Emission Spectra, Soft X-rays, and Metal Clusters in Biology

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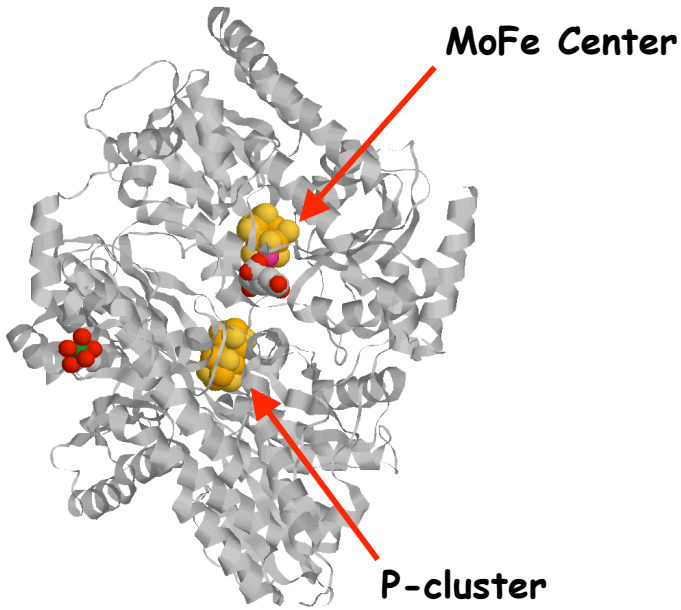
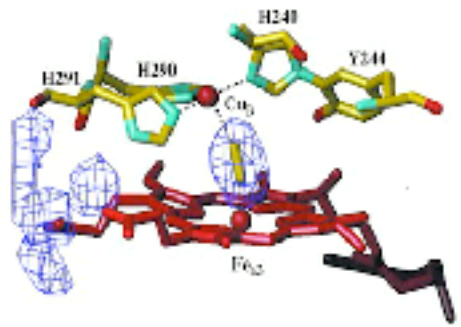
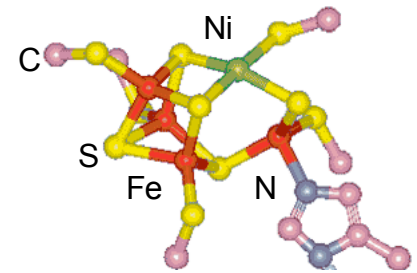
Metal Clusters in Biology





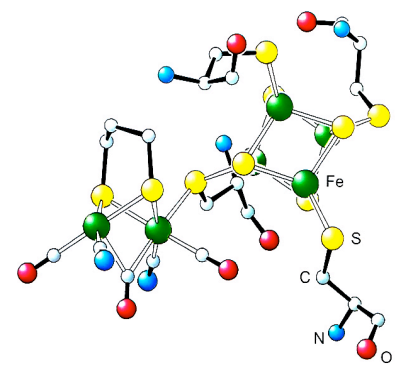
$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$

Cu-Fe Site in
Cytochrome Oxidase



$CO + H_2O \longrightarrow CO_2 + 2H^+ + 2e^-$

NiFe C-cluster in CODH



$H_2 \longrightarrow 2H^+ + 2e^-$

Fe Hydrogenase H-cluster

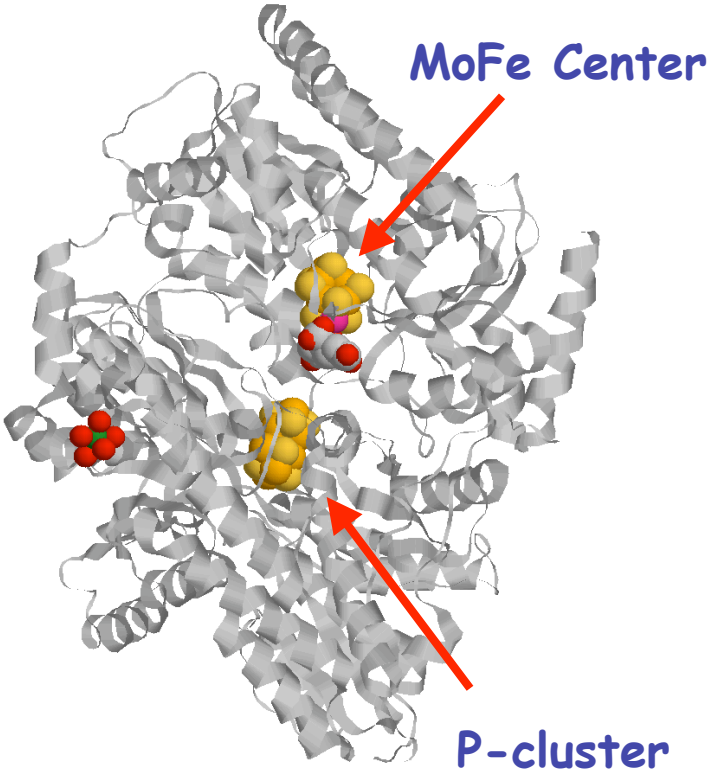
- Catalyze many fundamental life and environmental processes
- Involved in 30% of all enzymes

MoFe Nitrogenase

- Enzymatic reduction of Dinitrogen
- Bacterial enzyme - soil, root nodules
- Processes 3 x 10^{11} kg/year worldwide

