How biological catalysts make and break bonds - What X-ray spectroscopy can teach us…

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Max-Planck-Institut für Chemische Energiekonversion
& Department of Chemistry and Chemical Biology,
Cornell University

EUCALL Workshop: Biology at Advanced Laser Light Sources
30 November - 01 December 2017

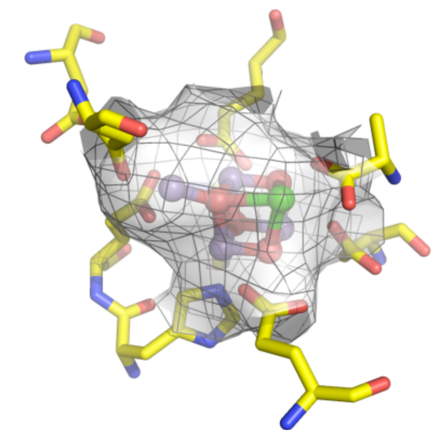
Venue: European X-ray Free-Electron Laser Facility
Holzkoppel 4, 22869 Schenefeld, Germany

Motivation: From Biological to Chemical Catalysis

Taking inspiration from Nature....

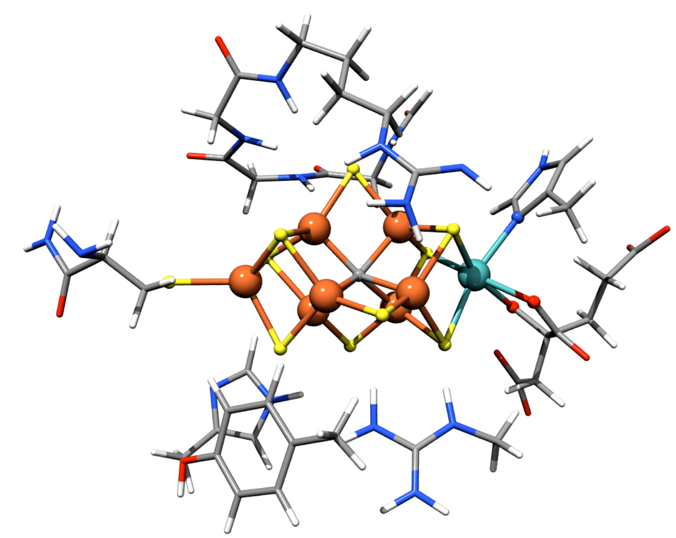
Photosystem II [Mn]

$$2\ H_2O \longrightarrow O_2 + 4H^+ + 4e^-$$



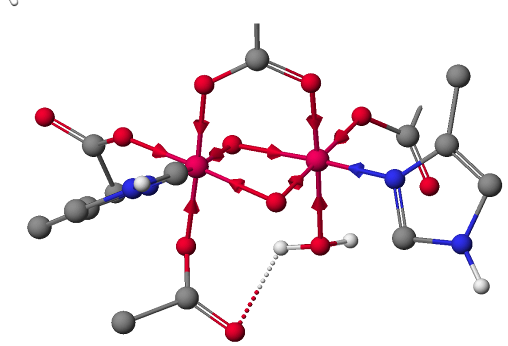
Nitrogenase [Fe,Mo]

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3$$



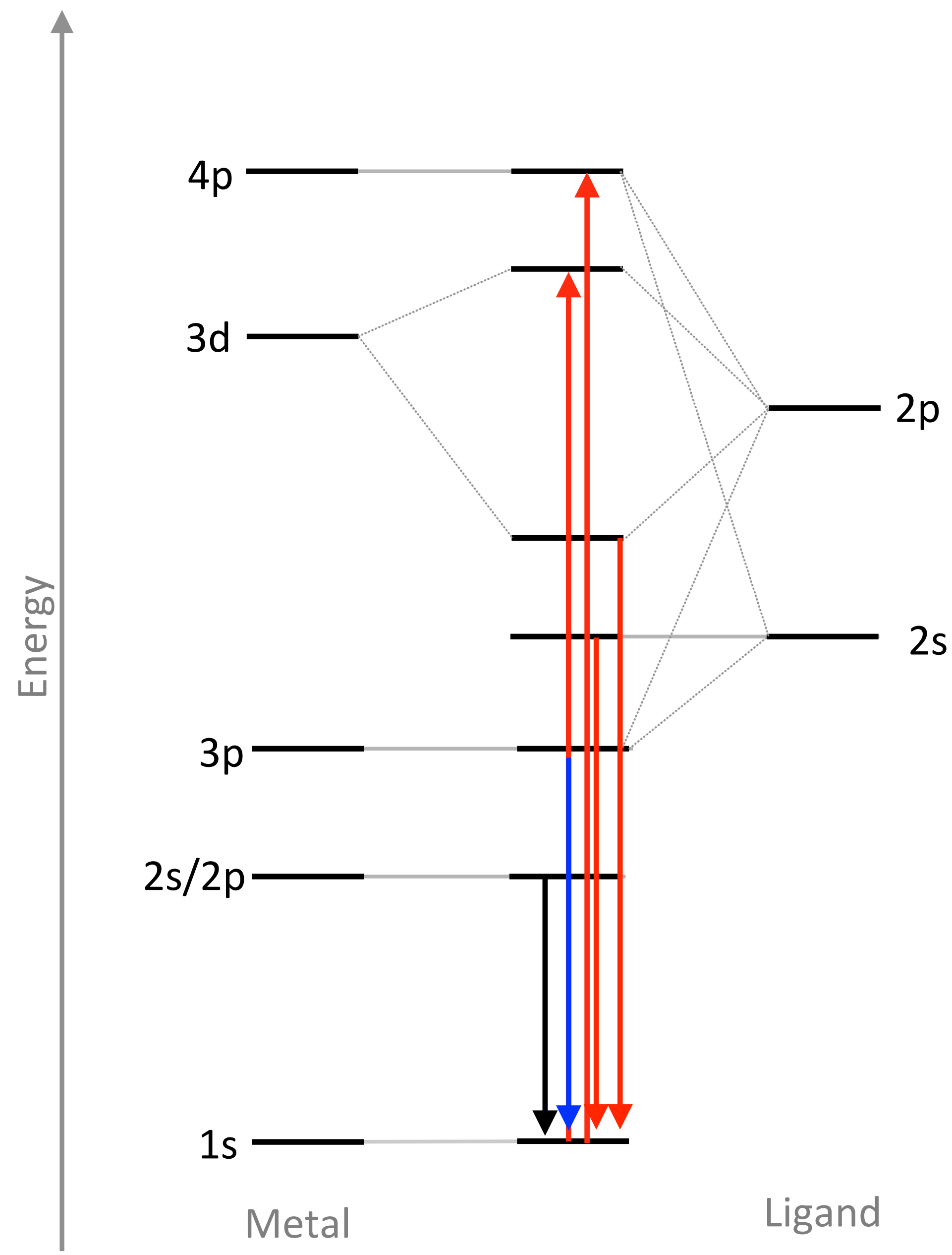
Methane Monoxygenase [Fe,Cu]

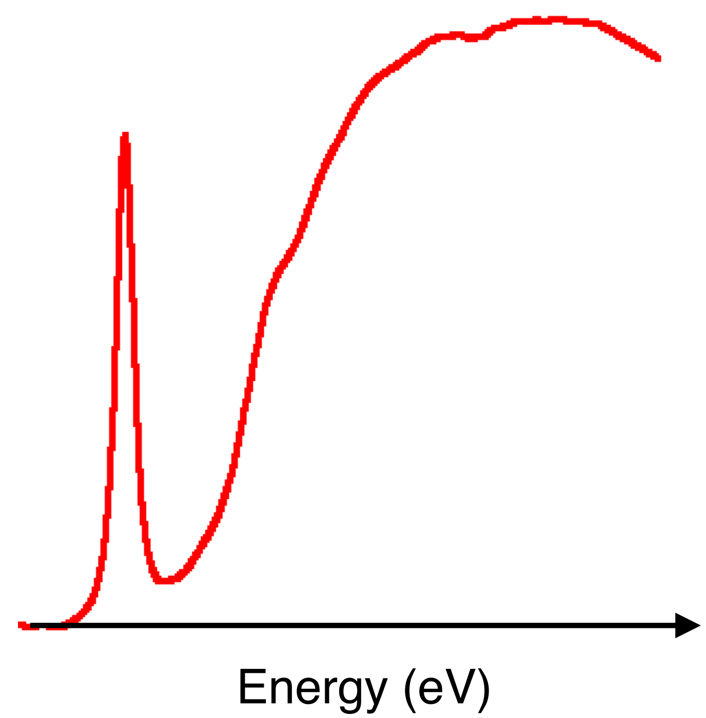
$$2CH_4 + O_2 \longrightarrow 2H_3COH$$



Goal: To obtain fundamental mechanistic insights that can be more broadly applied.

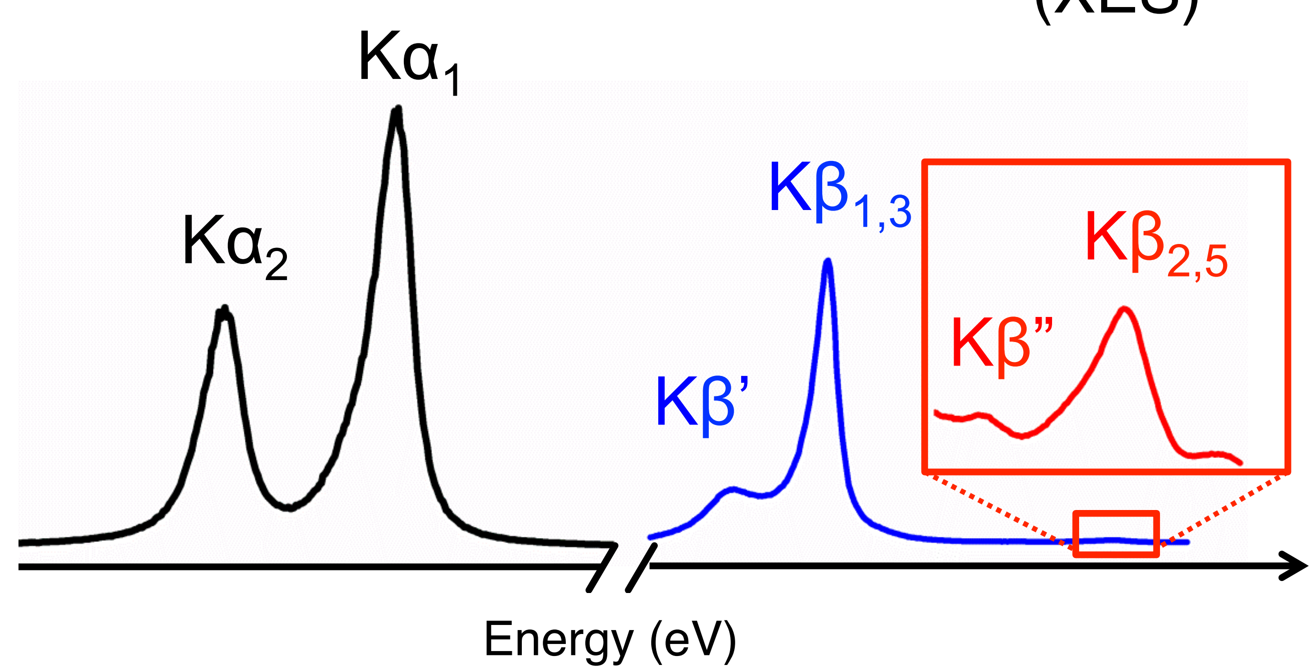
X-ray Spectroscopy



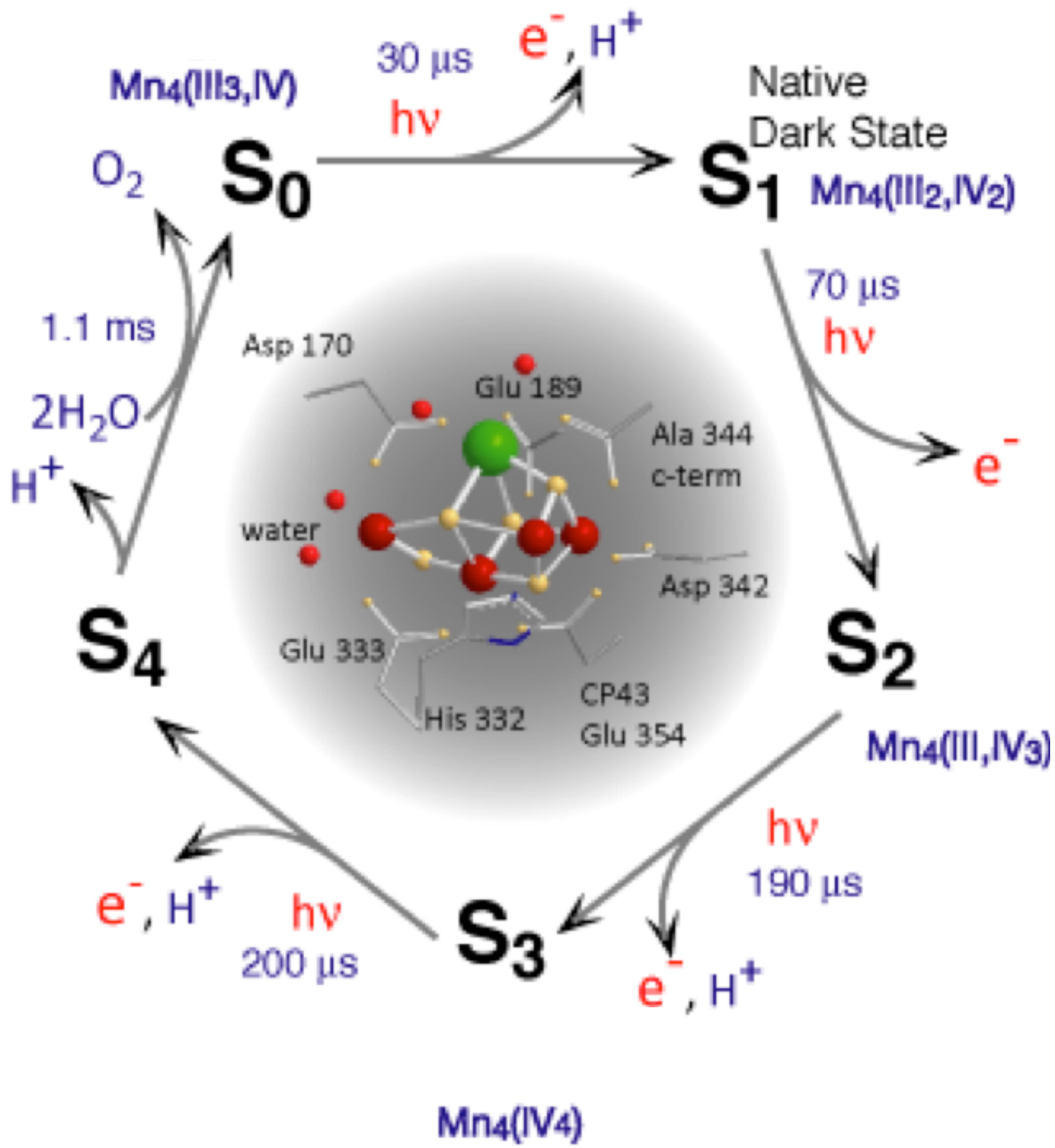


X-ray Absorption Spectroscopy (XAS)

X-ray Emission Spectroscopy (XES)



