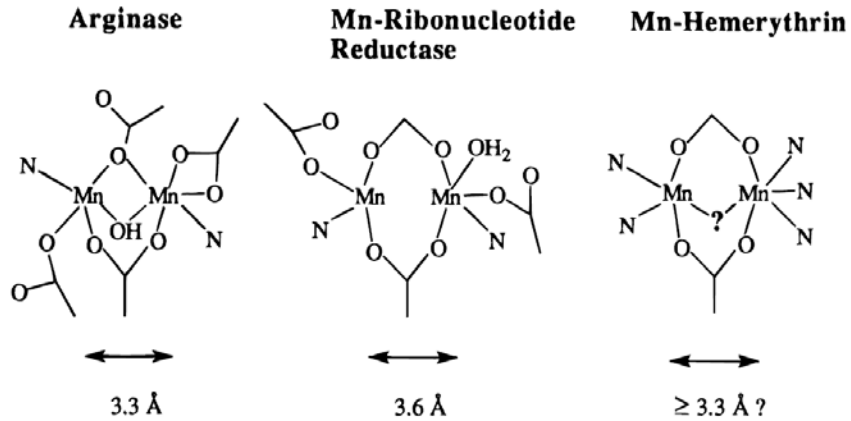




**Without data from other methods, not seeing a metal-metal interaction in a known dinuclear active site can be an exercise in negative evidence!**

*Inorg. Chem.* **1994**, *33*, 1589-1593

# Detecting Metal-Metal Scattering in Biology




**Mn—Mn scattering only clearly detected for Mn catalase, which also happens to be the only one with a strong magnetic interaction between metals & a bridging ligand**

**A good quote from the paper:** "It appears that at least one single-atom bridge is necessary to observe EXAFS detectable metal-metal scattering, and there is ample evidence that a μ-OH ligand can fulfill this role…"

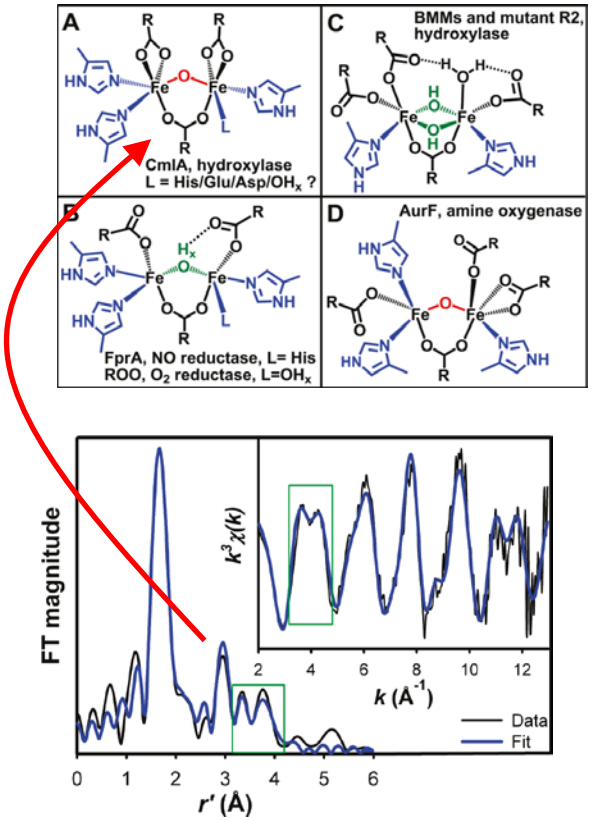
Table 6: Comparison of EXAFS Fitting Results with Averaged Crystallographic Parameters

| sample | | Mn−O | | Mn−N | | avg Mn−(O/N)[c] | | Mn···Mn | | number of His residues/Mn |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $R^a$ | CN[b] | $R^a$ | CN[b] | $R^a$ | CN[b] | $R^a$ | CN[b] | |
| $Mn_2$RR | (EXAFS) | 2.15 | 5.0 | | | 2.15 | 5.0 | nd[f] | nd[f] | nd[f] |
| | crystal | 2.20 | 3.5 | 2.25 | 1.0 | 2.21 | 4.5 | 3.6 | 1.0 | 1.0 |
| Arg | (EXAFS) | 2.17 | 3.5 | | | 2.17 | 3.5 | 3.41 | 0.5 | 0.5 |
| | crystal | 2.27 | 4.5 | 2.15 | 1.0 | 2.25 | 5.5 | 3.3 | 1.0 | 1.0 |
| $Mn_2$Hem | (EXAFS) | 2.10 | 3.0 | 2.26 | 2.0 | 2.16 | 5.0 | nd[f] | nd[f] | 1.0 |
| $Fe_2$Hem[d] | crystal | 2.14 | 3.0 | 2.22 | 2.5 | 2.18 | 5.5 | 3.32 | 1.0 | 2.5 |
| Cat | (EXAFS) | 2.11 | 3.0 | 2.27 | 1.0 | 2.15 | 4.0 | 3.54 | 0.5 | 1.0 |
| | crystal[e] | | | | | | | 3.6 | 1.0 | |