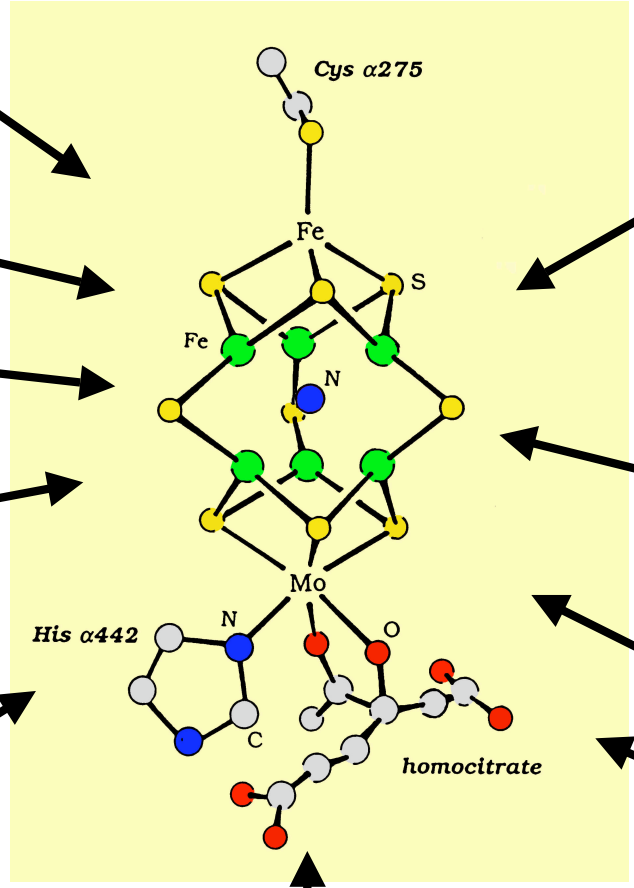
- N≡N : bond dissociation enthalpy = 945 $kJmol^{-1}$
- Unknown mechanism

- MoFe Center can be extracted into N-methyl Formamide (NMF) = FeMoco



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

The MoFe Center : Approaches



(Mössbauer)

(EPR/ENDOR)

EXAFS

RIXS

Soft X-ray XAS & XMCD

Infrared Resonance Raman (vibrational)

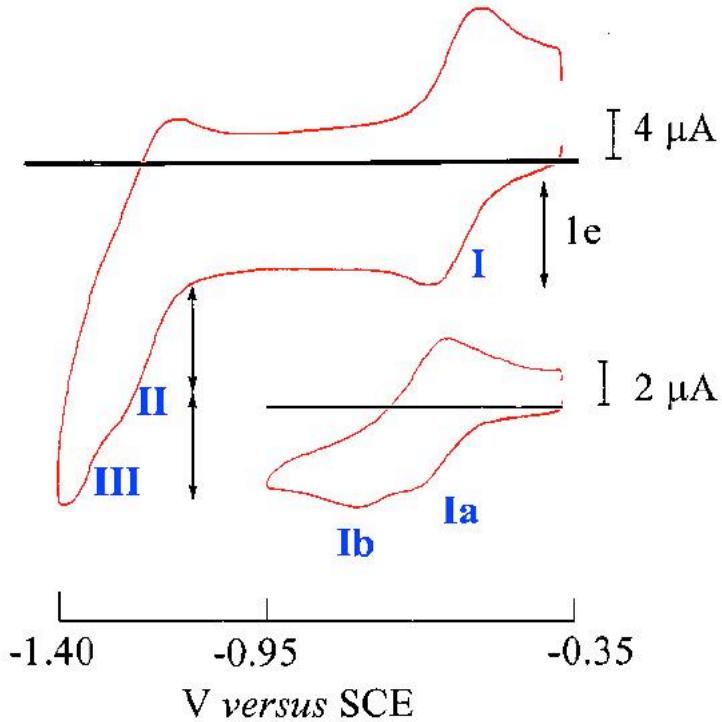
NRVS (vibrational)

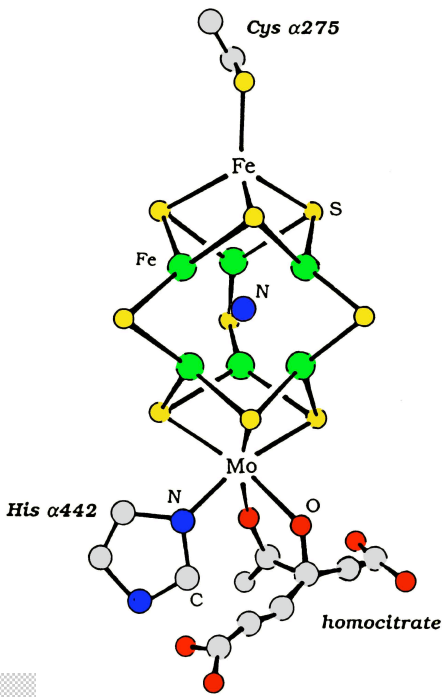
(NMR)

(Crystallography)

(Site Directed Mutagenesis)