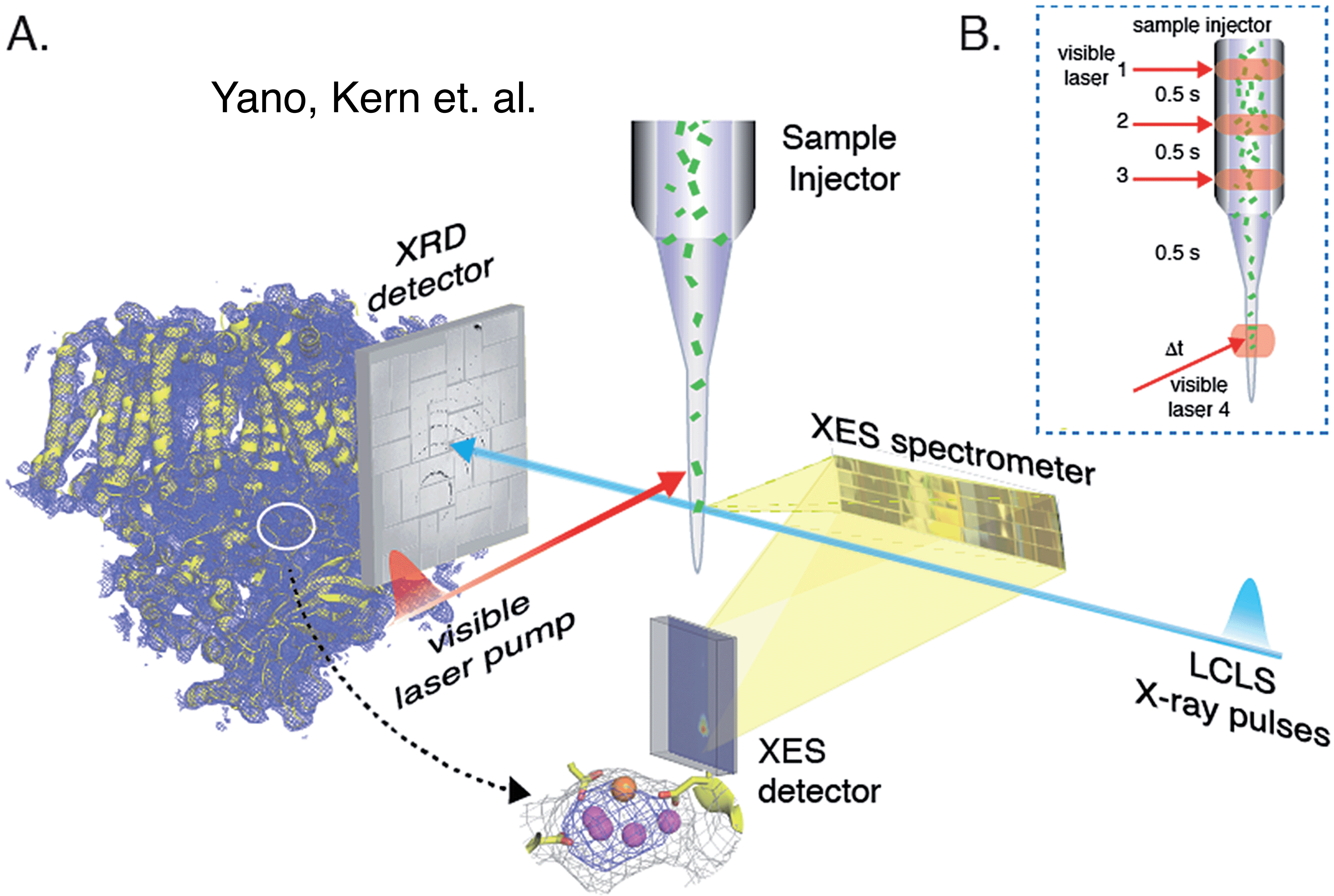
PSII: Mechanistic Understanding from Spectroscopy



Adapted from:
Yano et. al.

- The OEC of PSII by far the most studied biological catalyst at XFELs (and elsewhere)
- Critical bond making and breaking processes on the order of micro to milliseconds
- Importance of XFEL due to damage rates
- S-states can be generated by laser flashing.

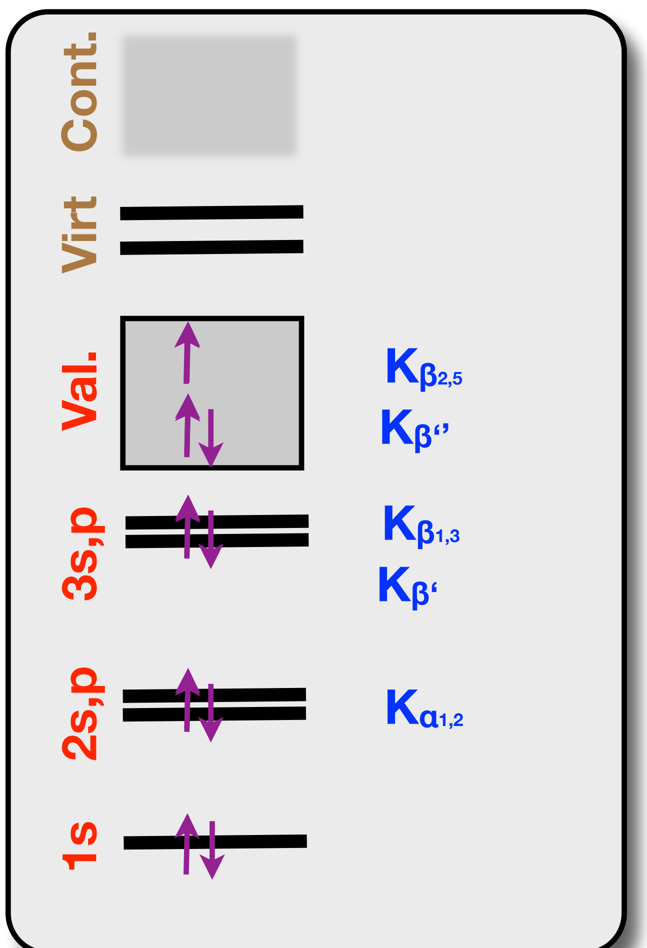
PSII: Combined XRD and XES

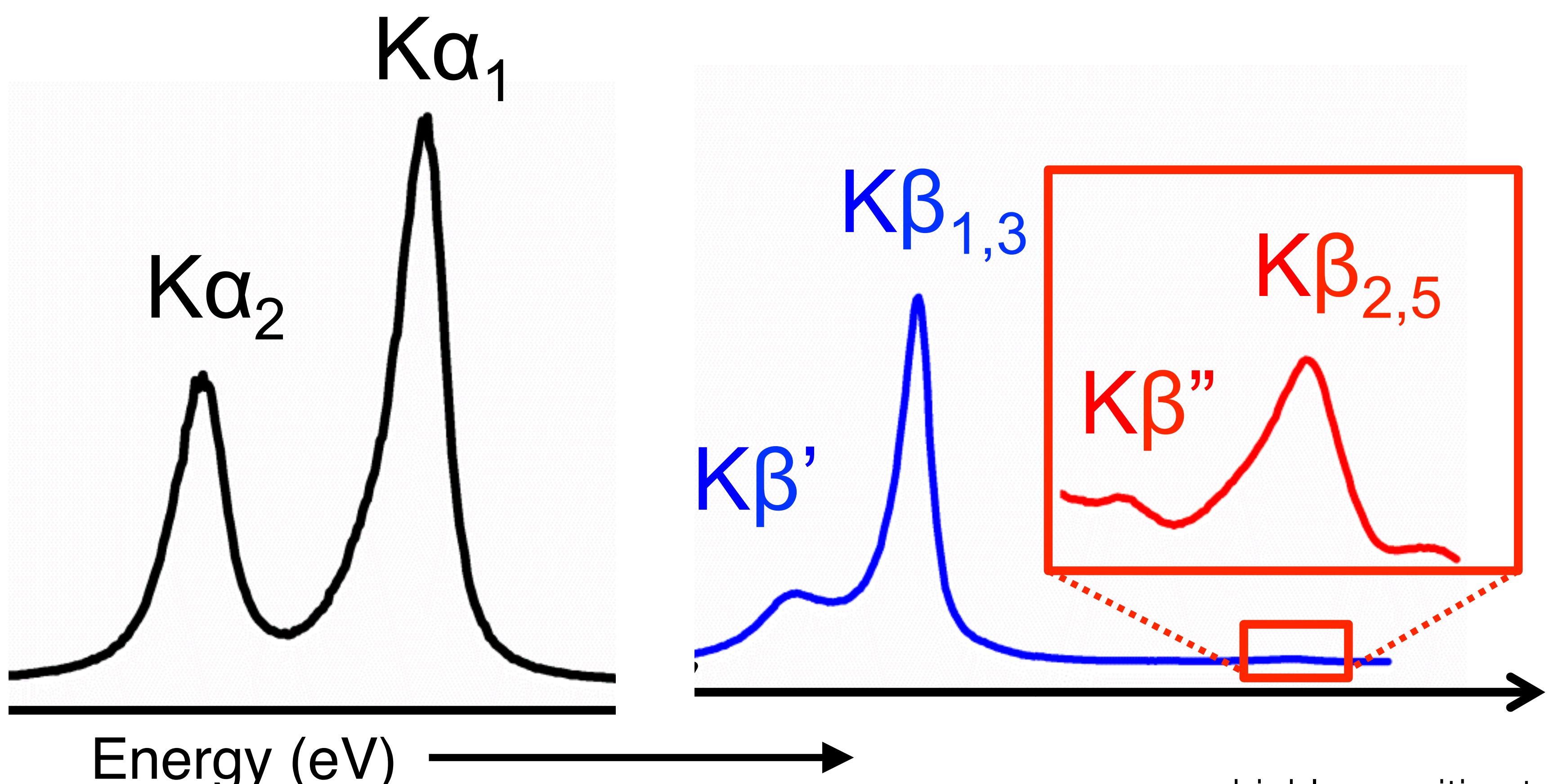


Applications to other biological catalysts??
But first, more on XES….

X-Ray Emission Spectra

N. Lee, T. Petrenko, U. Bergmann, F. Neese, S. DeBeer, J. Am. Chem. Soc., 2010, 132, 9715-9727.



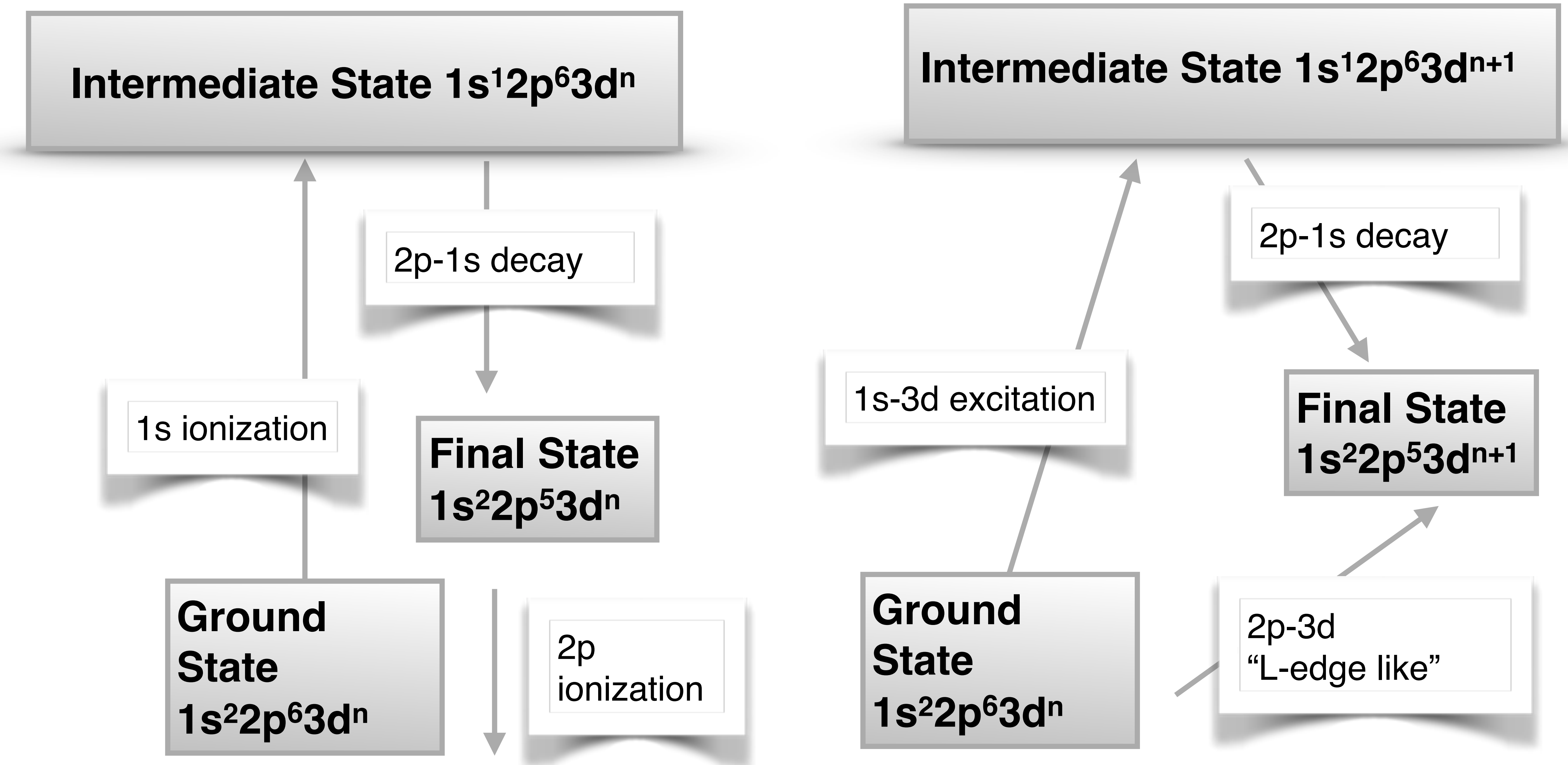


- dominated by 2p spin orbit
- K-alpha detected XAS (1s2p RIXS) enables higher resolution

- dominated by 3p-3d exchange
- spin state marker
- enables oxidation state and spin dependent XAS

- highly sensitive to ligand environment
- measure of ligand identity, ionization potential, and protonation state
- enables ligand selective XAS

Non-resonant Kα XES *vs.* Resonant Kα XES

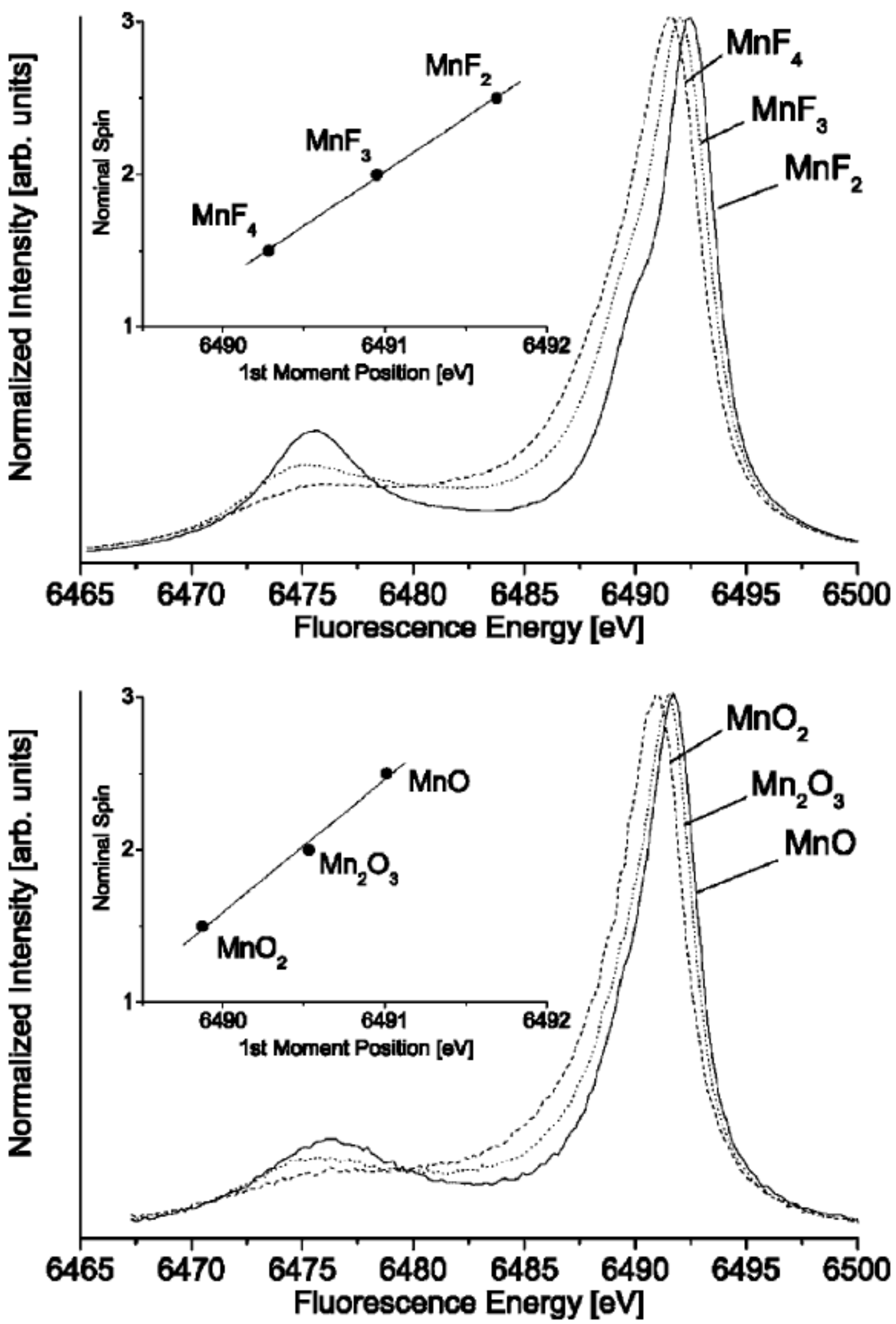


- incident beam at fixed energy above absorption edge
- core excitations into the continuum
- spectra effectively independent of incident energy

