Applications for X-Ray Spectroscopy

Jordan Planillo

Rensselaer Polytechnic Institute

Dr. Jerry Seidler

University of Washington

Abstract

X-ray crystallography via X-ray diffraction has been a reliable method method for determining crystal structure and composition. For complex crystals, as in the case of metalloproteins, X-ray diffraction fails to provide such information. X-ray spectroscopy gives better insight to the structure and composition of such complex crystal. The metalloprotein of interest is nitrogenase, the enzyme that allows for nitrogen fixation in plants and bacteria. This report details the experimental procedures for analyzing nitrogenase, specifically: experimental apparatus, simulation of nitrogenase with Fe(VI)N, and data analysis.

Introduction

With respect to metalloproteins, one of the hardest technical issues is to identify the existence and species of light elements bonded to catalytically active metal sites. X-ray diffraction cannot be used to obtain such information due to practical and experimental limitations. In terms of practical limitations, large amounts of high quality crystallized material are required. Such large crystals place experimental limitations such as crystal defects and the inability to detect light elements due to the presence of heavier elements.

Nitrogenase is one such metalloprotein of scientific interest as it is the only know method for nitrogen fixation and the exact details as to how nitrogenase performs this function are still unknown. The understanding of this function will allow for the efficient production of man-made fertilizers. The following chemical reaction details nitrogen fixation:

$$\mathbf{N_2 + 8\ H^+ + 8\ e^- + 16\ ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i}$$

Although the reaction of $N_2 + H_2 \rightarrow NH_3$ is energetically favorable, the N_2 triple bond must first be broken. The Mo-Fe co-factor in nitrogenase is the active catalytic site which strips electrons one at a time from N2, thus allowing it to dissociate and react with H2 to form NH3. Einsle, et al, Science (2002) (Fig.1), proposes a new model for the structure of the catalytically-active Mo-Fe cofactor where there is a light atom (C, N, O, or S) in a central cavity of the metal cluster, it is suspected to be nitrogen as it can play the role of a charge reservoir.

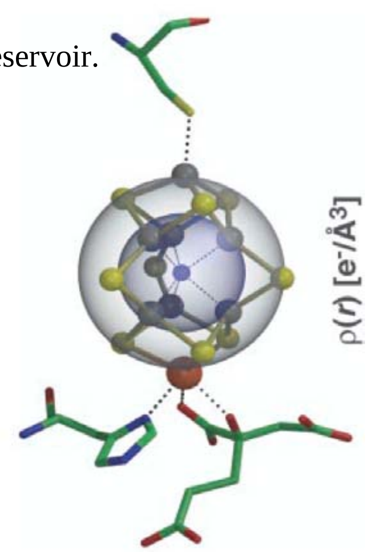


Fig.1
Mo-Fe cofactor
Color scheme:
Red-Mo
Grey-Fe
Yellow-S
Blue-Unknown

X-ray spectroscopy is the method of choice for studying metalloproteins because the x-ray emission spectra from metal atoms allow for the identification of light ligands. The emission of interest is when semicore electrons of the ligand fill in the 1s hole in the metal- the Kβ'' emission. The Kβ'' emission energy is directly correlated to the chemical species of the light ligand, however it is very hard to detect (Fig. 2).