X-ray Spectroscopy of FeMoco





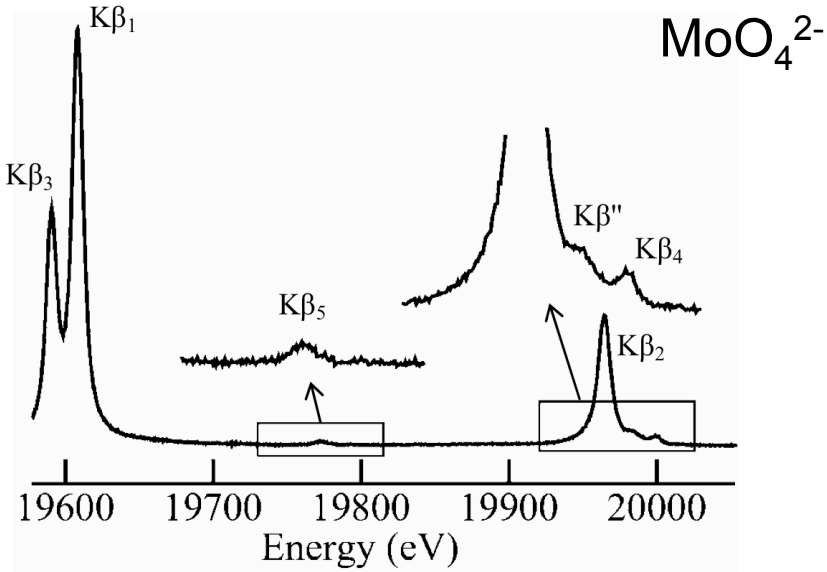
Nitrogenase MoFe Center

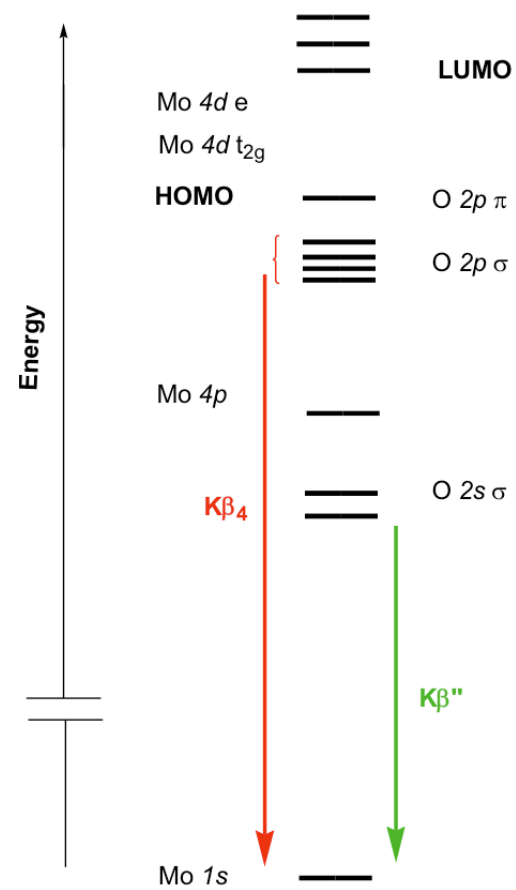
- Reductions I and II → Fe Core
  Reduction III → Mo Site

  (Pickett et al (2003) Chem Eur J 76-87)

- Study with Mo M-edges, Fe L-edges, S L-edges

Mo Kß Emission Spectroscopy

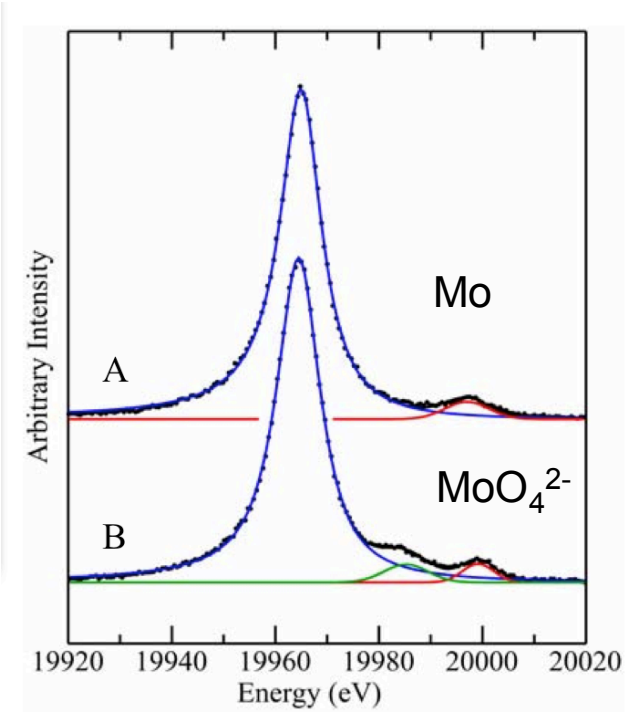




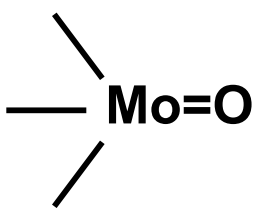
- **$K\beta_{1,3}$: Mo 2p, $K\beta_2$: Mo 4p, $K\beta_5$: Mo 3d**
  - insensitive to chemical environment

- **$K\beta_4$: Mo 4d / O 2p**
  Kß" : O 2s
  - sensitive to chemical environment

Mo Kß Emission Spectroscopy



	Mo-O (Å)	Energy (eV)		Emission rate	
Species		$K\beta_4$	$K\beta''$	$K\beta_4/K\beta_1$	$K\beta''/K\beta_1$
Mo Foil		19997.0	—	0.0125	—
$MoO(S_2CNEt_2)_2$	1.664	19997.4	19981.7	0.0109	0.0118
$MoO_2(S_2CNEt_2)_2$	1.703	19998.6	19983.8	0.0162	0.0135
K_2MoO_4	1.763	19999.3	19984.4	0.0160	0.0164



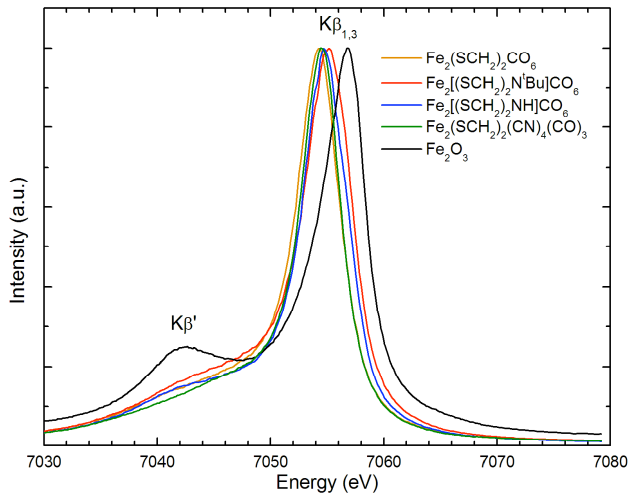
- Kß" - crossover in MoO compounds
  - O 2s character
- potential probe of bond length and ligand type
- Kß" normalized intensity ~ Mo-O bond length

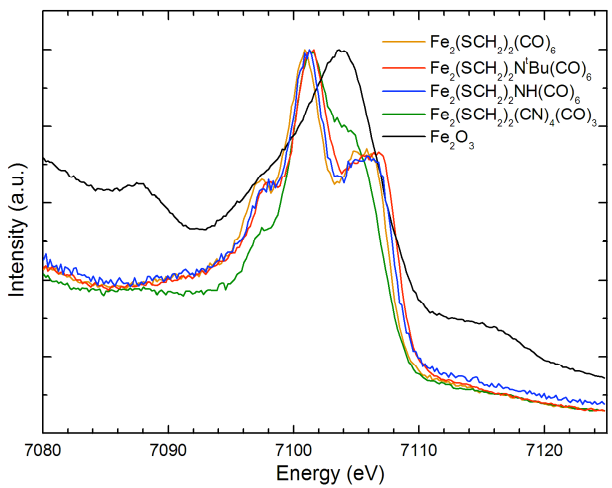
C.J. Doonan *et al.* (2005) Inorg. Chem 44, 2579-2581