- incident beam tuned to excite into the absorption edge of interest
- excitations into bound states
- spectra highly energy dependent

K-Beta Mainlines as a Probe of Spin/Oxidation State

Mn(IV) d^3, Mn(III) d^4, Mn(II) d^5

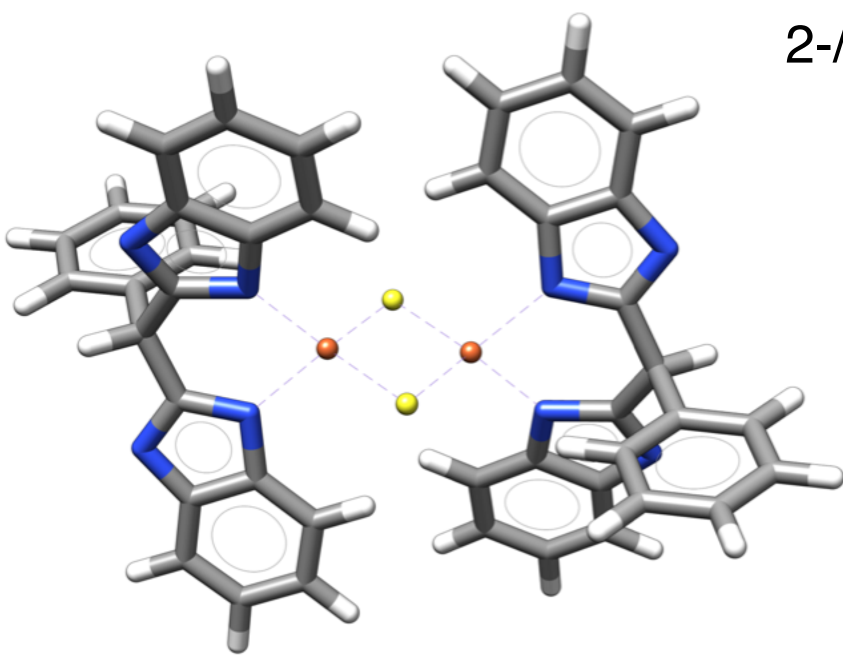


- Kβ lines dominated by 3p-3d exchange interaction
- Kβ$_{1,3}$ and Kβ' move closer together with decreasing spin state (i.e as 3p-3d exchange interaction decreases)
- Kβ lines reflect number of unpaired d electrons
- Can be applied to any metal.
- Has been used to "establish" damage free XRD data collection….

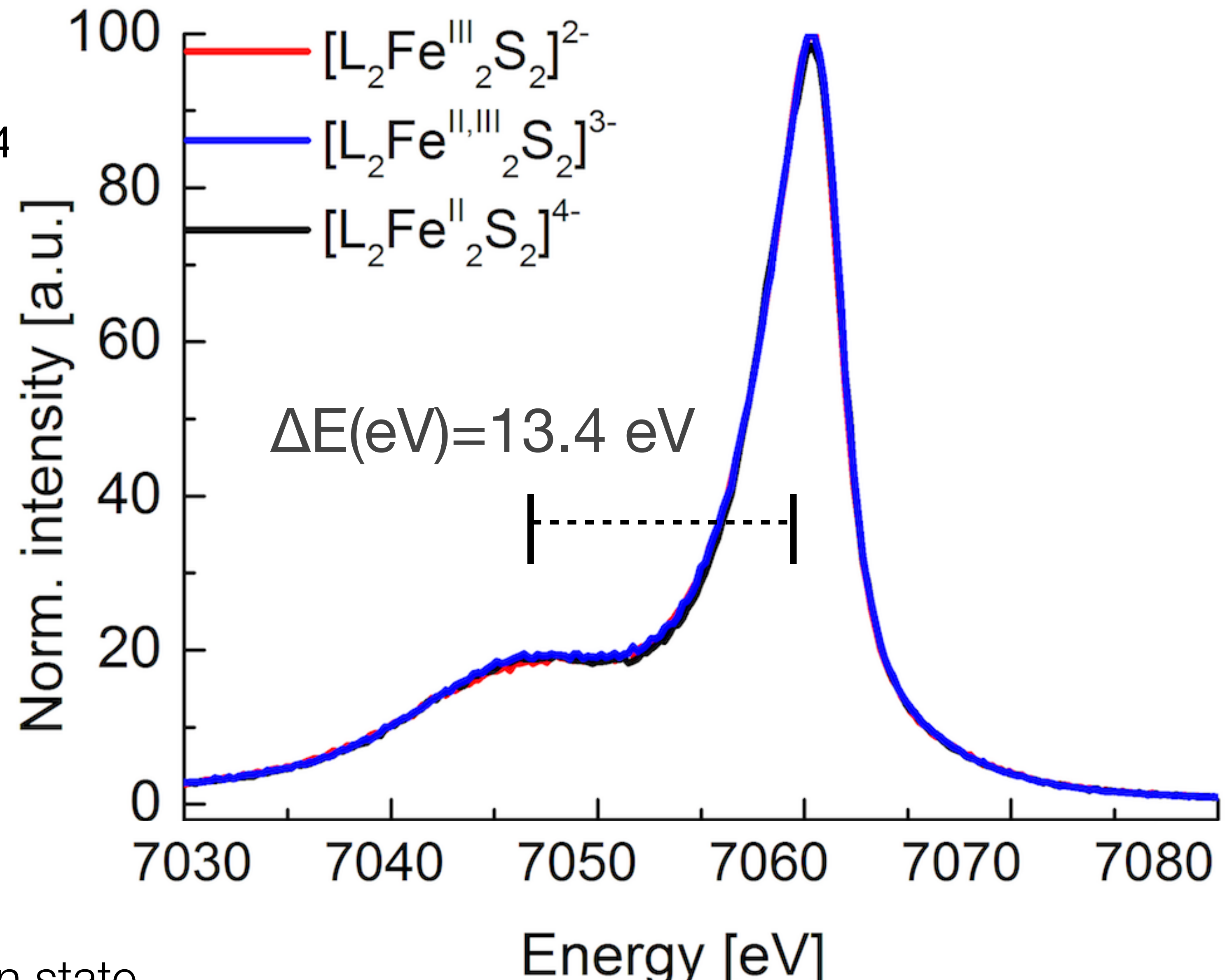
Glatzel, P.; Bergmann, U. Coord. Chem. Rev **2005**, 249, 65.

Lessons from Model Studies

In Collaboration with
F. Meyer (Göttingen)



2-/3-/4



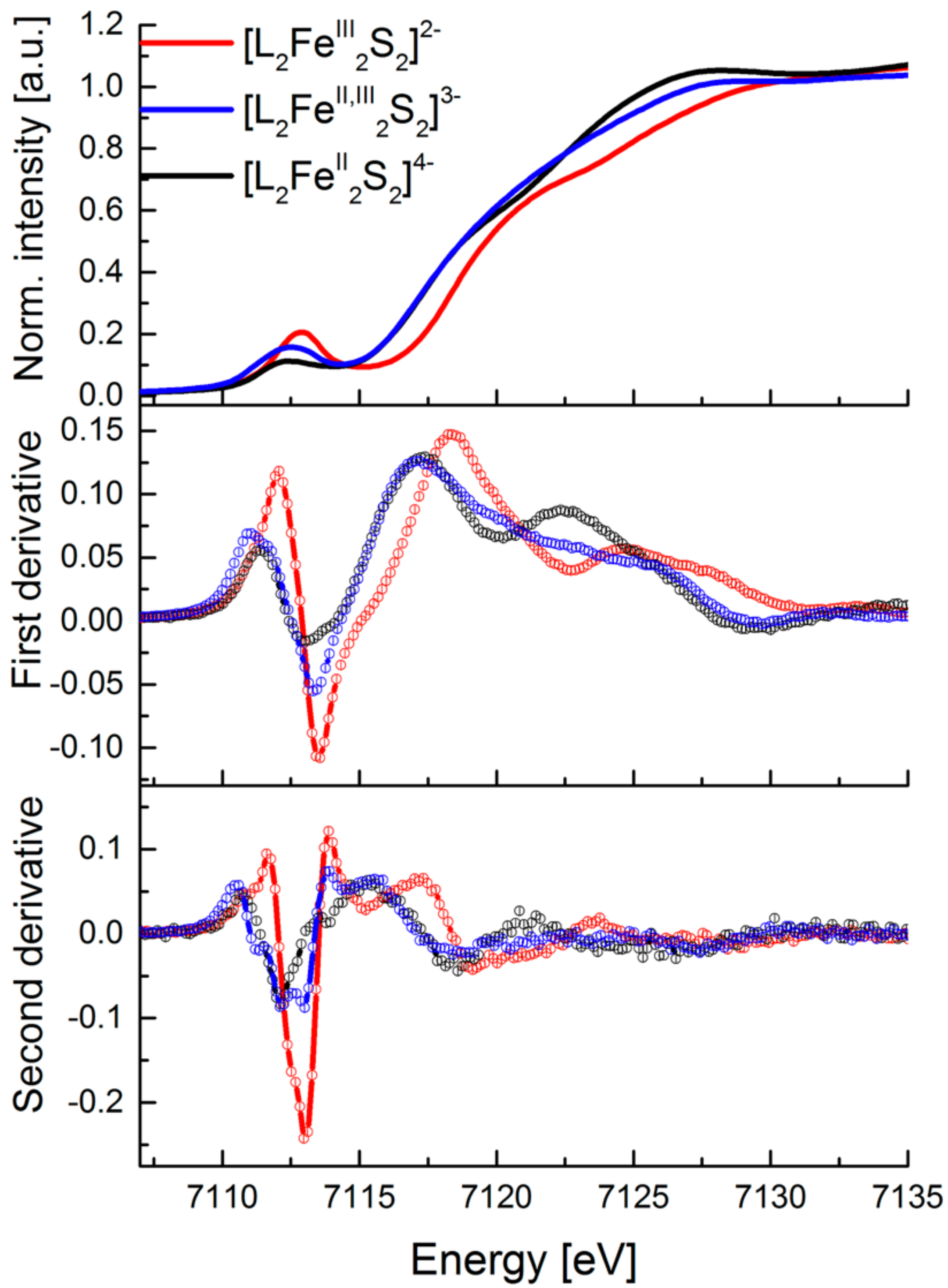
A. Albers *et al., Angew. Chem. Int. Edit.*, **2011**, *9191* (39)

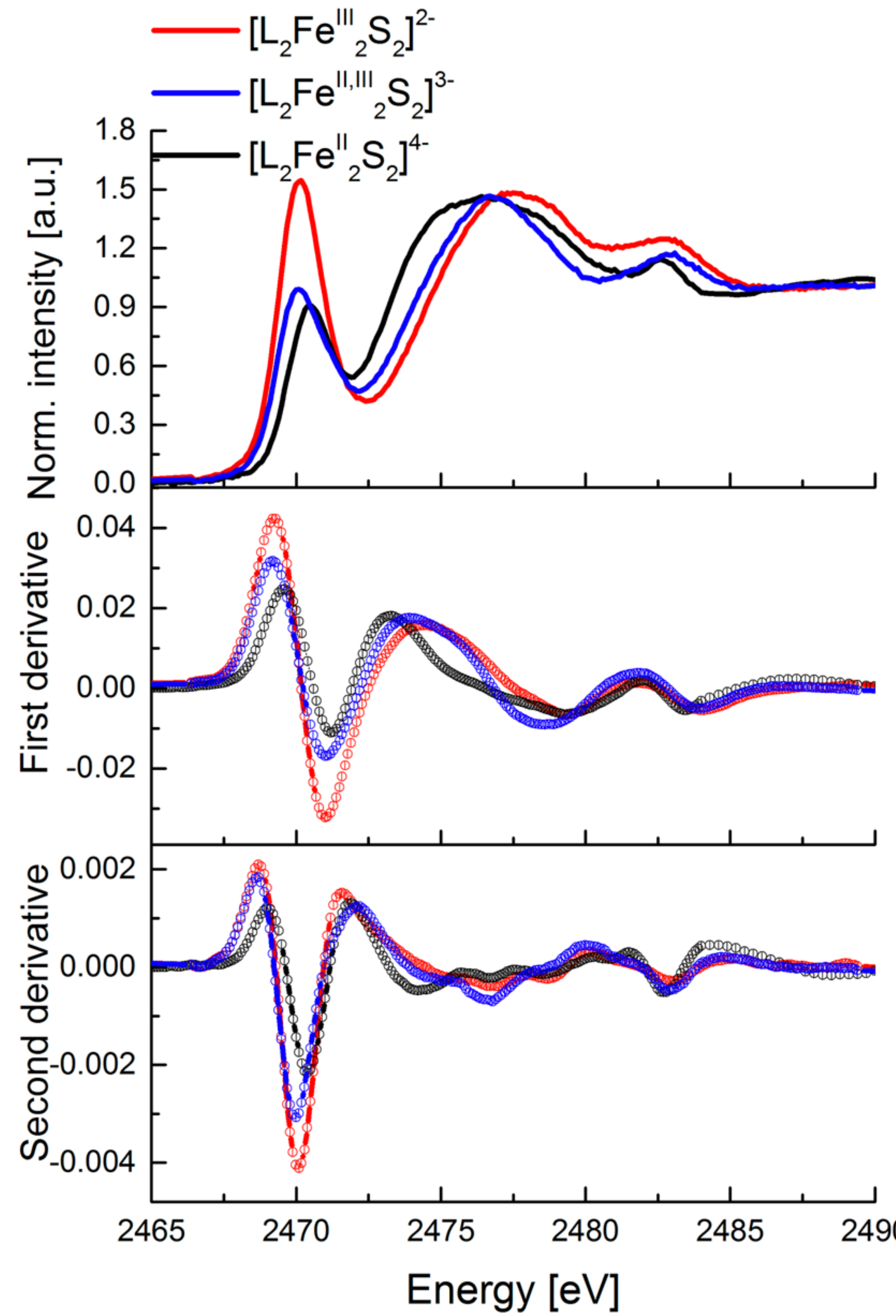
A. Albers *et al., J. Am. Chem. Soc.*, 135, **2013**, *1704.*

J. Kowalska *et al., Inorg. Chem.*, 55, **2016,** 4485.

- Contributions due to change in oxidation state and covalency completely cancel!
- ***A cautionary note on assigning oxidation states from KB mainlines!***