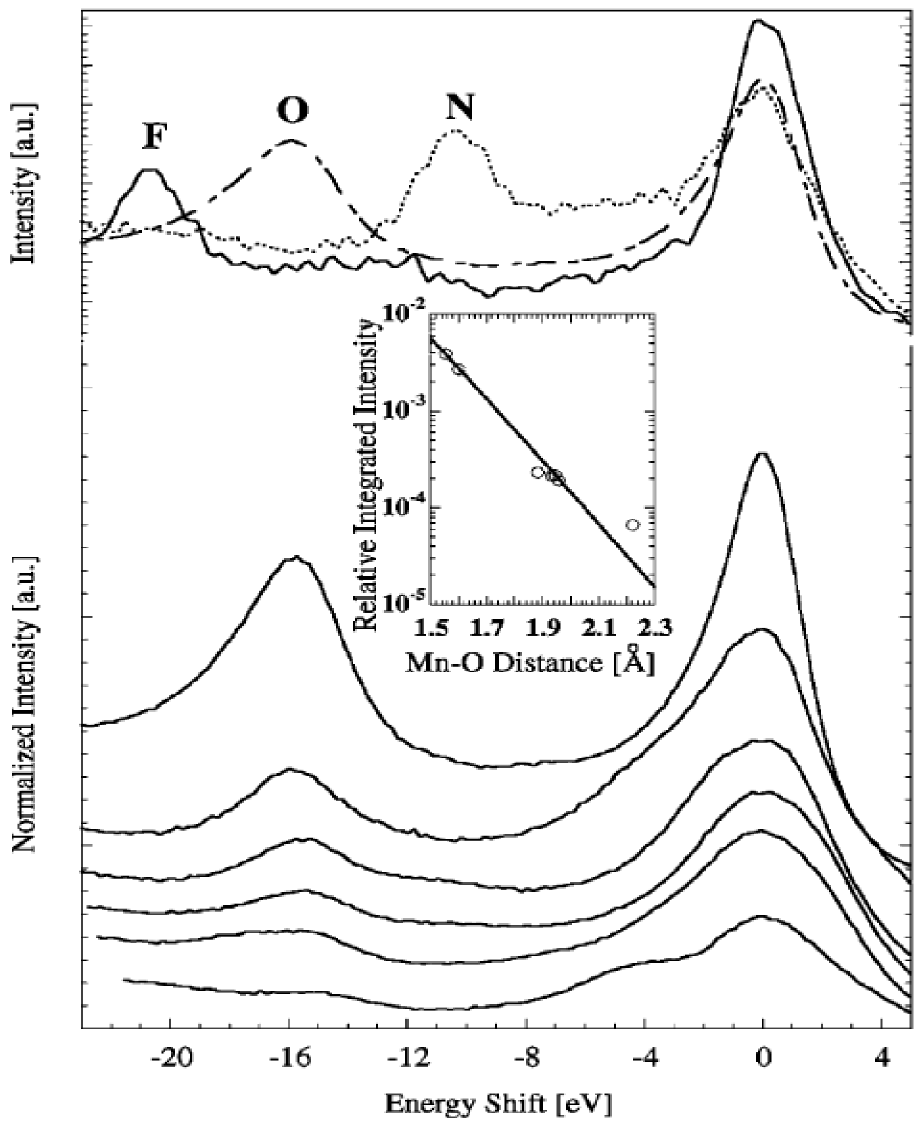


Fig .2

U. Bergmann, C.R. Horne, T.J. Collins, J.M. Workman, S.P. Cramer, Chem. Phys. Lett. 302 (1999) 119.

Experimental Setup

Before any measurements can be taken, calculations must first be made. One critical calculation is determining the correct crystal for the photon emission energy of interest. This is obtained using Bragg's law:

$$2d\sin(\theta) = n\lambda$$

From this, a table relating emission energies to analyzer crystals was compiled (Table 1). For the case of nitrogenase, the emission energy of Fe Kβ is 7057.980 eV with the corresponding crystal of Ge(620).

Another critical piece of experimental hardware that I was heavily involved in, was the sample box. Special design considerations included the ability for the sample box to contain a He gas environment and accommodate a homologated sample holder. The sample box was manufactured by milling aluminum square tubing.