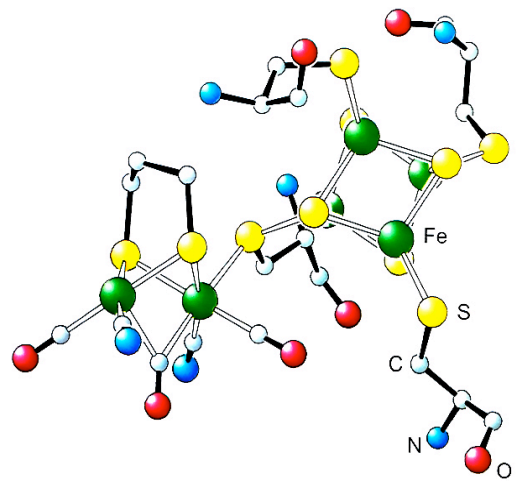
Mo Emission Spectroscopy - Where Next?

- More model work - better understand valence behavior
- Proteins:
  - Nitrogenase
  - Mo Hydroxylases (Xanthine Oxidase, Aldehyde Oxidase)
- Higher order edges
  - Mo L (2.5keV)
  - Mo M (Soft x-ray)

Fe Kß Emission of H-cluster Models

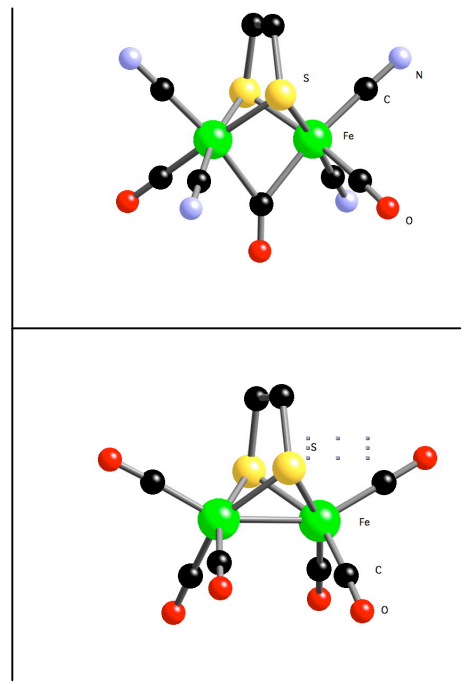






$H_2 \longrightarrow 2H^+ + 2e^-$

Fe Hydrogenase H-cluster

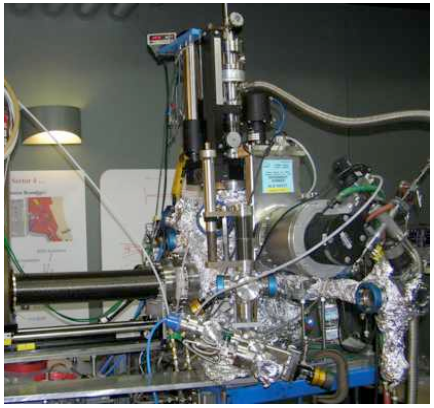


- **Kß' - sensitive Fe(I)**
- **Interesting Valence Region**

Y. Guo, S.J.George, S.P.Cramer unpub

Biological Soft X-ray Spectroscopy at ALS 4.0.2 (ABEX)

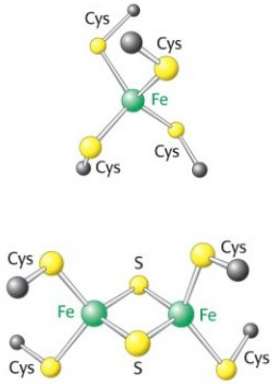
- L-edge XAS
  - Fluoresence Detected - 36+ element STJ (10 eV resolution)
    - 30 element Ge (~160 eV)
- L-edge XMCD
  - Paramagnetic Experiment - need 6 T, 2 K etc



Biological Soft X-ray Spectroscopy at ALS 4.0.2 (ABEX)

- L-edge XAS
  - Fluoresence Detected - 36+ element STJ (10 eV resolution)
    - 30 element Ge (~160 eV)
- L-edge XMCD
  - Paramagnetic Experiment - need 6 T, 2 K etc
- (Soft X-ray Emission Spectroscopy)
- Related Techniques
  - EXAFS, RIXS, NRVS
  - Infrared, EPR, Resonance Raman

Fe L-edge XAS and XMCD of FeS Proteins



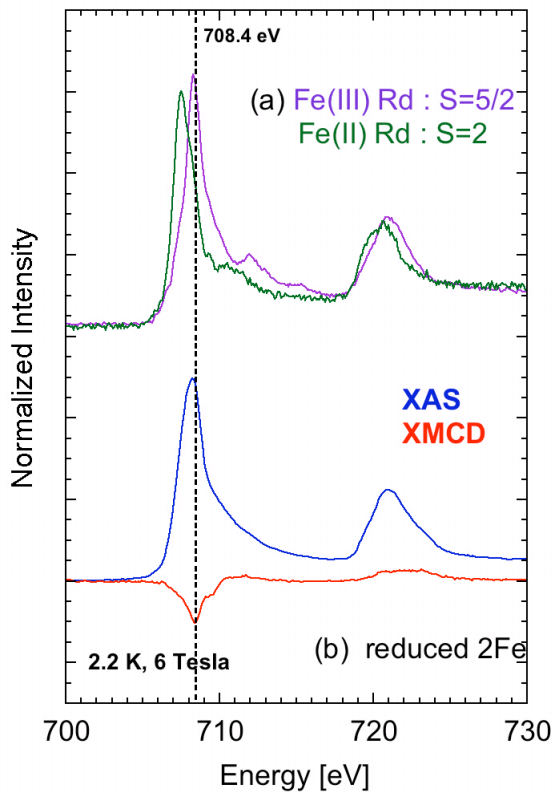
Fe(III) - Fe(III)

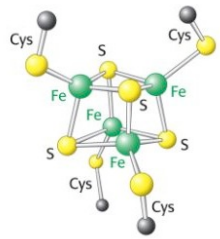
S=0

$\downarrow e^-$

Fe(III) - Fe(II)