[a] Bond length in angstroms. [b] Coordination number. [c] Averaged Mn−nearest neighbor distance from all ligands. [d] Coordination information taken from deoxy hemerythrin (Holmes et al., 1991). [e] Only the Mn···Mn separation, determined from a 5.0 Å crystal structure, is presently available. [f] nd = not determined, see text.

*Biochemistry* **1997**, *36*, 9847-9858

# Detecting Metal-Metal Scattering in Biology



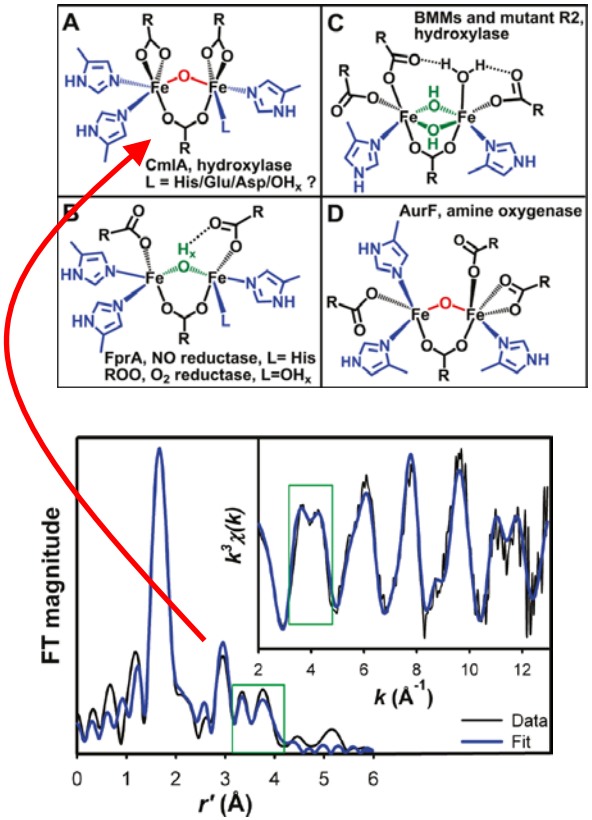

**Increasing prevalence of crystal structure data has made EXAFS analysis of *structurally uncharacterized* proteins w/ dinuclear active sites less common.**

**But it's still important to understand limitations!**

Metal-metal distances of 2.5 – 5 Å are typical.
Rare to detect r' > ~3.7 Å because such sites lack single atom bridges (there are exceptions).

*J. Am. Chem. Soc.* **2011**, *133*, 6938–6941

# Detecting Metal-Metal Scattering in Biology

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**But it's still important to understand limitations!**

Metal-metal distances of 2.5 – 5 Å are typical.
Rare to detect r' > ~3.7 Å because such sites lack single atom bridges (there are exceptions).

---

**I think I have a dinuclear site but I can't see it by XAS.**
**What can I do?**

**EXAFS analysis issues:**

1. Is your fit model a good one?
2. Are you considering His MS features, and how?

**Consider what evidence you have for there being two metals:**

1. Use spectroscopies that probe magnetic interactions, e.g., MCD, EPR, Mössbauer.
2. Change conditions, e.g., pH to affect bridging $H_2O$ or OH, or add compounds that might bind to both metals. Do spectroscopic properties of metal change?

For an older, but still relevant, discussion on pitfalls in detecting metal-metal interactions in biological systems, see: Pamela J. Riggs-Gelasco, Timothy L. Stemmler, and James E. Penner-Hahn, "XAFS of dinuclear metal sites in proteins and model compounds." *Coord. Chem. Rev.* **1995**, *144*, 245-286.