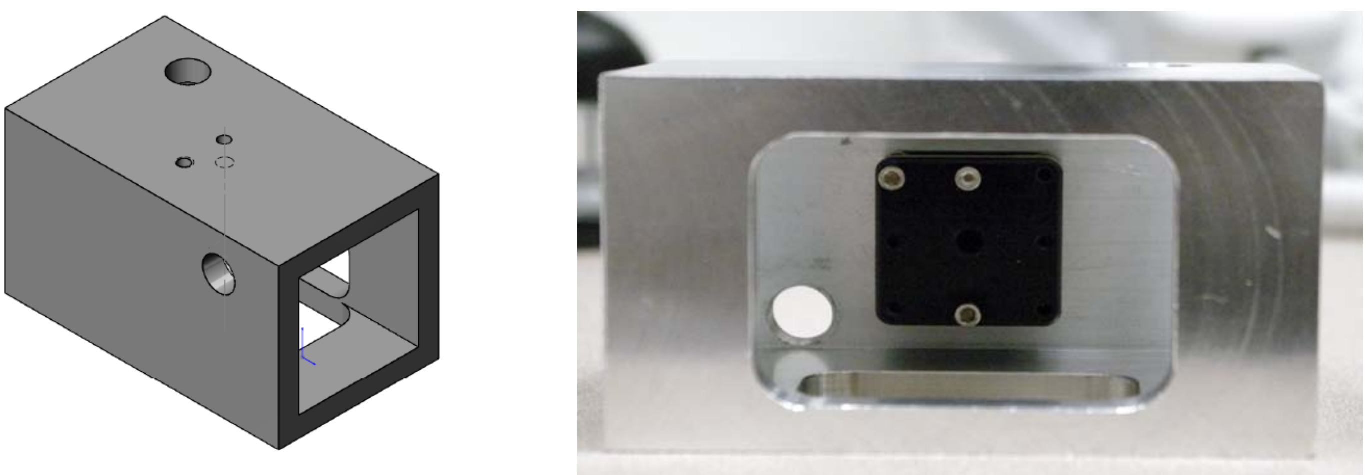


Fig. 3: Sample box (Design and Construction)

Additional preparation (assembly/sample preparation/sealing/sheilding)took place on-site at the Advanced Photon Source at Argonne National Laboratory.

Fig.4: Spectrometer Assembly

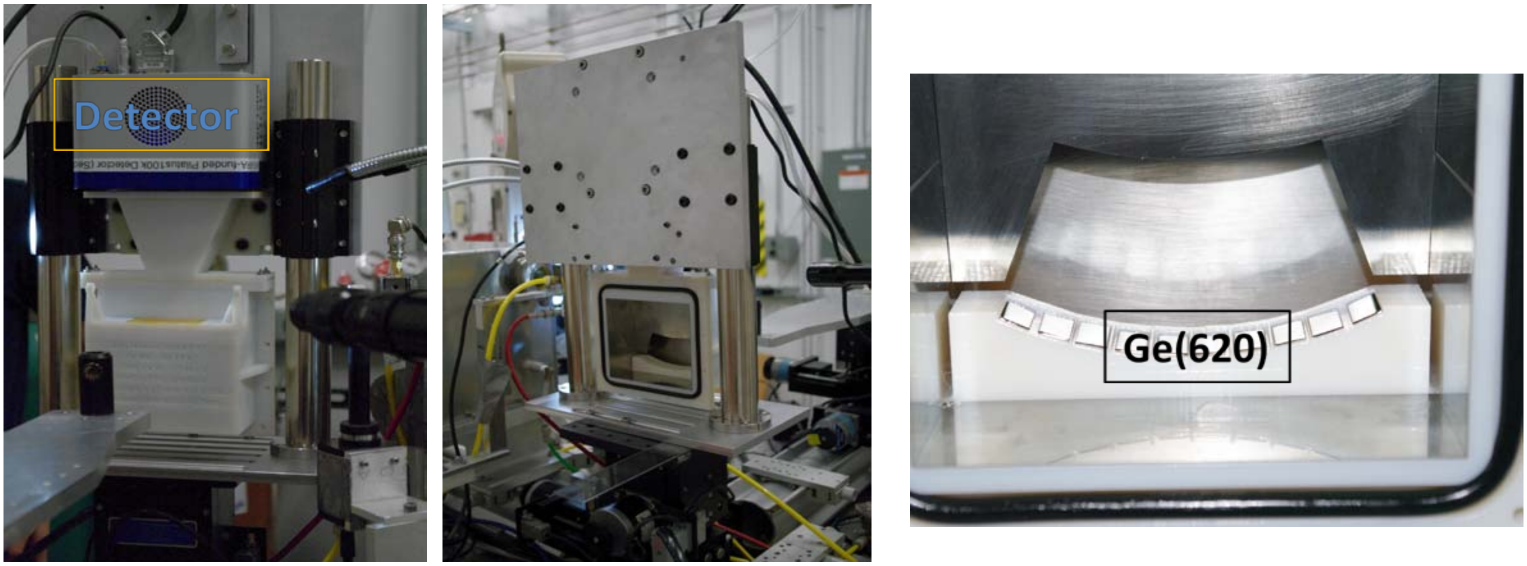


Fig. 5:Noise Reduction

He gas environment in the spectrometer (left), in sample box (center), Lead shield (right)