S=1/2





2Fe(III) - 2Fe(II)

S=0

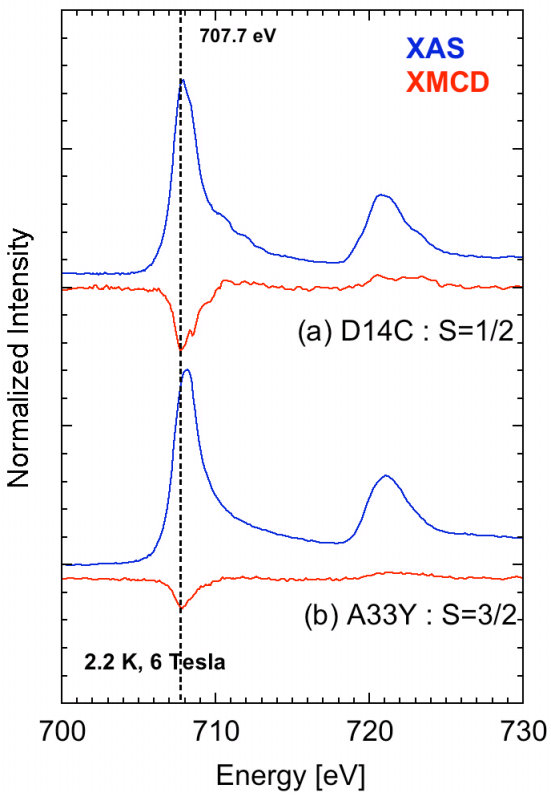
$\downarrow e^-$

Fe(III) - 3Fe(II)

S=1/2

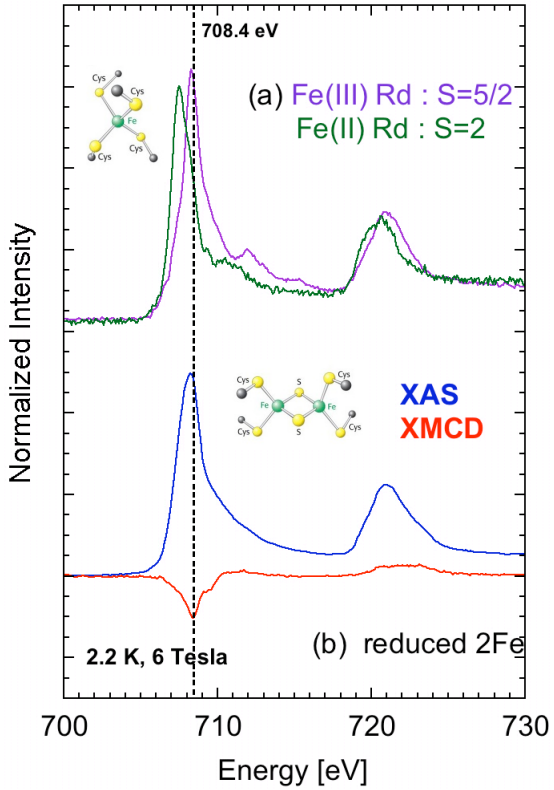
or

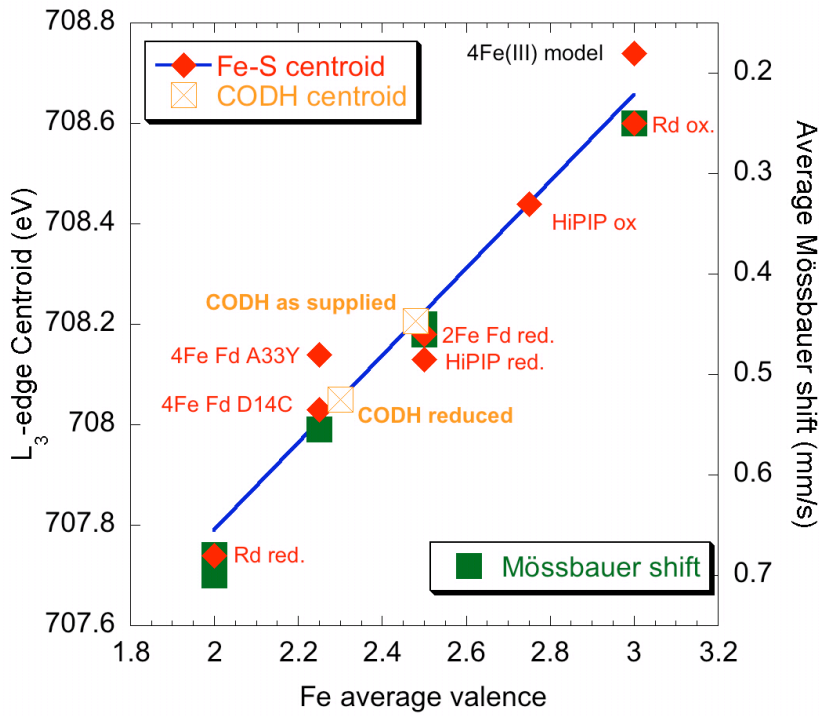
S=3/2



- [2Fe-2S] - XMCD on Fe(III) side : [4Fe-4S] - Fe(II), BUT S=3/2 smaller XMCD than S=1/2 (!)
- Analysis with LMFT + Spin Hamiltonian