*J. Am. Chem. Soc.* **2011**, *133*, 6938–6941

# Some Concluding Remarks

**Keys to success in biological XAS (also true for other areas)**

1. Pose a specific question that you want to answer and use data from other methods.

2. You can get as much or as little geometric data as you want/need out of the data.

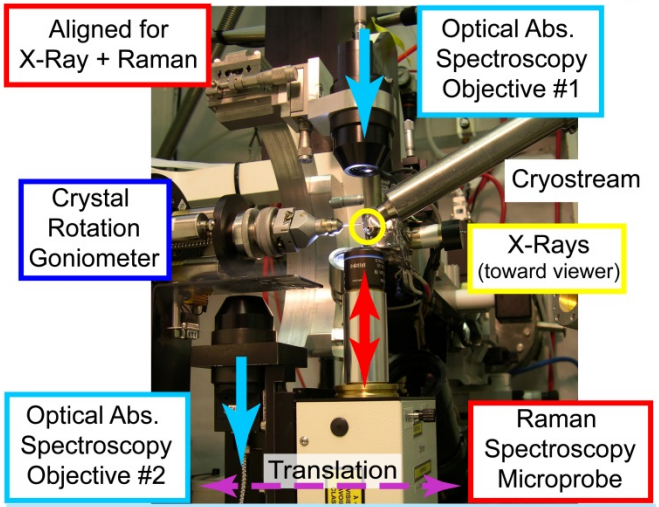
3. Think about how you are analyzing the data – model limitations? constraints?

Don't over-interpret results to get the result you "want."

**Real data may look ugly, but it's still data!**

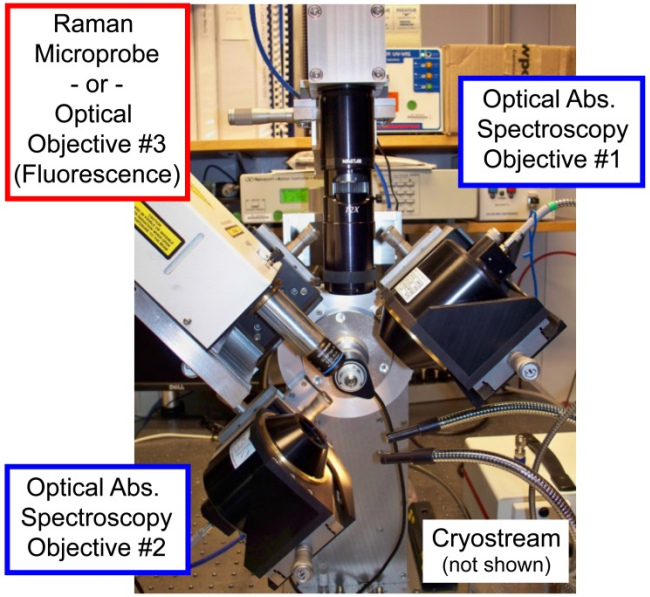
**To close: A brief look at NSLS-II SM3**

NSLS X26-C On-Line Capability w/ X-Ray




X-Ray Crystallography with or without
(Polarized) Electronic Absorption Spec. (200 – 1000 nm)
(Polarized) Resonance Raman Spec. w/ Four Lasers
(785 nm, 633 nm, 532 nm and 473 nm)
Probe Diameters: 90, 36, 25, or 18 μm

NSLS X26-C Off-Line Capability w/o X-Ray




# SM3 Capabilities & Operations

***Initial Scope* (a–d)**.

a) MX correlated w/ optical & Raman spectroscopy

b) Solution-based X-ray absorption spectroscopy (XAS) from macromolecules and model complexes of metalloproteins