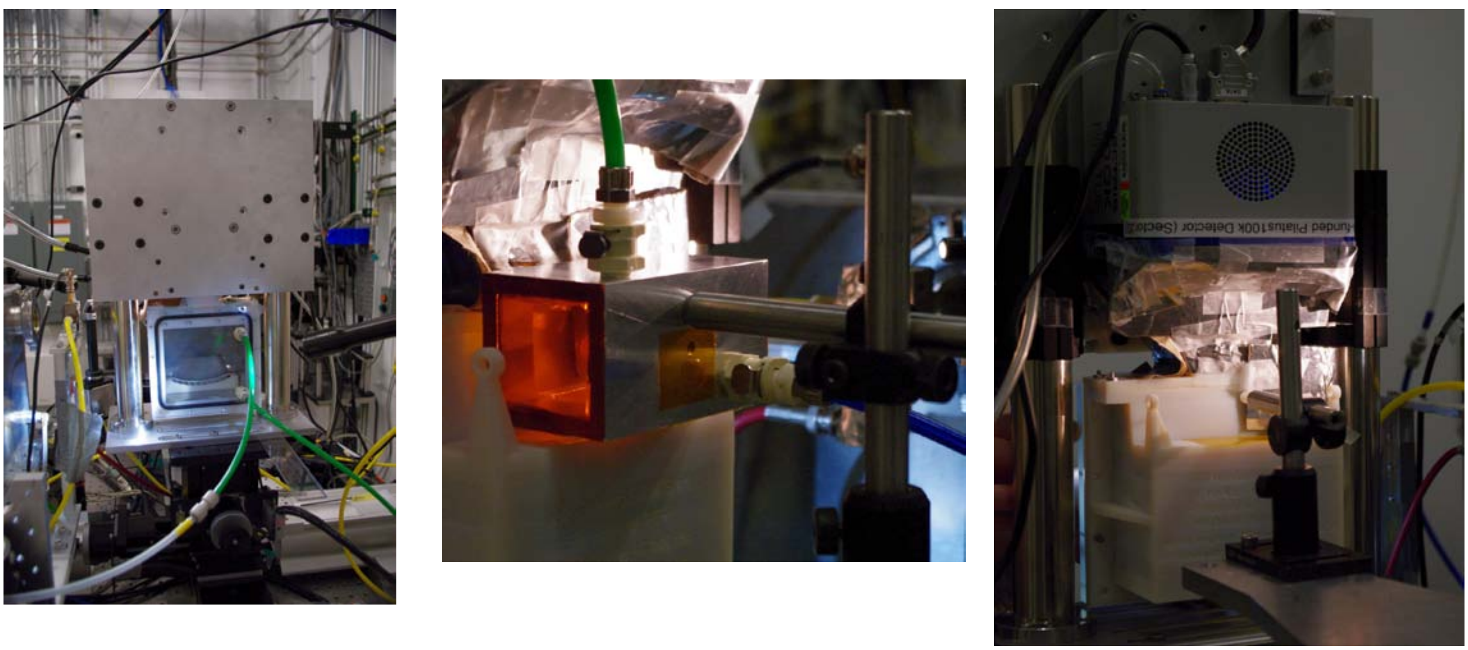


Fig. 6: Raw Spectrometer data

Fe Elastic scattering lines (bottom) and fluorescence lines (top)

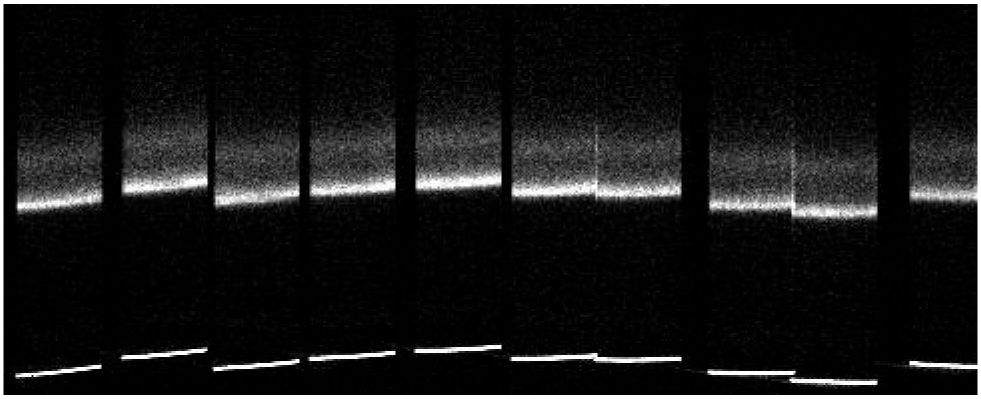


Fig. 7: Calibriation Matrix

Using the elastic scattering lines, the energies associated with each pixel can be assigned