Fe L-edge XAS and XMCD of FeS Proteins

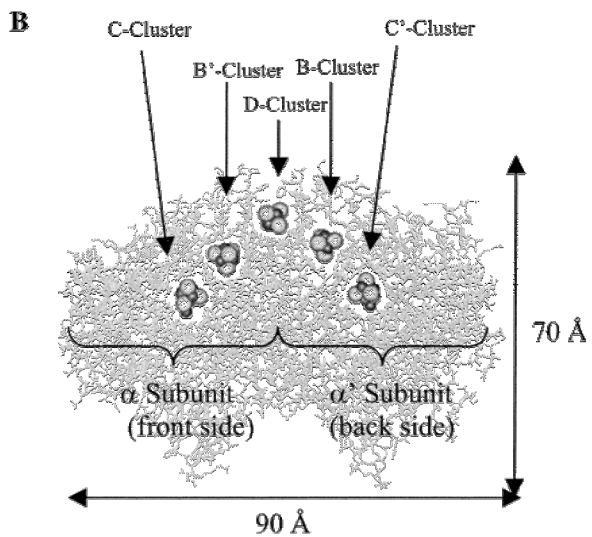


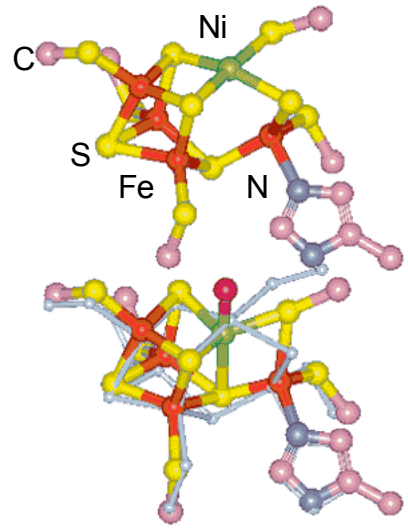


- **Empirical Measurement of FeS Fe(III):Fe(II) Ratio (redox state) from L_3 centroid.**

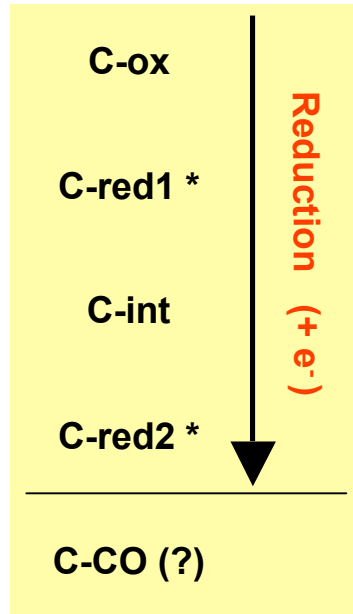
C. Piamonteze, S.J.George, S.P.Cramer unpub

CODH - Carbon Monoxide Dehydrogenase



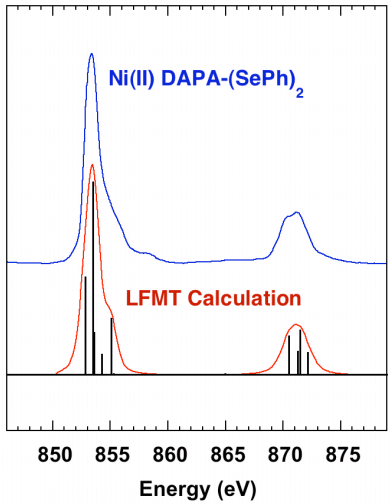


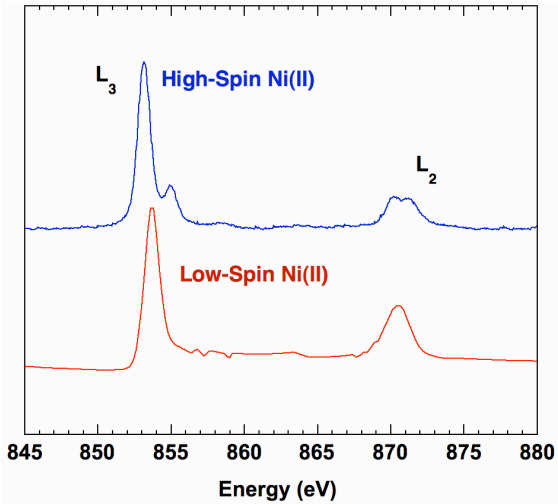
- **The C-cluster**

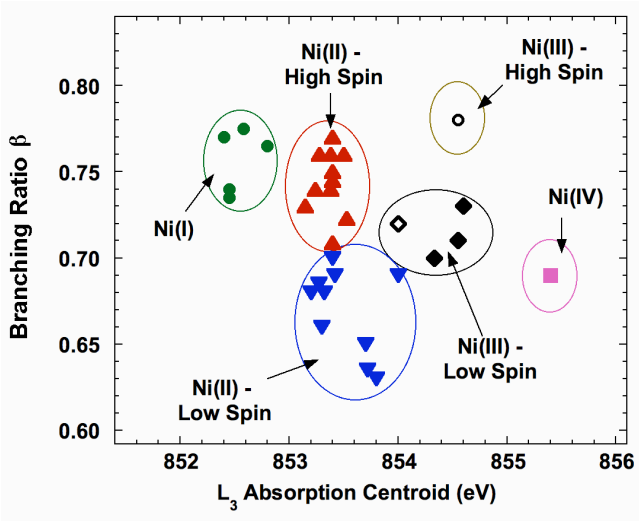


- Life on carbon monoxide
- $CO + H_2O \longleftrightarrow CO_2 + 2H^+ + 2e^-$
- Active site C-cluster is $NiFe_4S_x$ cluster
- C-cluster has many redox / chemical / conformational states