c) Polarized electronic absorption spectroscopy

d) Polarized (resonance) Raman spectroscopy

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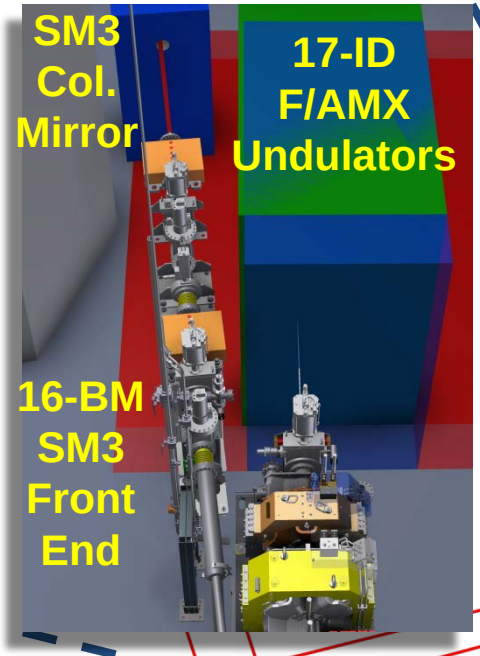
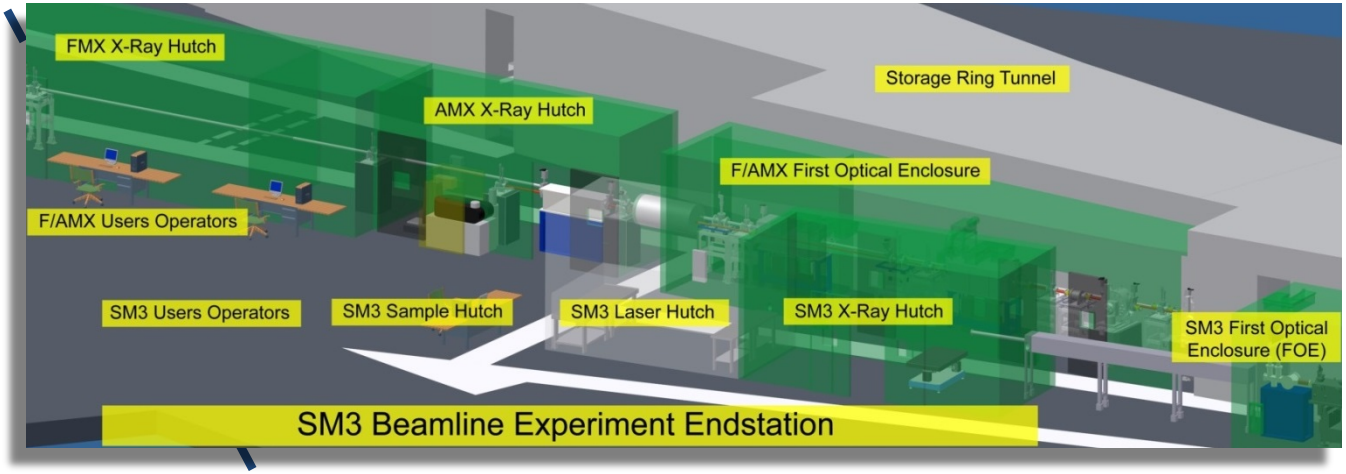
# SM3 Beamline at NSLS-II