Fe K- and S K-edges clearly different…

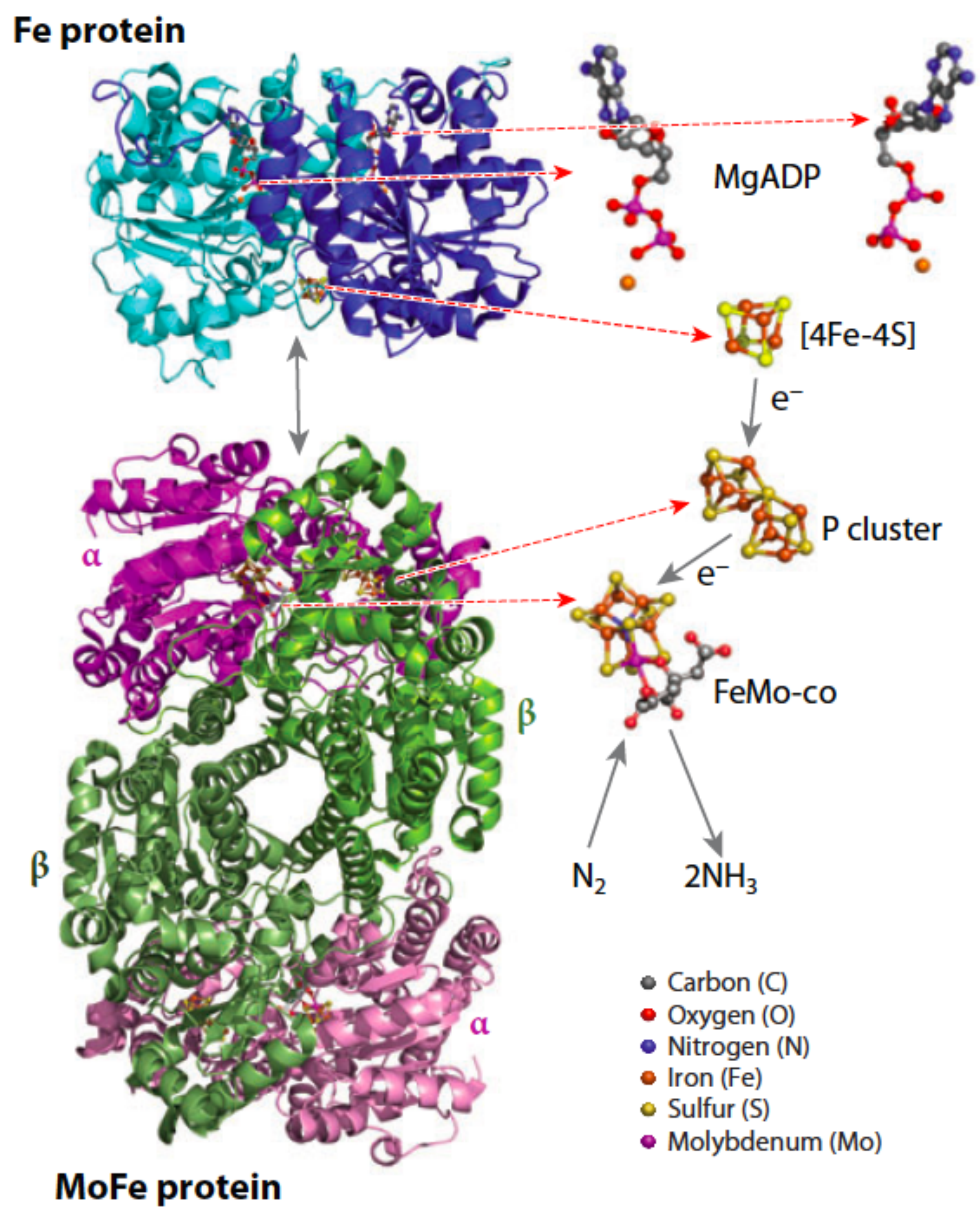




Nature's Machinery for N_2 Activation

Mo-dependent nitrogenases catalyze....
$N_2 + 8e^- + 8H^+ + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i$

Multicomponent protein (Fe protein (64 kDa) and MoFe protein (250 kDa))



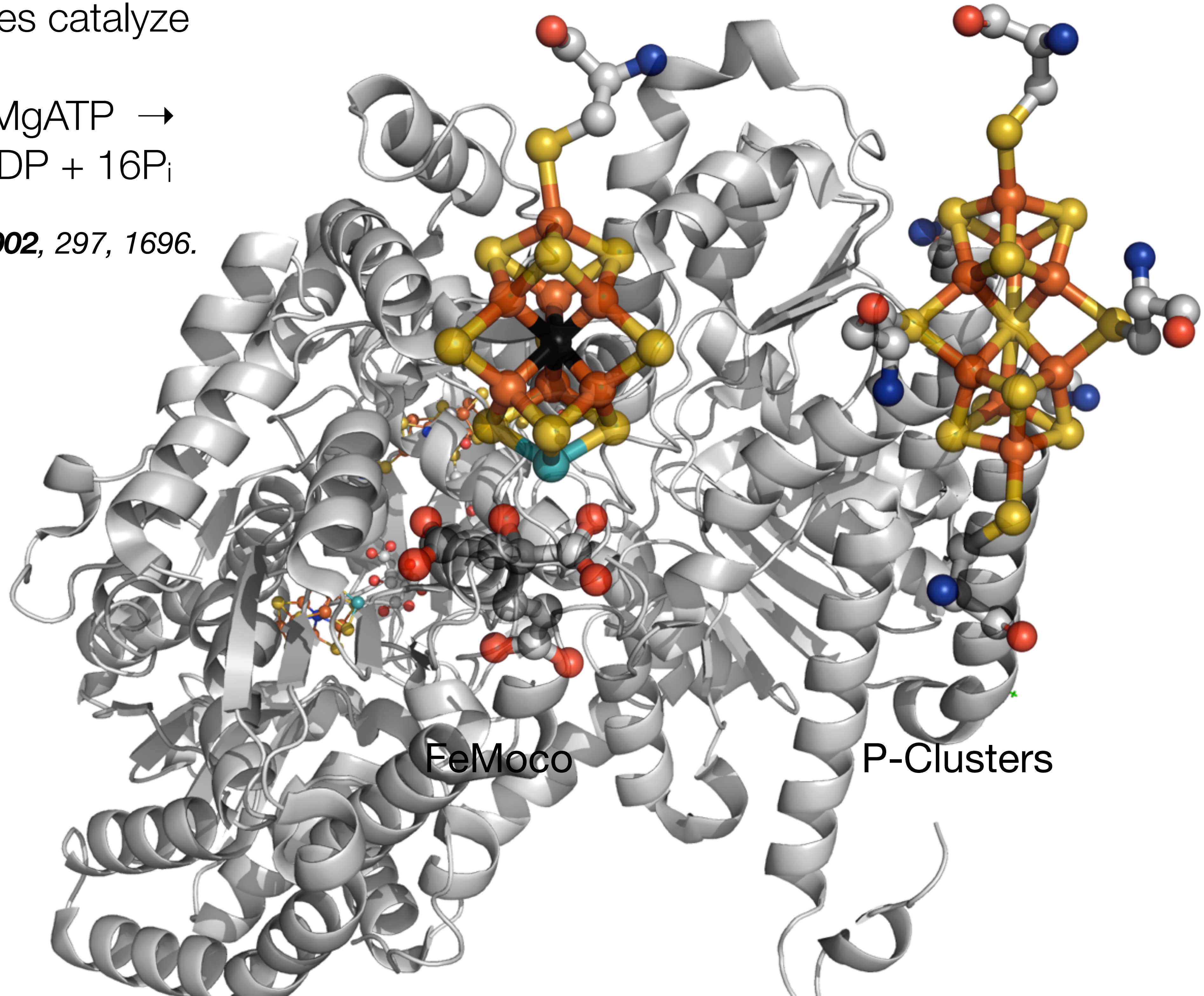
Seefeldt, Hoffman, Dean, Ann. Rev. Biochem. 2009

MoFe Nitrogenase

- Nitrogenase enzymes catalyze the reaction:

$N_2 + 8e^- + 8H^+ + 16MgATP \rightarrow$
$2NH_3 + H_2 + 16MgADP + 16P_i$

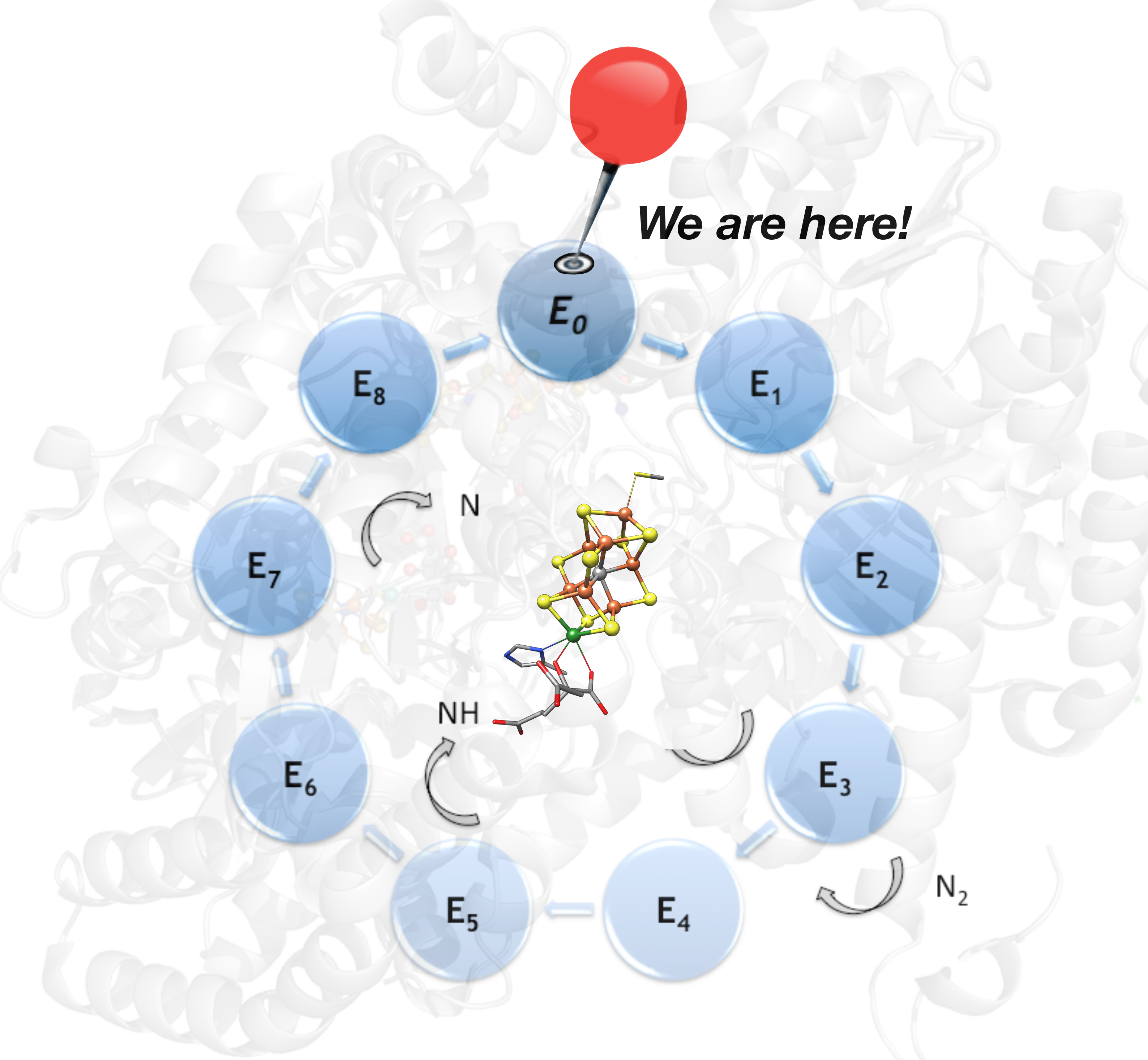
*Einsle et al, Science, **2002**, 297, 1696.*



Biological N_2 Reduction: Open Questions…

▸Molybdenum Nitrogenase enzymes catalyze the reaction:

$N_2 + 8e^- + 8H^+ + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i$



An understanding of the molecular level mechanism requires a detailed understanding of the electronic structure of the cofactors at each stage in the cycle.

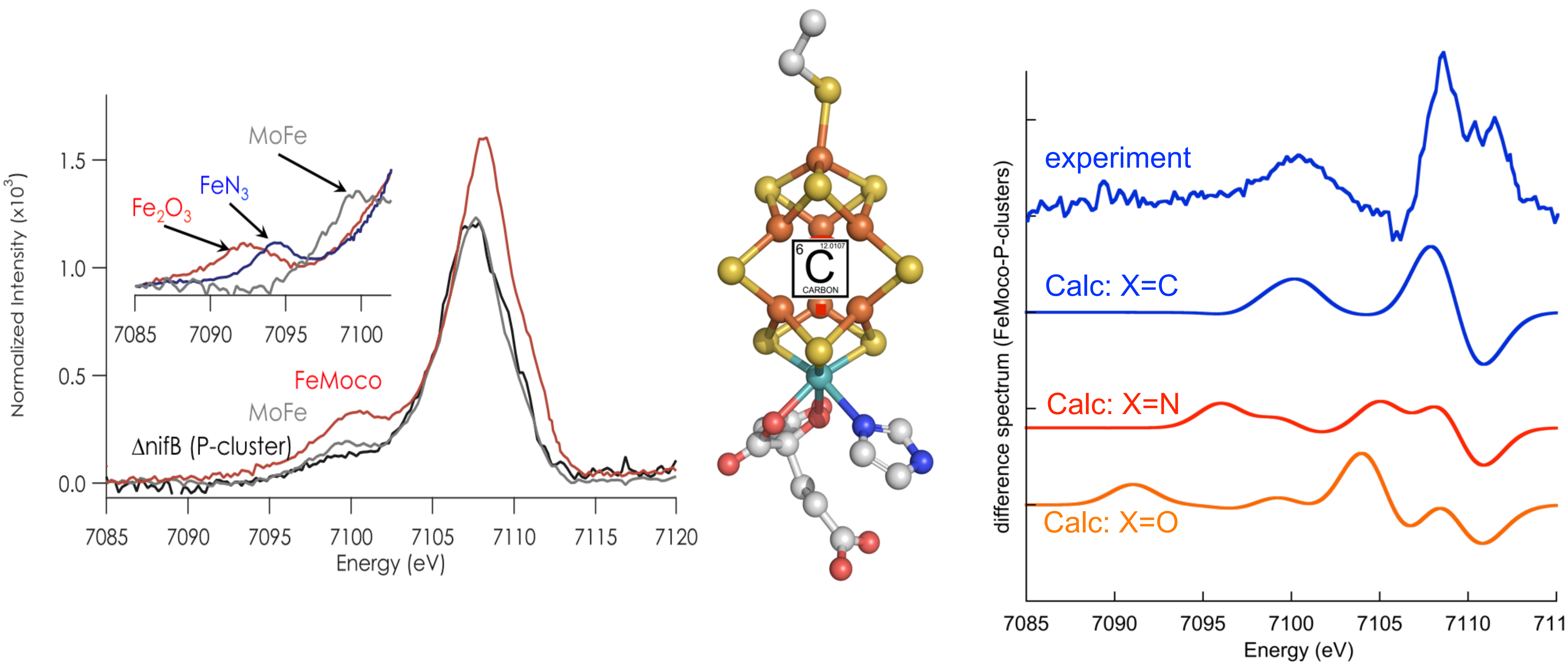
Requires knowledge of:

- Atomic composition
- Charge distribution
- Oxidation state distribution
- Magnetic coupling

The role of X-ray Spectroscopy…

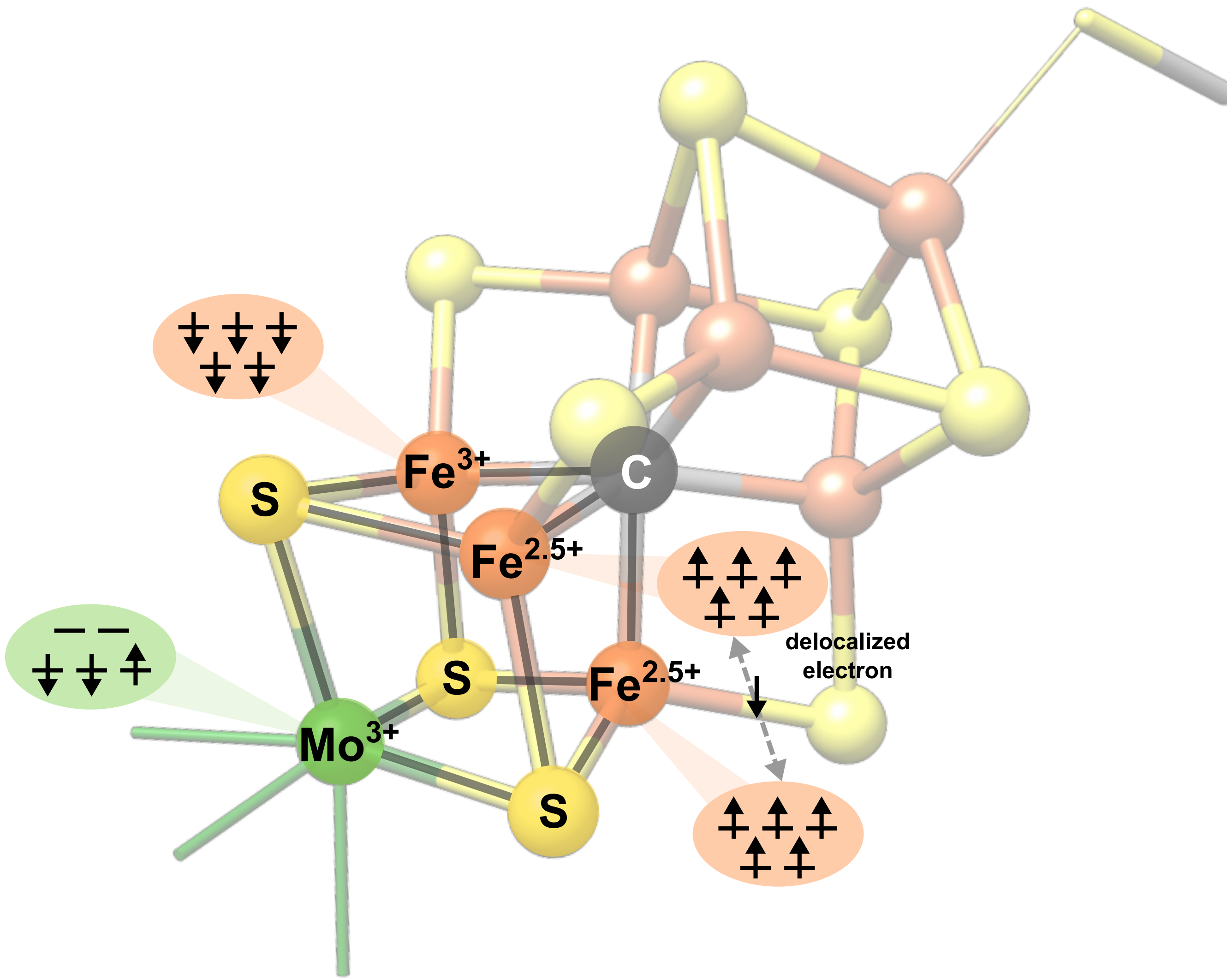
Identifying the Central Atom in FeMoco

Valence-to-Core: X-Ray Emission ⟷ **Quantum Chemistry**



Lancaster, K.M.; Roemelt, M.; Ettenhuber, P.; Hu, Y.; Ribbe, M.W.; Neese, F.; Bergmann, U.; DeBeer, S. *Science* **2011**, 334, 974-977.