Ni L-edge XAS

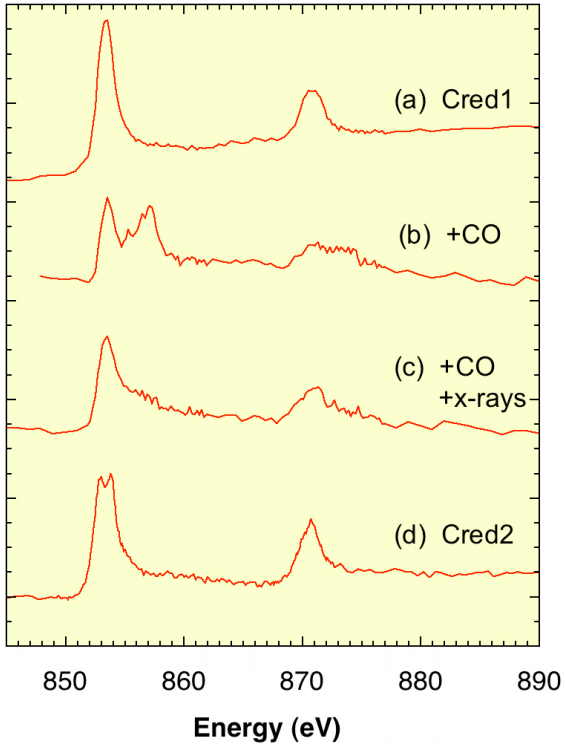


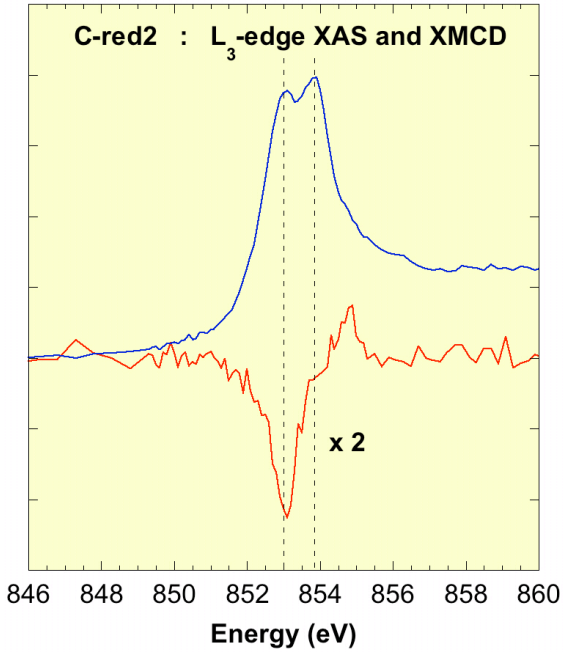


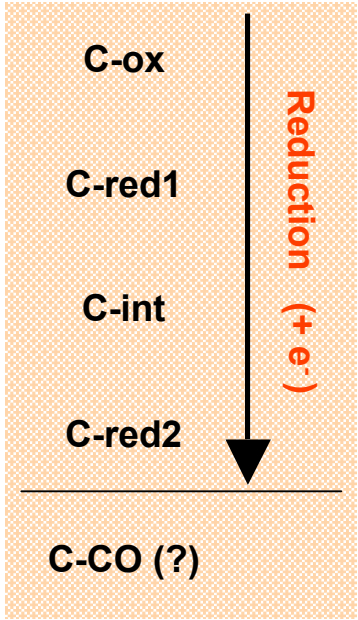


- LMFT (Ligand Field Multiplet Theory) ⟶ symmetry, 10Dq etc
- Peak position ⟶ oxidation state
- Branching ratio ⟶ spin-state
- Correlation Diagram
- XMCD - Paramagnetism

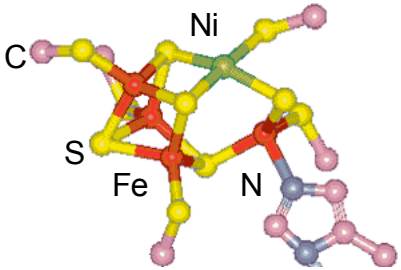
Soft XAS of CODH C-cluster





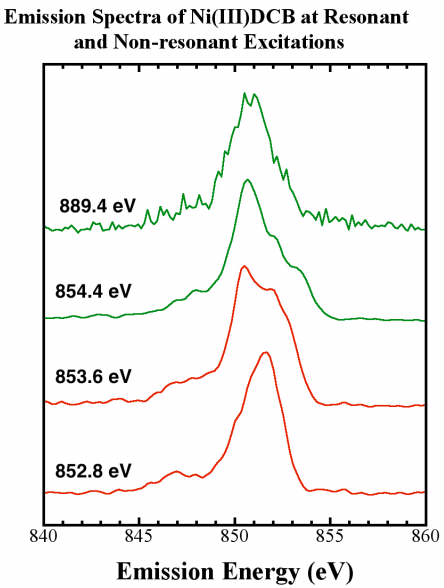


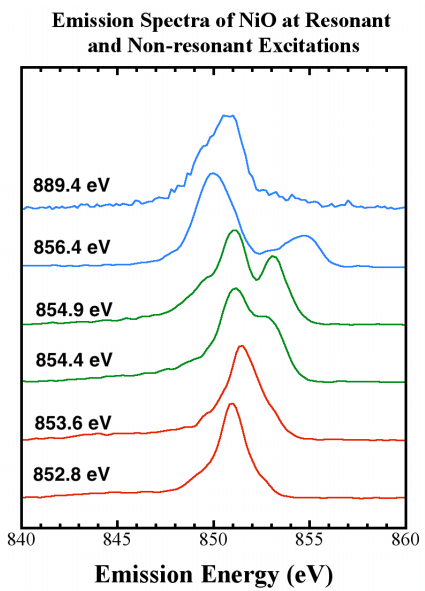
- Ni(II)
- Follow spin-state and (lack of) oxidation state changes.
- XMCD selects paramagnetic components