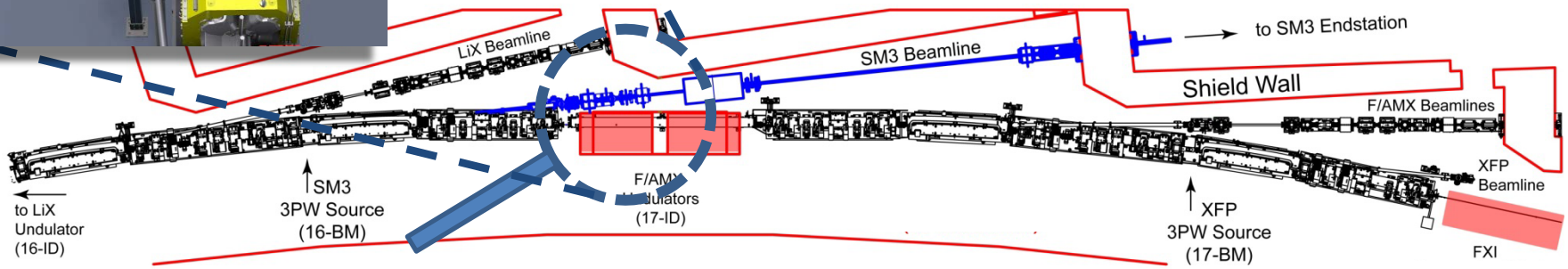



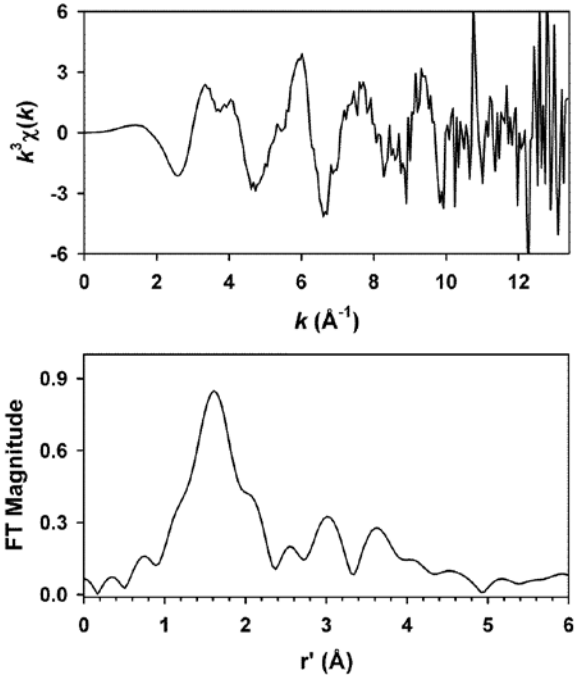

**Table 5: Performance Estimates for Beamline SM3 at NSLS-II**

| | Horiz. acc. | Vert. acc. | Vert. mono | X-ray Flux (photon/s) | | | Beam Size |
|---|---|---|---|---|---|---|---|
| | (mrad) | (mrad) | (μrad) | (5 keV) | (10 keV) | (20 keV) | (h,v μm²) |
| X3B at NSLS [a] | ~3.0 | 0.18 | | | **$9\times10^{11}$** | | 8000 x 1000 |
| X26C at NSLS | | | | | **$1.0\times10^{10}$** | | 200 x 200 |
| SM3 w/ collimating mirror inside shield wall | 3.0 | 0.25 | 2.3 | $5.4\times10^{12}$ | **$4.6\times10^{12}$** | $8.8\times10^{11}$ | 161 x 60 (MX)<br>8000 x 1000 (XAS) |

[a] X3B flux is the maximum achievable with a monochromator acceptance of 2x50 mm (V x H) and no downstream slits. In normal operation the monochromator acceptance is 1x25 mm, and downstream slits further aperture the beam.

# MISCELLANEOUS REFERENCE MATERIAL

# Some things to remember when dealing with biological XAFS data




Avg'd data for 25 scans on a 0.5mM Fe protein (NSLS X3B, 13 element detector)

**Even with our best efforts, sometimes the data we get is not phenomenal**

Taking $\Delta k \sim 1.5 – 10.5$, $\Delta r = 0.8 - 4.0$ (fairly aggressive), and applying the Nyquist criterion for $N_{idp}$: $2\Delta k\Delta r / \pi = \sim 18$

**18 variables is not a lot, especially for an analysis with several shells (and this estimate assumes "ideal" data!)**

Parameter constraints/restraints can be quite important during analysis (more on that from other lectures and during data analysis sessions)

## Model Building

Models are typically not built using ATOMS or similar programs to convert CIF files to FEFF-readable coordinates.

Instead, construct models using structure manipulation tools with geometry data from closely related/feasible structures (Cambridge CCSD or the Protein Data Bank are excellent resources), and bringing the coordinates into your feff.inp file. **This is an iterative process**, and it is very important to have physically reasonable geometries.

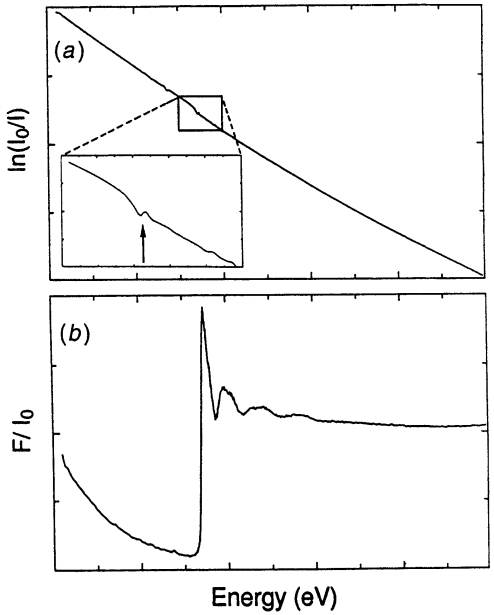
http://tanna.bch.ed.ac.uk/ - Curated database of metal coordination geometries in proteins; useful starting point.

# Some Technical Considerations for Biological XAS

A useful overview (if a little bit out of date) by Ascone, Meyer-Klaucke, and Murphy entitled "Experimental aspects of biological X-ray absorption spectroscopy" can be found in *J. Synchrotron Rad.* 2003, *10*, 16-22.