Current Picture of the E_0 state of FeMoco

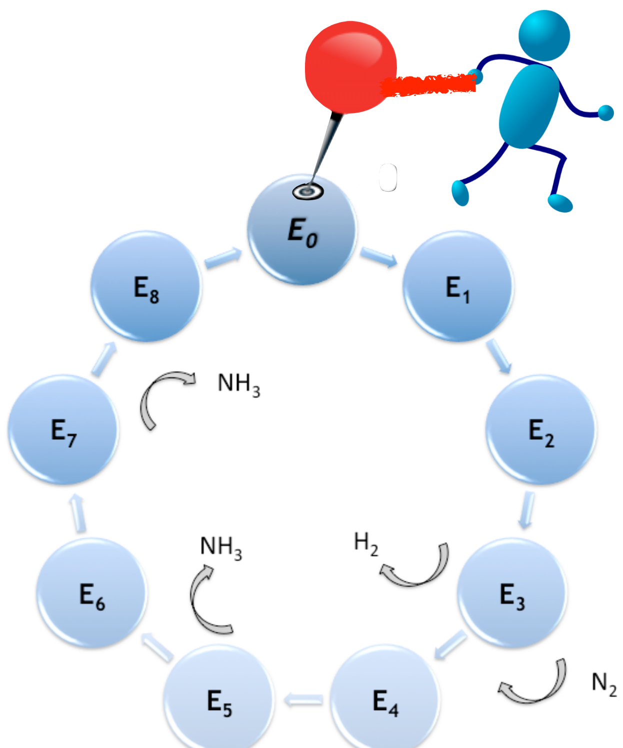


- ✓Based on:
- ✓VtC XES
- ✓Mo HERFD XAS
- ✓Fe HERFD XAS
- ✓Mössbauer
- ✓Fe/Mo L-edge XAS + XMCD
- ✓DFT correlated to spectroscopy

✓FeMoco: $[MoFe_7S_9C]^{1-}$: 4Fe(III)3Fe(II)Mo(III) g.s.

Lancaster, K.M.; Roemelt, M.; Ettenhuber, P.; Hu, Y.; Ribbe, M.W.; Neese, F.; Bergmann, U.; DeBeer, S. *Science* **2011**, 334, 974-977.
Lancaster, K. M., Hu, Y., Bergmann, U., Ribbe, M. W., and DeBeer, S. *JACS* **2013** *135*, 610-612.
Bjornsson, R., Lima, F. A., Spatzal, T., Weyhermueller, T., Glatzel, P., Bill, E., Einsle, O., Neese, F., and DeBeer, S. Chemical Science **2014** 5, 3096-3103.
Rees, J. A., Bjornsson, R., Schlesier, J., Sippel, D., Einsle, O., and DeBeer, S. *Angewandte Chemie* **2015**, *54,* 13249-13252
Bjornsson, R.; Neese, F.; Schrock, R. R.; Einsle, O.; DeBeer, S. *J. Biol. Inorg. Chem.* **2015**, *20*, 447-460.
Bjornsson, R., Neese, F., and DeBeer, S., *Inorganic Chemistry* **2017**, *56*, 1470.
Rees, J.A.; Bjornsson, R.; Kowalska, J.K.; Lima, F. A.; Schlesier, J.; Sippel , D.;Weyhermüller, T. Einsle, O.; Kovacs, J.A.;DeBeer, S. *Dalton Transactions*, **2017**, *46*, 2445.

Moving Beyond E_0: The Challenges in Trapping Intermediates

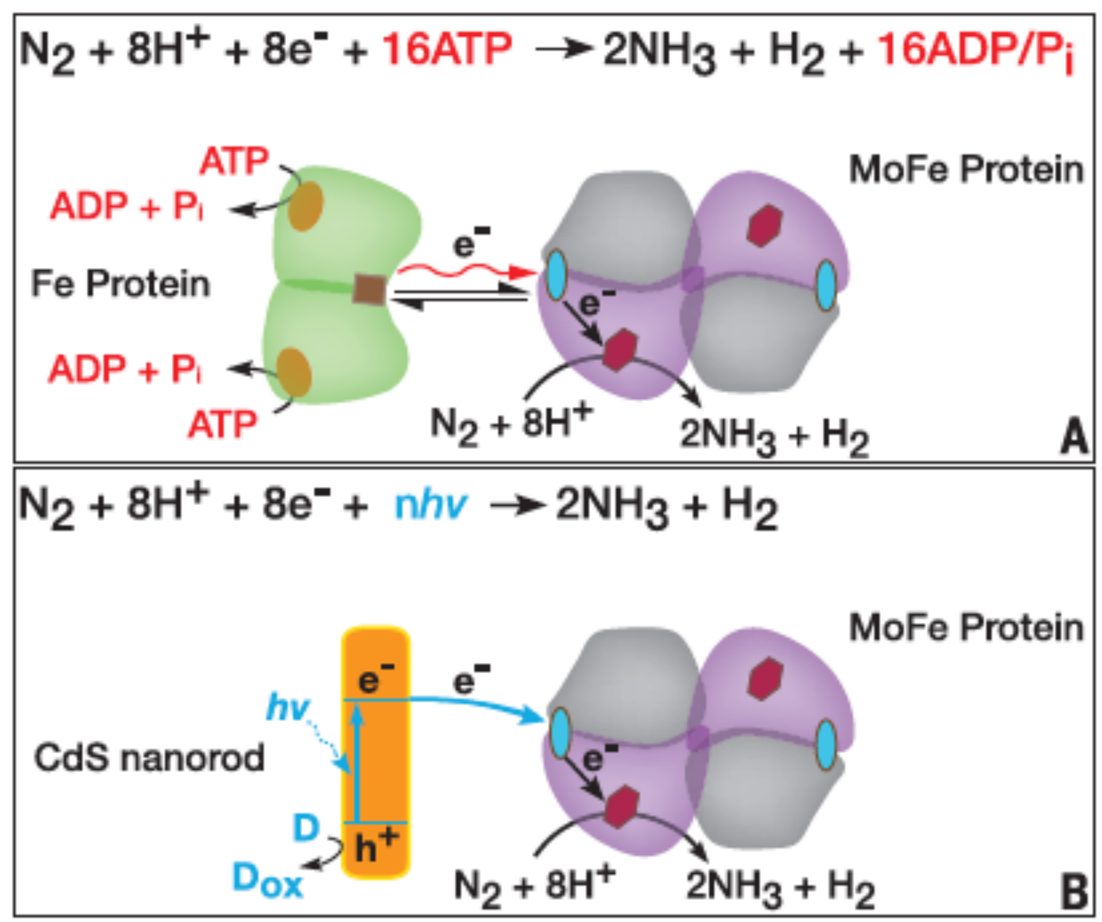


- Depends strongly on electron e- flux conditions (ratio of FeP to MoFe)
- Until very recently MoFe protein had been thought to requires native reductase
- CdS produces uncontrolled cascade of electrons….

Light-driven dinitrogen reduction catalyzed by a CdS:nitrogenase MoFe protein biohybrid

Katherine A. Brown,[1] Derek F. Harris,[2] Molly B. Wilker,[3*] Andrew Rasmussen,[2] Nimesh Khadka,[2] Hayden Hamby,[3] Stephen Keable,[4] Gordana Dukovic,[3] John W. Peters,[4] Lance C. Seefeldt,[2] Paul W. King[1]†

Science, 2016



J|A|C|S COMMUNICATIONS

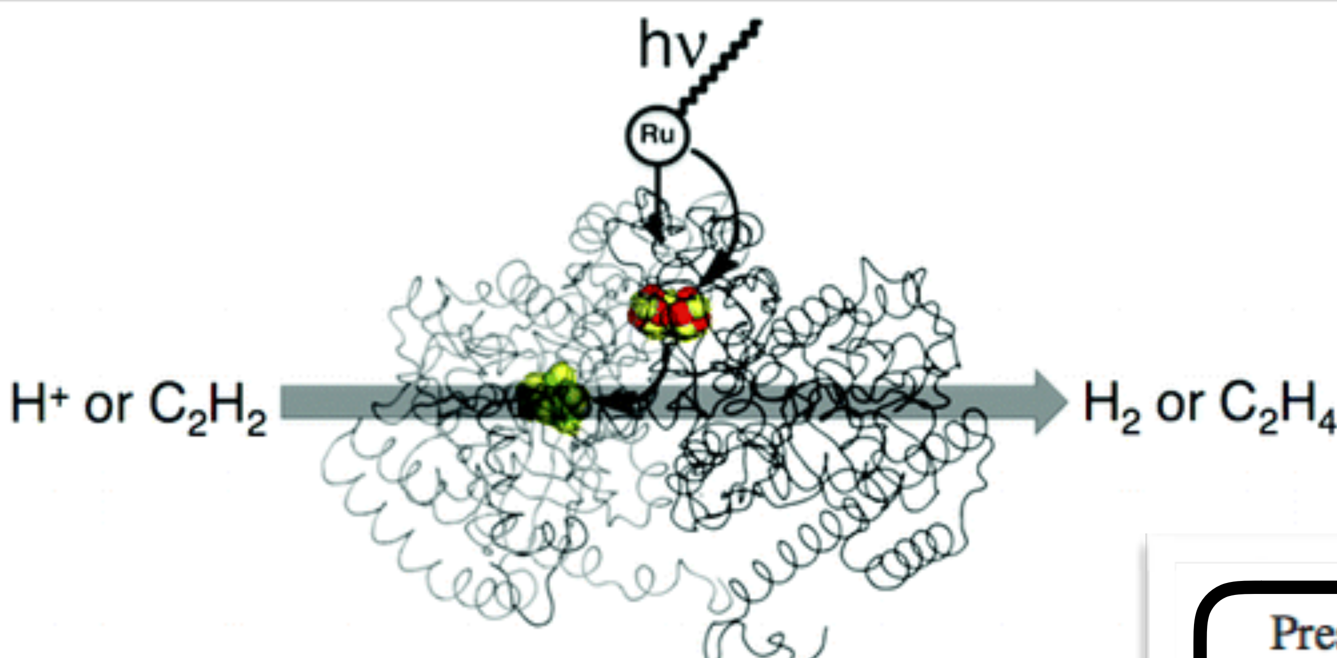
Published on Web 09/15/2010

ATP- and Iron−Protein-Independent Activation of Nitrogenase Catalysis by Light

Lauren E. Roth, Joey C. Nguyen, and F. Akif Tezcan*

University of California, San Diego, Department of Chemistry and Biochemistry, La Jolla, California 92093-0356

Received August 10, 2010; E-mail: tezcan@ucsd.edu



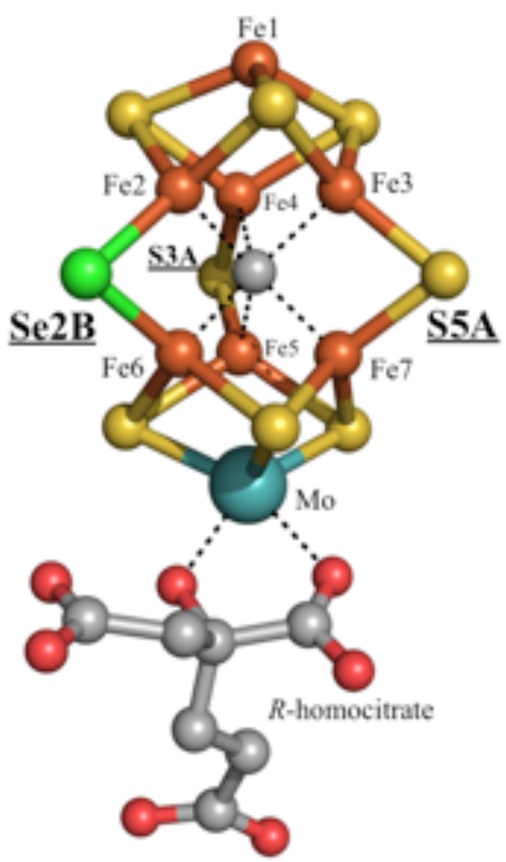
- Ruthenium tagged proteins could provide access to controlled "flashing" to generate intermediates. However….

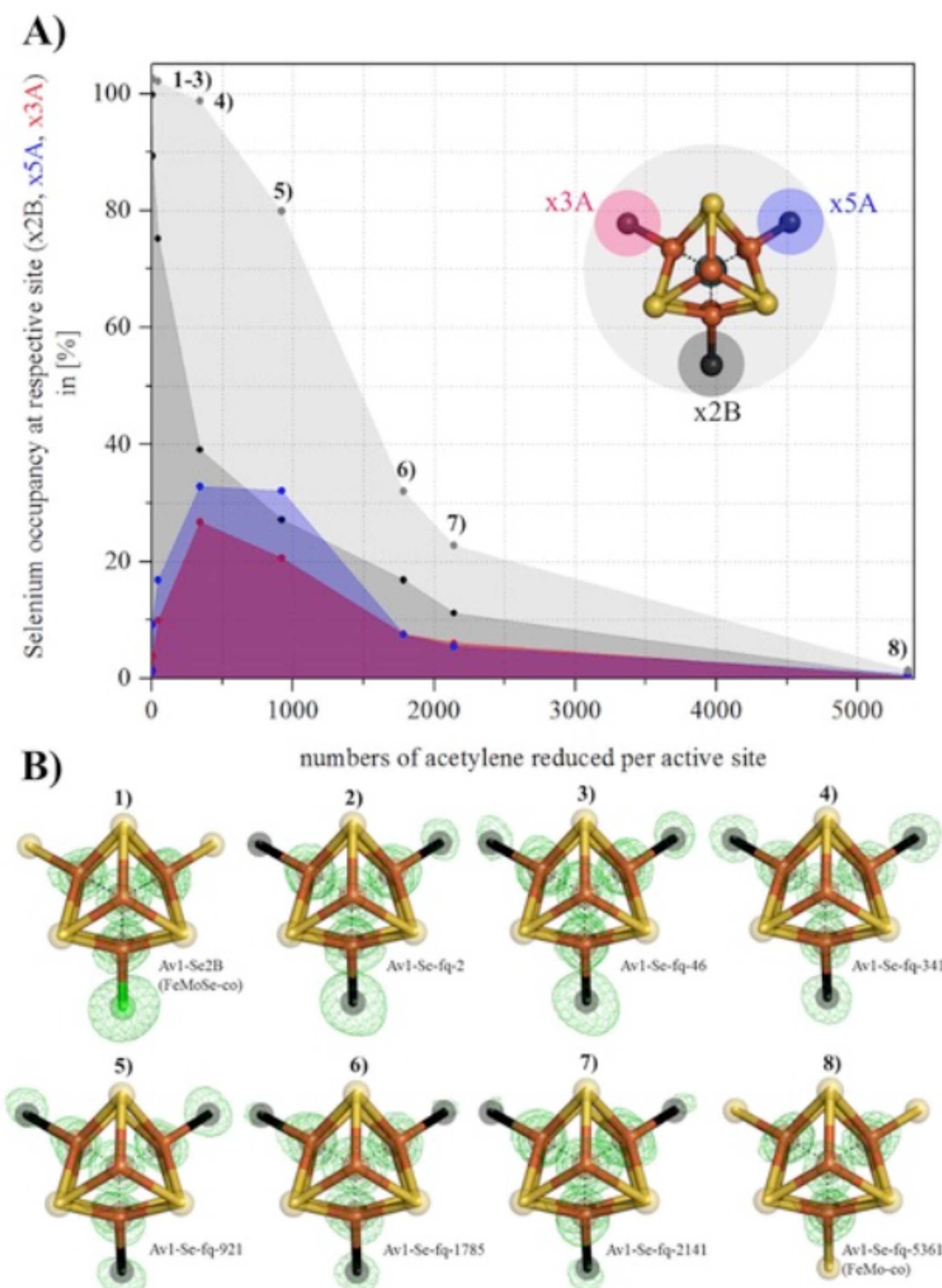
Presently, the quantum yield (ϕ = catalytically useful electrons/photons absorbed by $Ru^{II}BP$) of our light-driven system is <1%, which likely is the reason why Ru−C158 fails to produce significant levels of ammonia from N_2. We are currently pursuing the optimization of our system for increased yields of electron injection to potentially enable this 6-e^- process. Ultimately, however, our experiments show that ATP hydrolysis and FeP are not absolutely essential for substrate reduction by nitrogenase as commonly assumed.