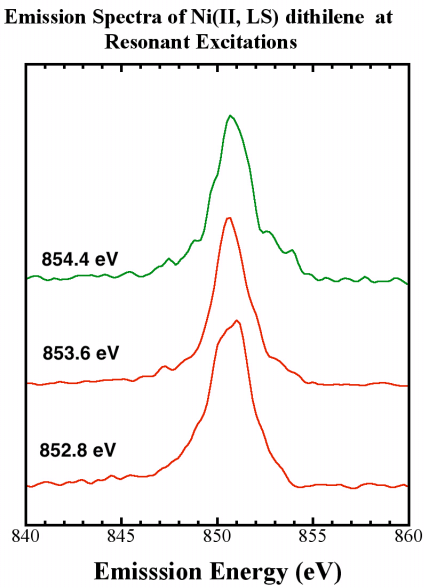


C. Piamonteze, S.J.George, S.P.Cramer unpub

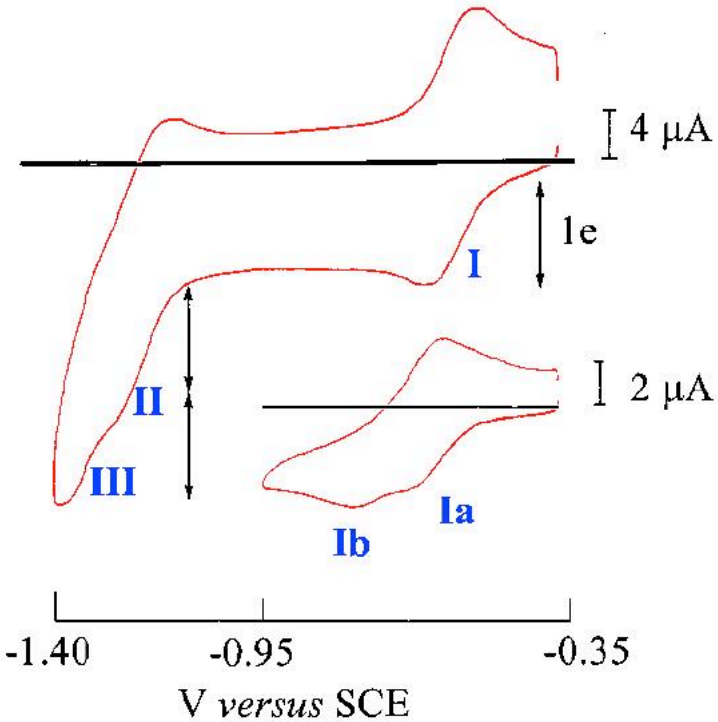
Ni L-Emission Spectra

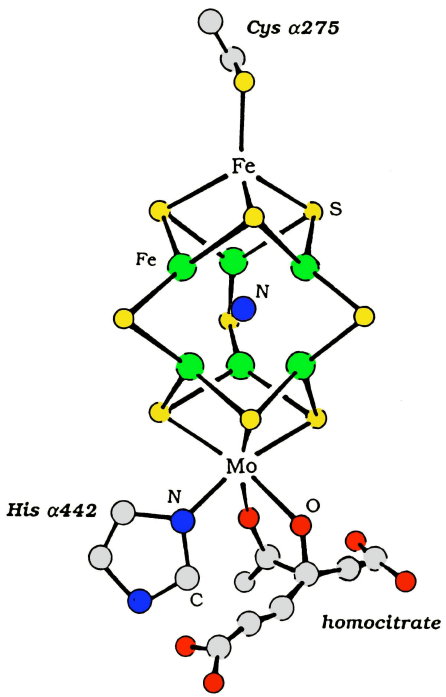






Soft X-ray Spectroscopy of FeMoco

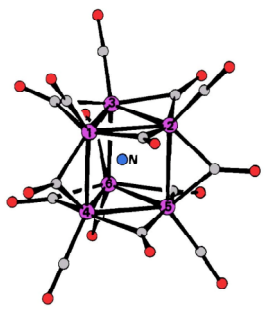




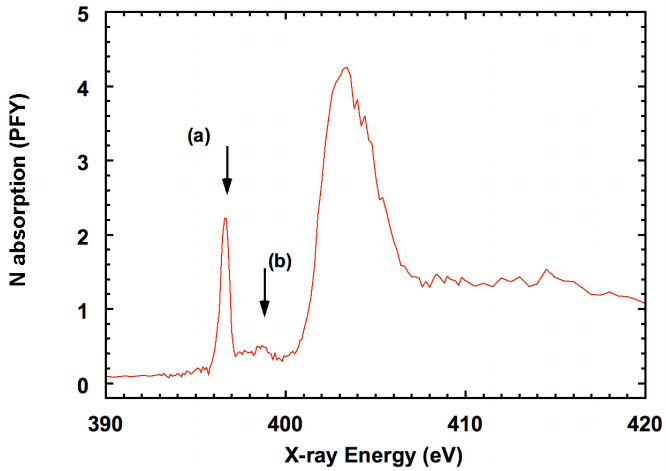
Nitrogenase MoFe Center

- Study with Mo M-edges, Fe L-edges, S L-edges
- But what about the central atom …….

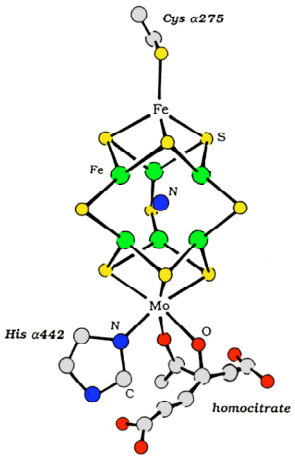
FeMoco : Nitrogen K-edges



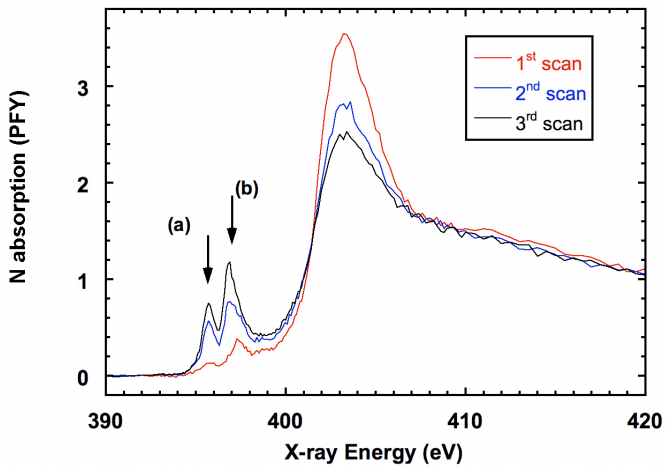
$[Fe_6N(CO)_{15}]^{3-}$



- FeMoco - active center of Nitrogenase.
- Proposed to contain interstitial Nitrogen atom.
- (FeMoco prepared in acetone - not N-methylformamide.)



FeMoco



- N K-edge of $[Fe6N(CO)15]^{3-}$ has 2 sharp bands.
- Analogous bands in FeMoco edge on photoreduction.
- N K-edge EXAFS should reveal any shell of 6 Fe-atoms.

***S.J.Georgeet al.* unpub**

The Future

- Soft X-ray emission - at least on model systems
  - Transition metals
  - Light element K-edges

- If beamline and analyzer mono resolution can be high enough
  - Resonance enhanced x-ray Raman Spectroscopy = site specific vibrational technique.

Acknowledgements

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Steve Singer

U. Nebraska, Lincoln
Steve Ragsdale
Javier Seravilli

U. Saskatchewan
Graham George
Christian Doonan

John Innes Centre
Barry Smith

Caltech
Jonas Peters
Steven Brown

$$: NIH, NSF, DOE OBER

***hv* : ALS, APS**