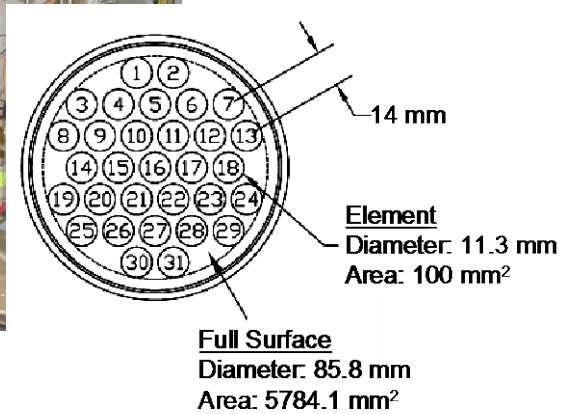
## Sample considerations

- Almost always in aqueous solution. $H_2O$ is a difficult solvent (solvated $e^-$, ice, lots of scattering); many samples have glassing agents.
- Work at low temperatures ($LN_2$ or LHe temperatures) to minimize radiation damage & vibrational disorder
- Proteins usually can't be concentrated very much or obtained in large quantities: low volumes (< 100 μL) at mM or μM concentrations (< 3 mM unless you are very lucky!). Easy to start scraping up against the lower limits of detection for difficult samples.
- Metal contamination by sample cells/beamline components/tape, etc is a consideration (plastics are not necessarily a panacea!)




2mM Ni protein
(top) transmission
(bot) fluorescence

# Some Technical Considerations for Biological XAS




## Detectors

A significant amount of published biological XAS data is obtained using multi-element solid state energy discriminating fluorescence detectors, typically based on Ge (Canberra Industries).

### Advantages:

- Large detector active areas to collect emitted sample fluorescence
- Excellent sensitivity relative to other types of fluorescence detectors (e.g. Lytle detectors)
- Fairly good energy discrimination & excellent for hard X-ray XAS
- In use at numerous beamlines worldwide with various array geometries (Ge13, Ge30, Ge100)

### Disadvantages

- Count-rate limited, with poorer energy resolution at high count rates (big issue at high flux beamlines)
- Very expensive & delicate ($400k - $1.5 million for modern 30-100 element setup); require $LN_2$ cooling.
- Not as good for soft X-ray XAS.

## The Future

Other detector technologies may overcome some of these limitations . . .

see ISS @ NSLS-II

# Reading Material & Resources

**XAFS of Photosystem II OEC:**
Discrepancies between existing crystal structures:*Chem. Eur. J.* **2007**, *13*, 5082 – 5089
Radiation damage of PS II OEC crystals by XAS: *PNAS* **2005**, *102*, 12047–12052
Single-crystal polarized XAFS of PS II OEC: *Science* **2006**, *314*, 821-825
A general review of PS II OEC XAFS: *Inorg. Chem.* **2008**, *47*, 1711-1726.

**RFQ XAS:**
TNF$\alpha$: *PNAS* **2007**, *104*, 4931-4936
Methane Monooxygenase: *Science,* **1997***, 275,* 515-518, *Acc. Chem. Res.* **2007,** *40***,** 475–483
PS II OEC: *Science* **2005**, *310*, 1019-1021 (pump-probe experiment at single X-ray energies)

**Analysis of Mononuclear N/S Zn(II) sites:**
*J. Am. Chem. Soc.* **1998,** *120***,** 8401-8409; *Coord. Chem. Rev.* **2005,** *249,* 161–177
Another approach to identifying Zn sites in proteins, using a database of input geometries and XANES calculations:
*J. Synchrotron Rad.* **2010**, *17*, 41–52 (Applicable to other metals and sample types)

**Histidines & Metal-Metal Interactions in Metalloprotein XAFS:**
One of the earliest treatments of His multiple scattering: *J. Am. Chem. Soc.* **1987**, *109*, 7157-7162
Analysis of dinuclear Ni(II) urease: *Inorg. Chem.* **1994**, *33*, 1589-1593
Review on Detecting Metal-Metal XAFS: *Coord. Chem. Rev.* **1995**, *144*, 245-286
Analysis of dinuclear Mn(II) proteins: *Biochemistry* **1997**, *36*, 9847-9858
Reduced MMO (highlights importance of FEFF input model): *Inorg. Chem.* **2004**, *43*, 4579-4589
Simulation of angular His parameters: *J. Am. Chem. Soc.* **2008**, *130*, 17826–17835
A new dinuclear Fe(III) enzyme: *J. Am. Chem. Soc.* **2011**, *133*, 6938–6941 (good example of an analysis being built up from first shell to outer shell and then to multiple scattering)

Curated database of metal coordination data for proteins, including typical bond lengths: http://tanna.bch.ed.ac.uk/
2005 University of Sydney XAS Course (bio focused): http://www.umich.edu/~jphgroup/XAS_Course/index.htm