Se HERFD to Interrogate Intermediates

T. Spatzal *et al., ELife*, **2015**, 4:e11620

In collaboration with Renee Arias, Doug Rees (Caltech)

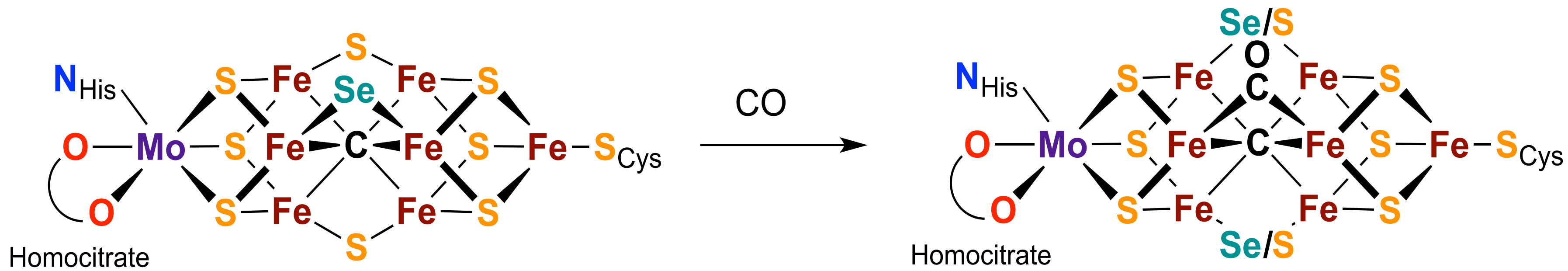


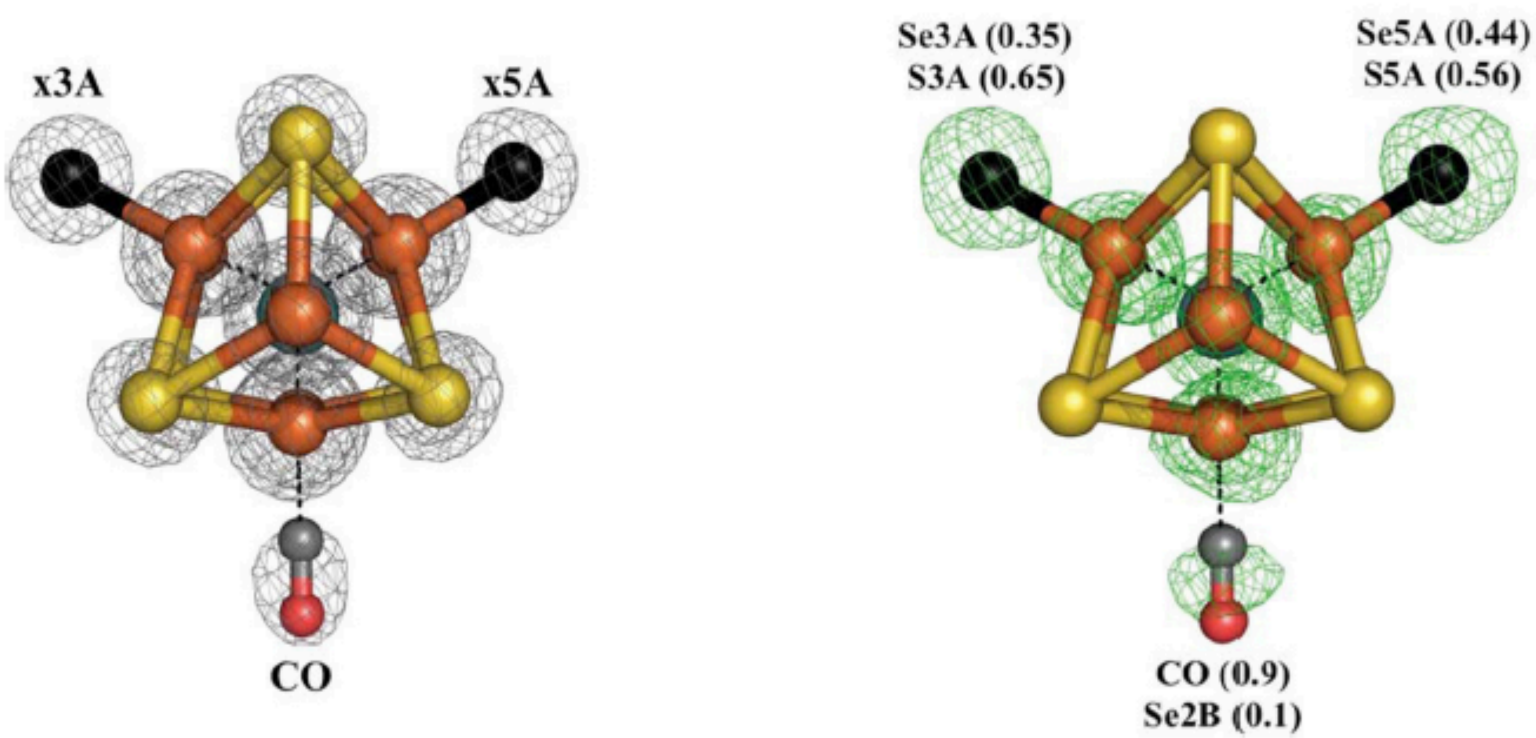


- Se can place the S2B belt sulfur
- Se migrates to S3A and S5A at later stages of catalysis
- FeMoco may be more dynamic than previously thought
- The central carbon, may provide structural stability

Displacement of Se by CO

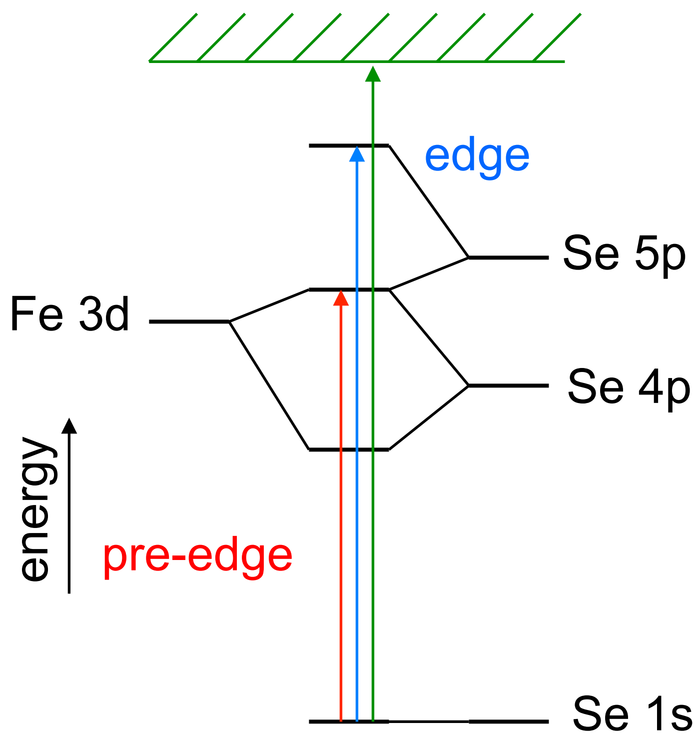
T. Spatzal *et al., ELife*, **2015**, 4:e11620

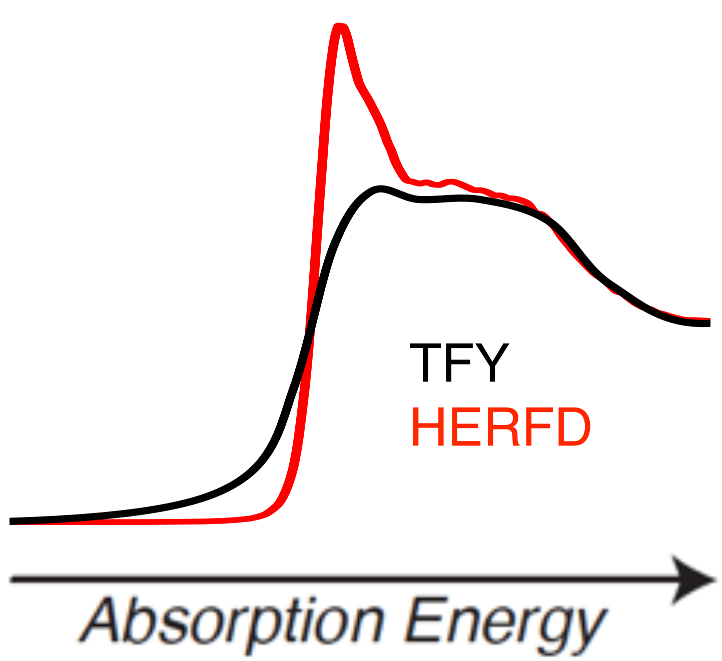




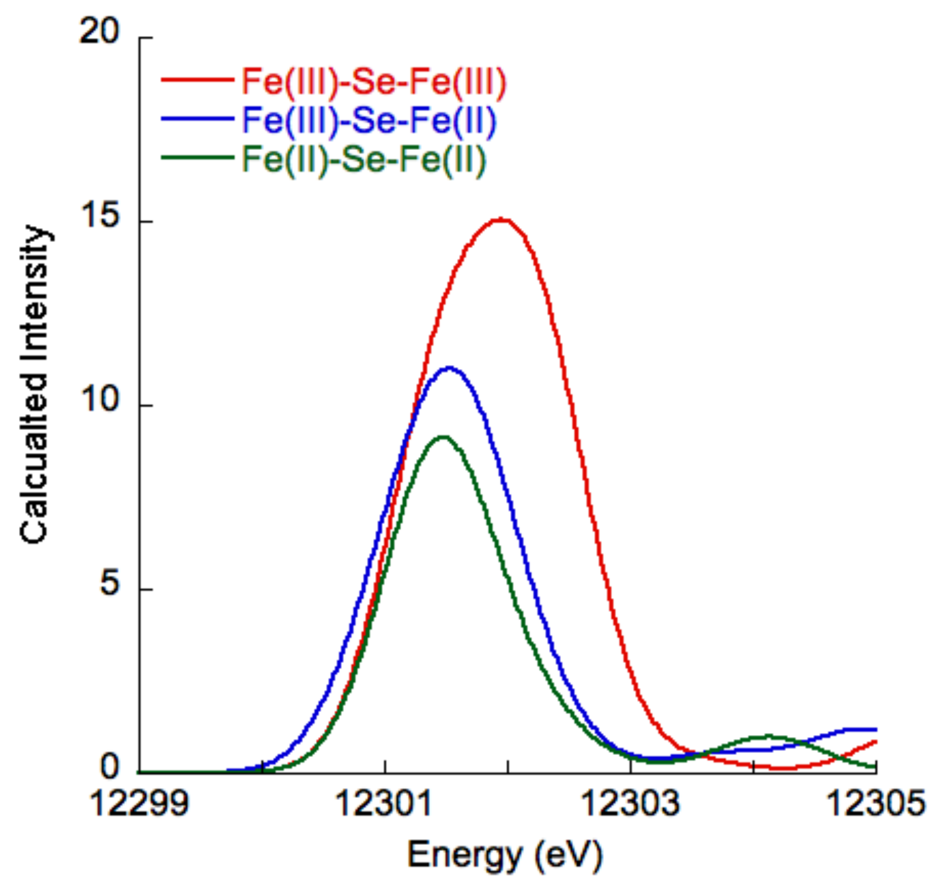
- In the presence of CO, CO will displace the S2B Se and Se migrates to S3A and S5A positions
- Electronic structure of CO bound form is unknown, though presumably corresponds to a more reduced FeMoco species. (S=1/2 E_2 state, however only ~30% of the EPR signal)
- ***Should allow for X-ray spectroscopic characterization of a reduced substrate-bound intermediate of FeMoco.***

Se HERFD XAS

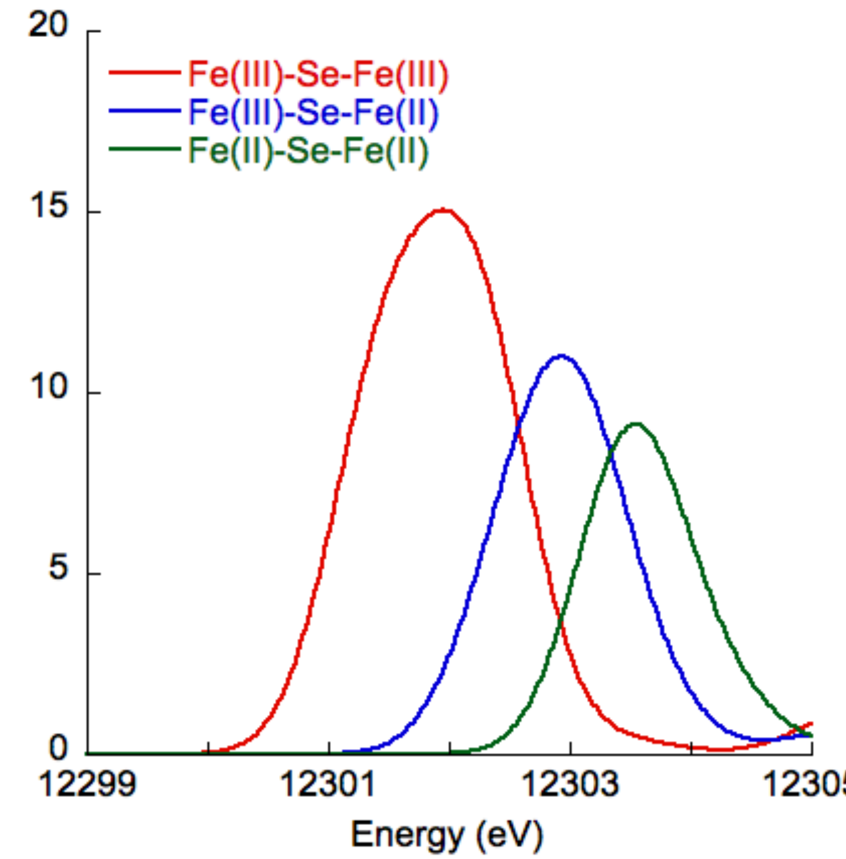




TDDFT calculated - no energy shift



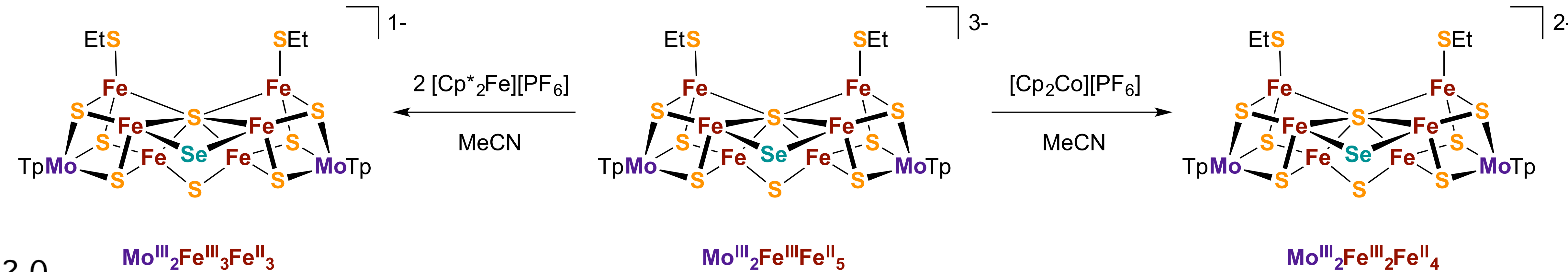
TDDFT calculated - aligned to Se 5p

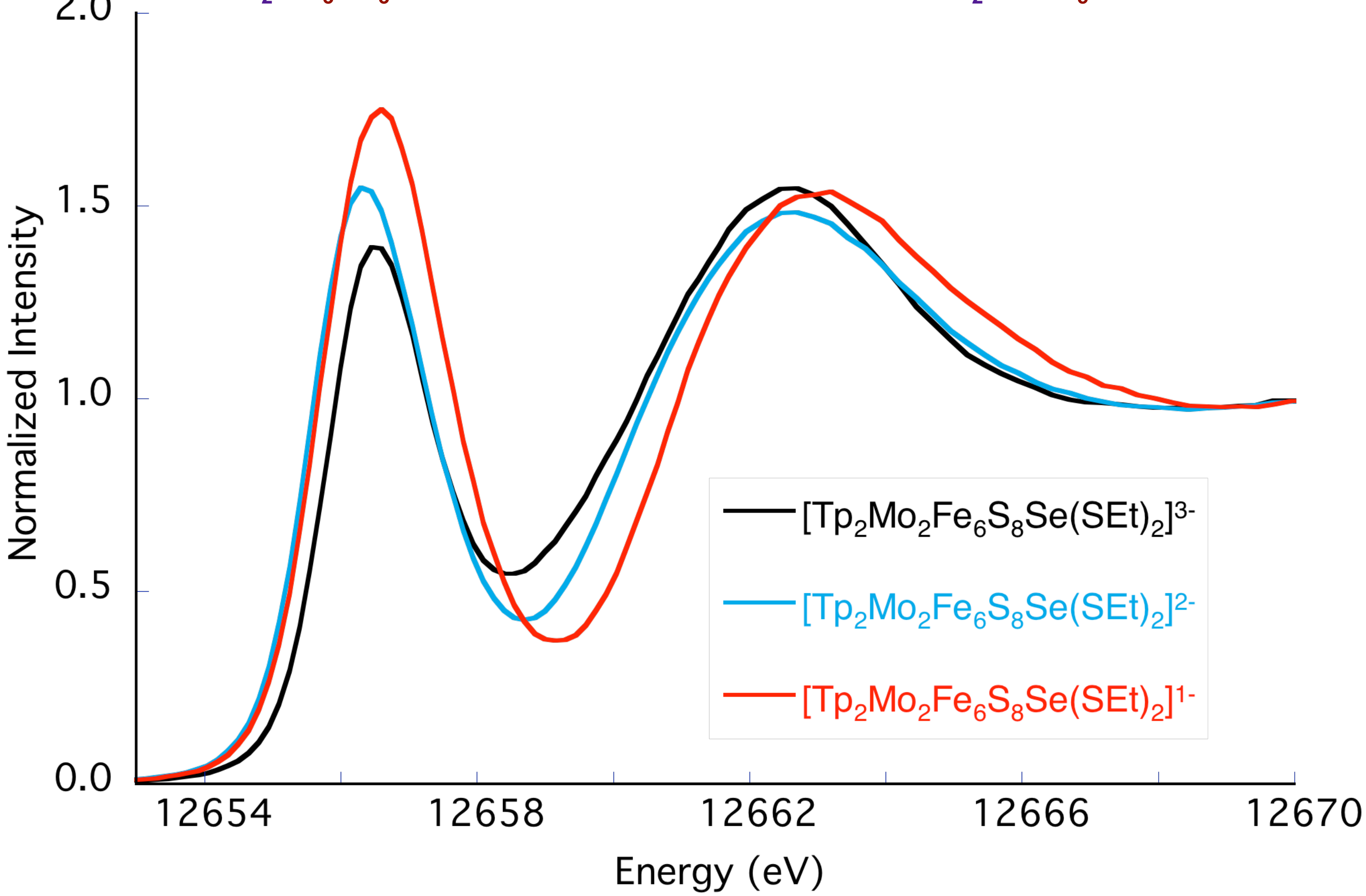


- Se ligand K-edge XAS applications limited due to large core hole lifetime broadening at Se.
- Increased resolution of a HERFD XAS measurement allow for well resolved pre-edge to be observed.
- Se pre-edge provides a probe of Fe-Se covalency
- Utilize Se as a spectator probe of Fe oxidation state.

Se HERFD of Se-bridged N2ase Models

Berlinguette, C.P; Holm, R.H. *J. Am. Chem. Soc.*, **2006**, *128*, 11993–12000.

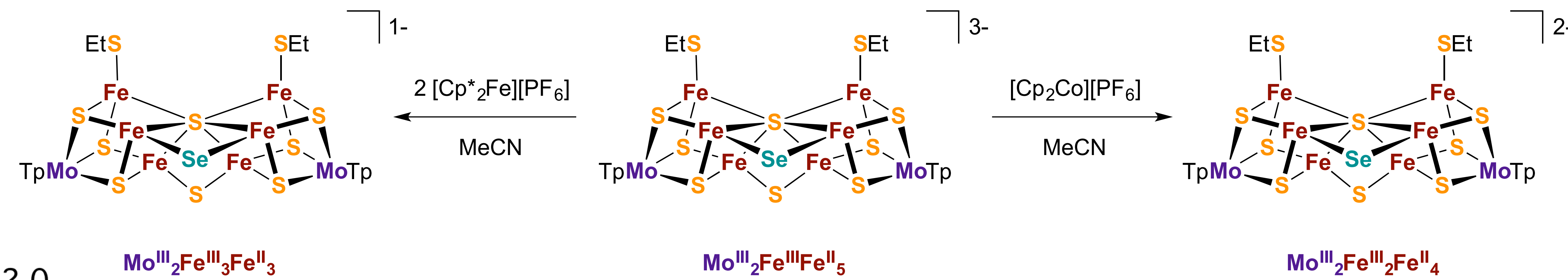


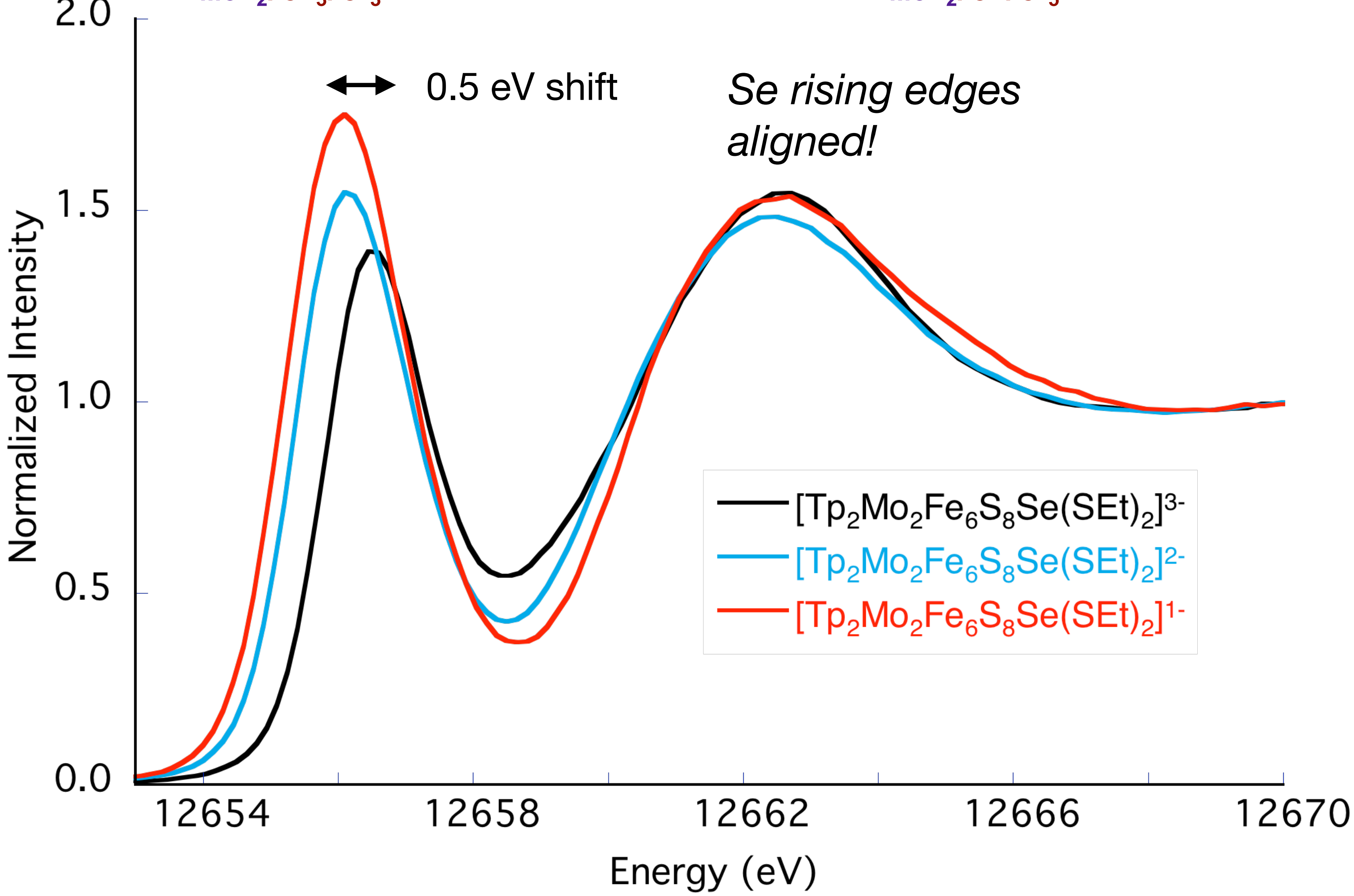


Justin Henthorn

Se HERFD of Se-bridged N2ase Models

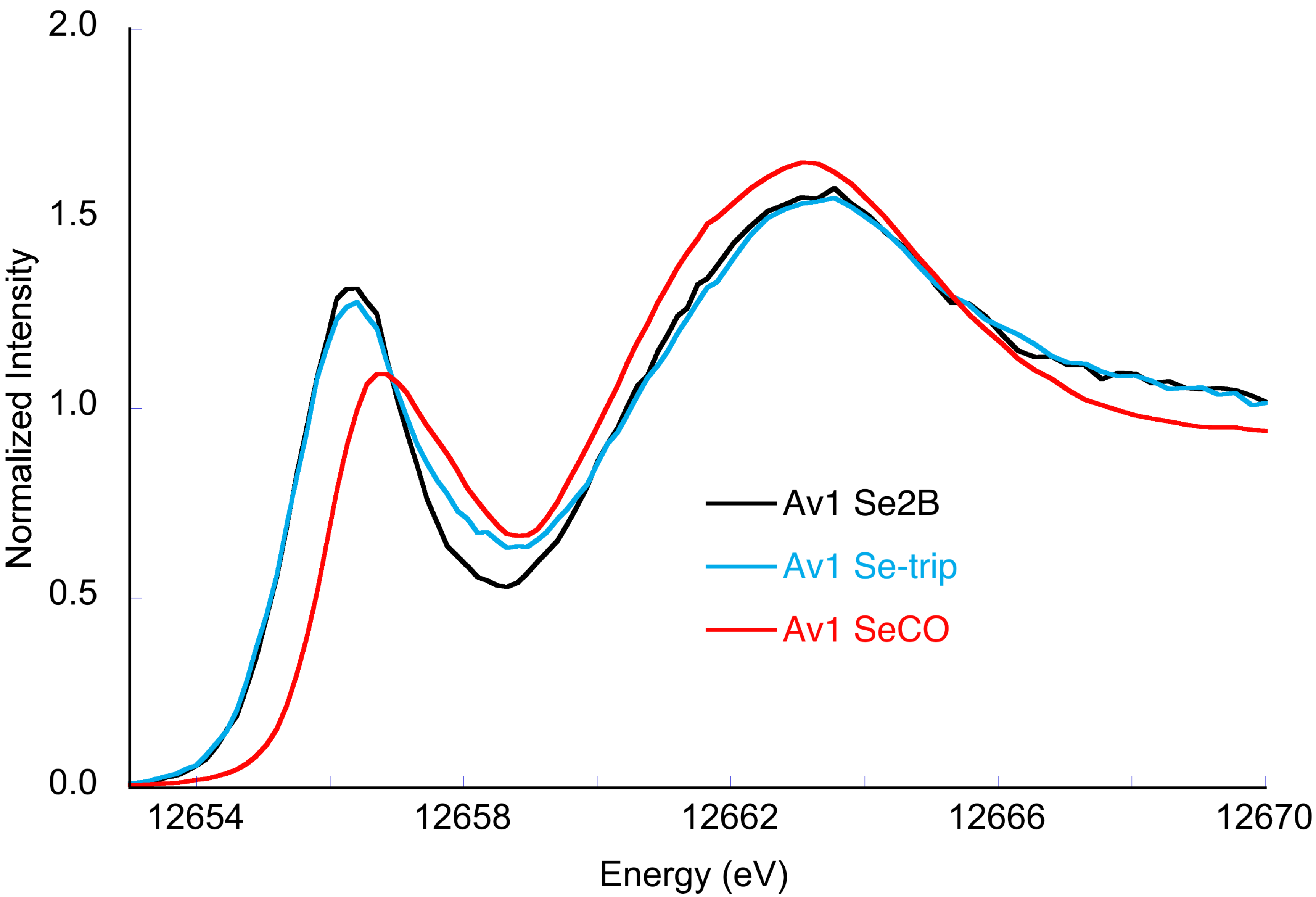
Berlinguette, C.P; Holm, R.H. *J. Am. Chem. Soc.*, **2006**, *128*, 11993–12000.

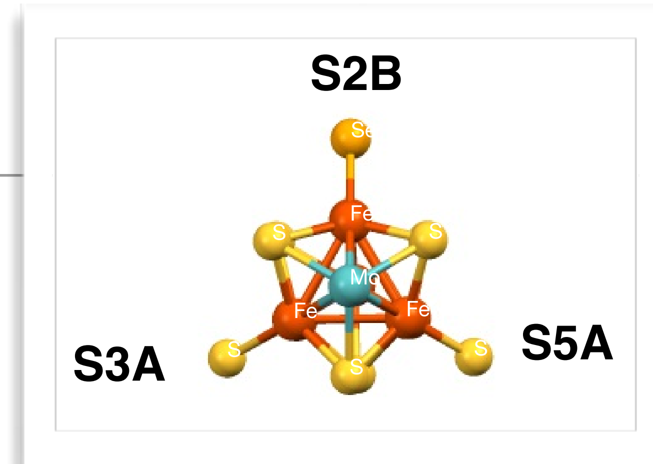




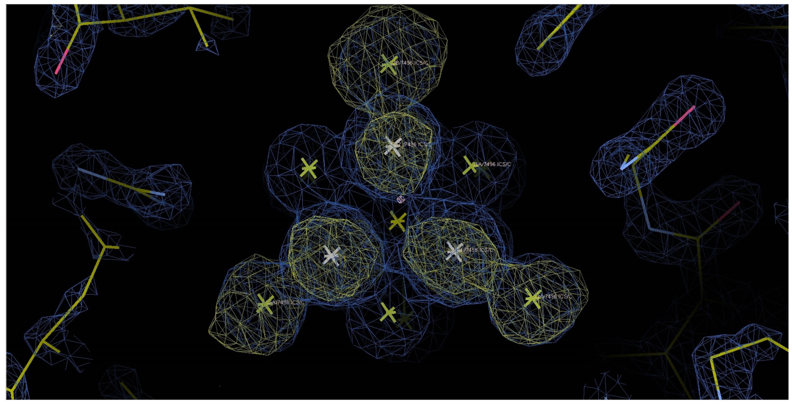
- Spectra aligned to Se rising edge in order to compensate for changes in Se donation.
- Now pre-edge clearly reflects shift to Zeff at the Fe increasing
- Reduction of 2 out of 6 irons gives rise to 0.5 eV pre-edge shift
- Demonstrates utility of Se HERFD as a spectator probe for cofactor charge

Se HERFD of MoFe



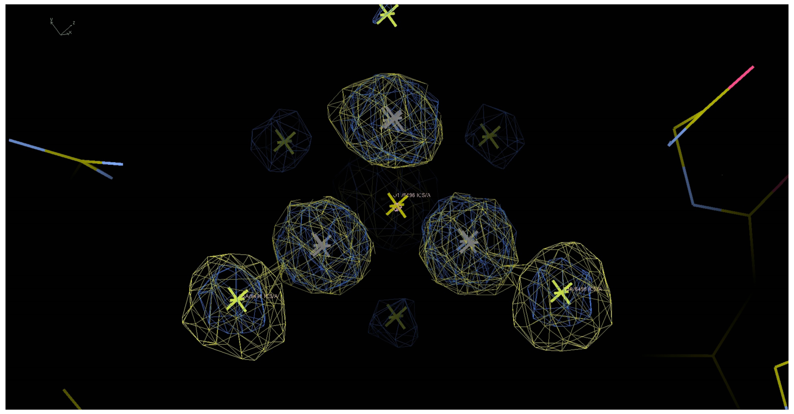


Av1 Se2B



S2B: 87.7% S3A: 49.2% S5A: 48.5%

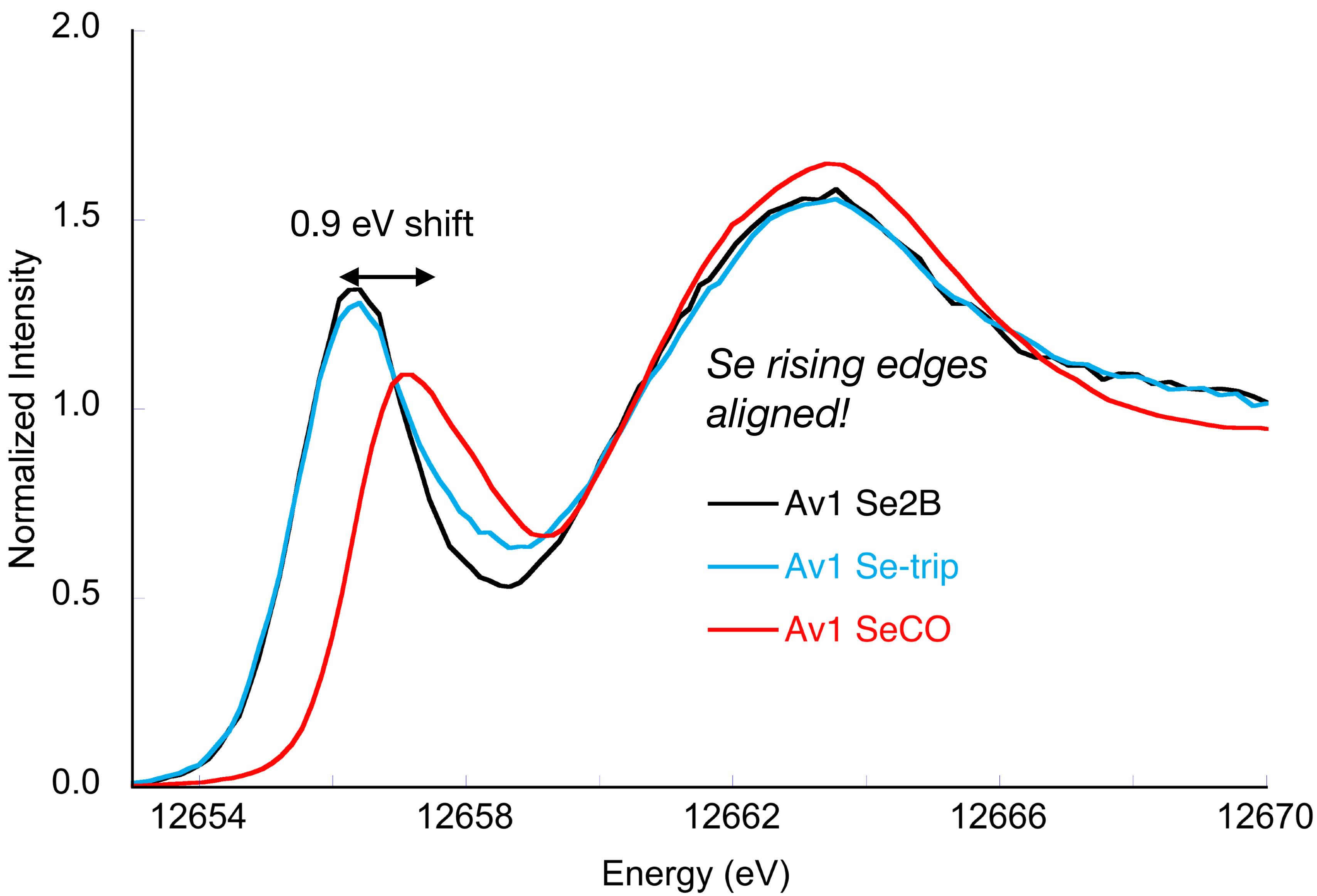
Av1 CO

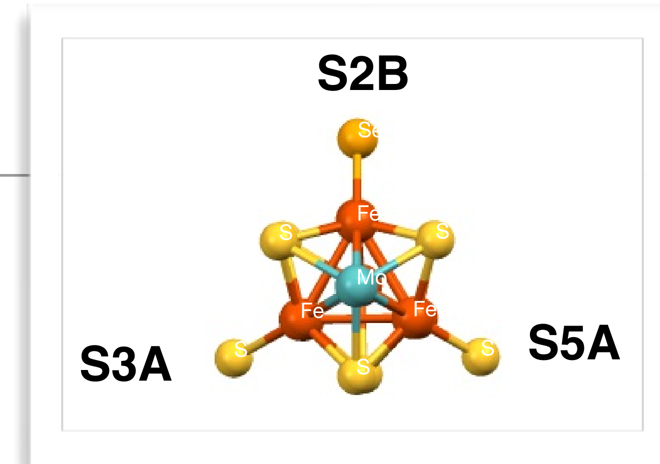


S2B: 12.5% S3A: 49.4% S5A: 48.5%

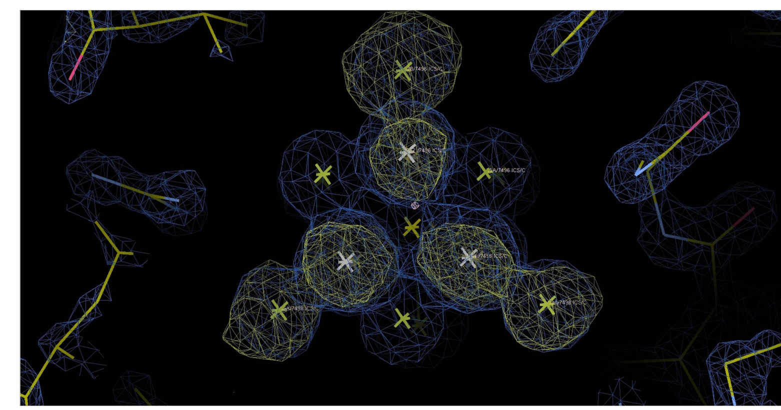
- Changes upon CO binding clearly more pronounced
- Reflects iron oxidation states interacting with S3A and S5A
- Indicates overall more reduced cluster

Se HERFD of MoFe





Av1 Se2B



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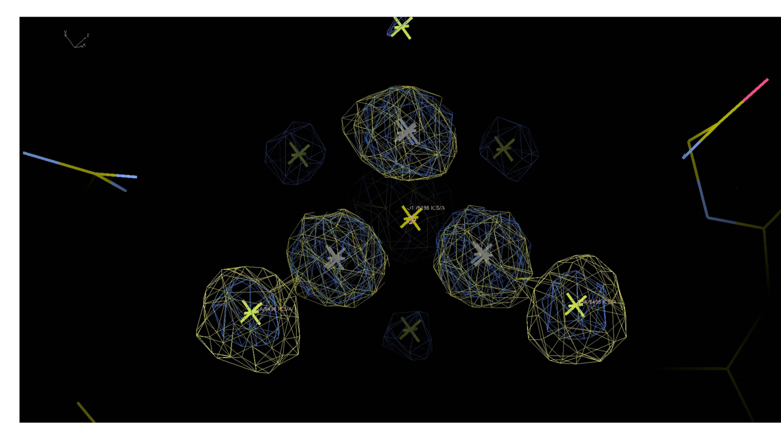
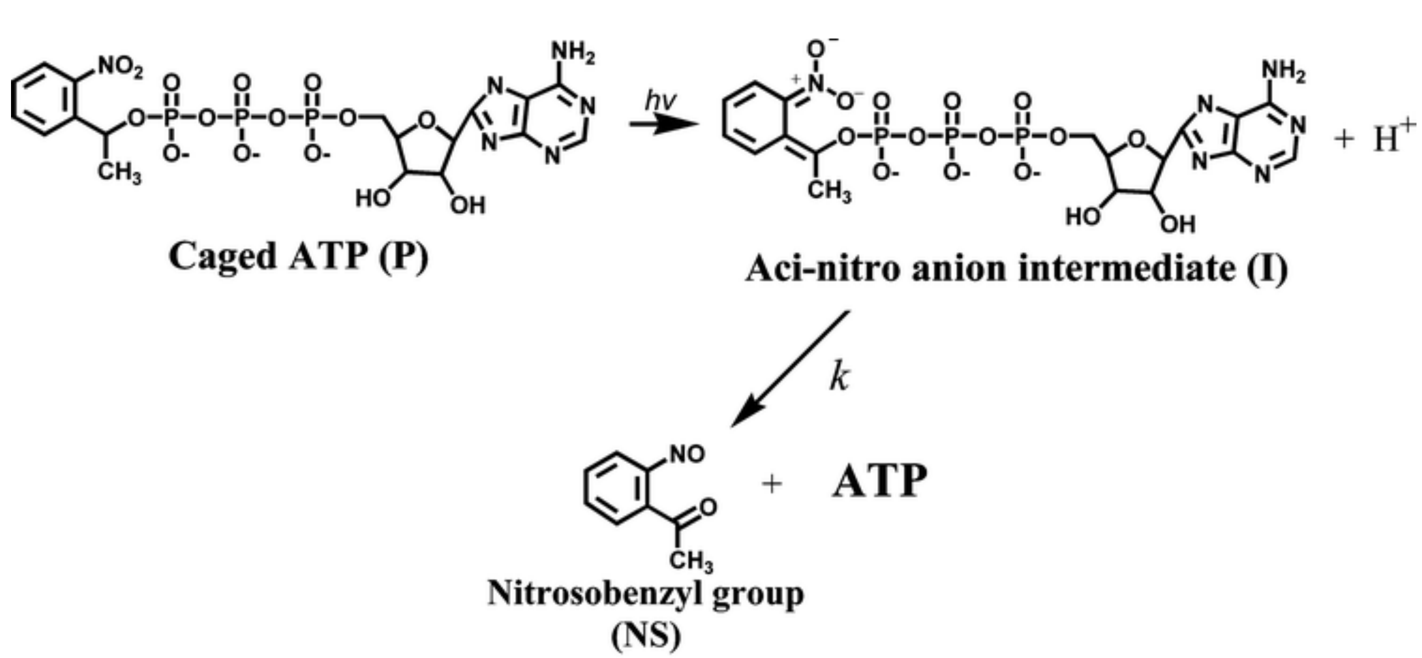


S2B: 12.5% S3A: 49.4% S5A: 48.5%

- Alignment of rising edge shows average d-manifold position shifts by almost 1 eV in CO bound protein
- Typically assumed that CO binds at two-electron reduced E2 state, with S=1/2 EPR (but only 30% by EPR spin quantitation)
- Is CO binding to other integer spin reduced states?
- Correlations to theory in progress…

Time-resolved studies of Nitrogenase - Outlook

- Further developments are needed to enable controlled access to reaction intermediates in nitrogenase
- The presence of the iron protein, P-cluster, and FeMoco makes evaluation of iron spectra more challenging. Selective tools are needed!
- Selenated nitrogenase provides access to a more selective probe. Se HERFD in progress…
- Additional photo triggers should be explored, such as caged ATP…



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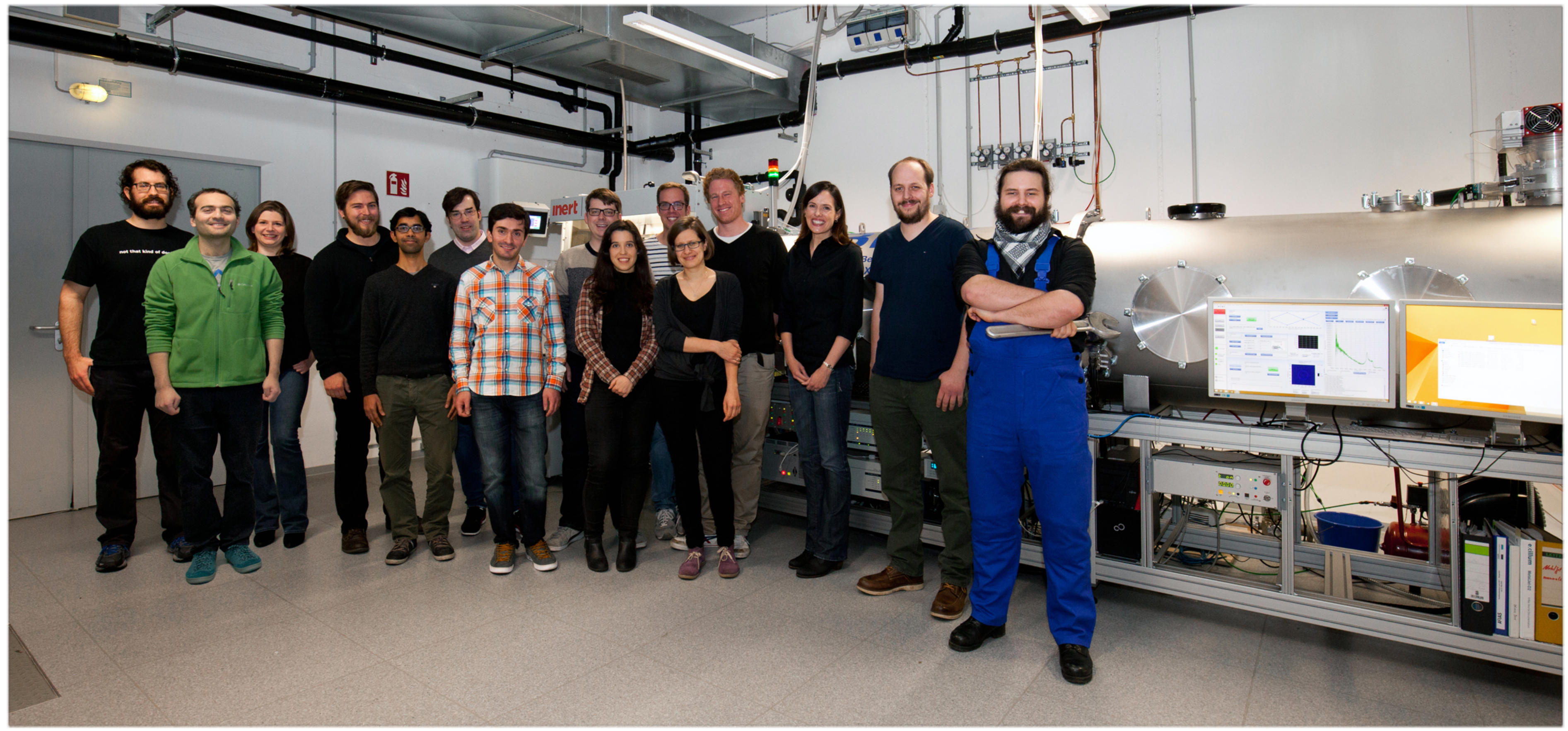

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Vijay Chilkuri
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Anselm Hahn
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Joanna Kowalska
Sergey Peredkov
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Casey van Stappen
Aleksandra Wandzilak

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$ DFG
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$ IMPRS (RGC and CV)
Beam time: ESRF, CHESS, SOLEIL, SSRL

Thank you for your attention!