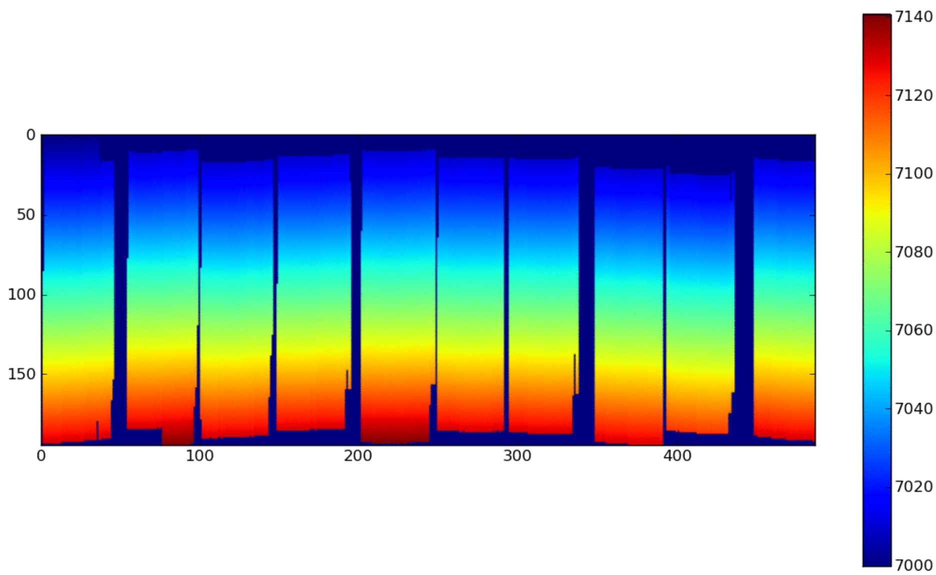


Fig. 8: Nitrogenase Simulation with Fe(VI)N

The purpose of this measurement is to observe the Fe Kβ’’ in a sample similar to nitrogenase and determine systematic considerations for nitrogenase in future experiments.
The initial plot (blue line) is the initial 30s exposure.

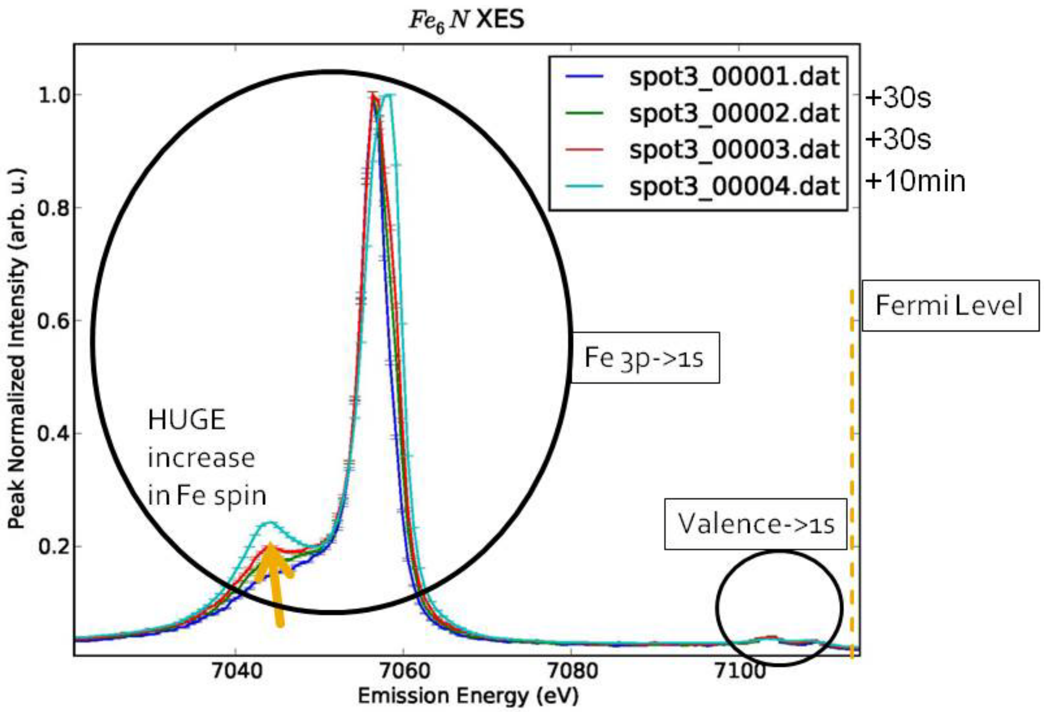
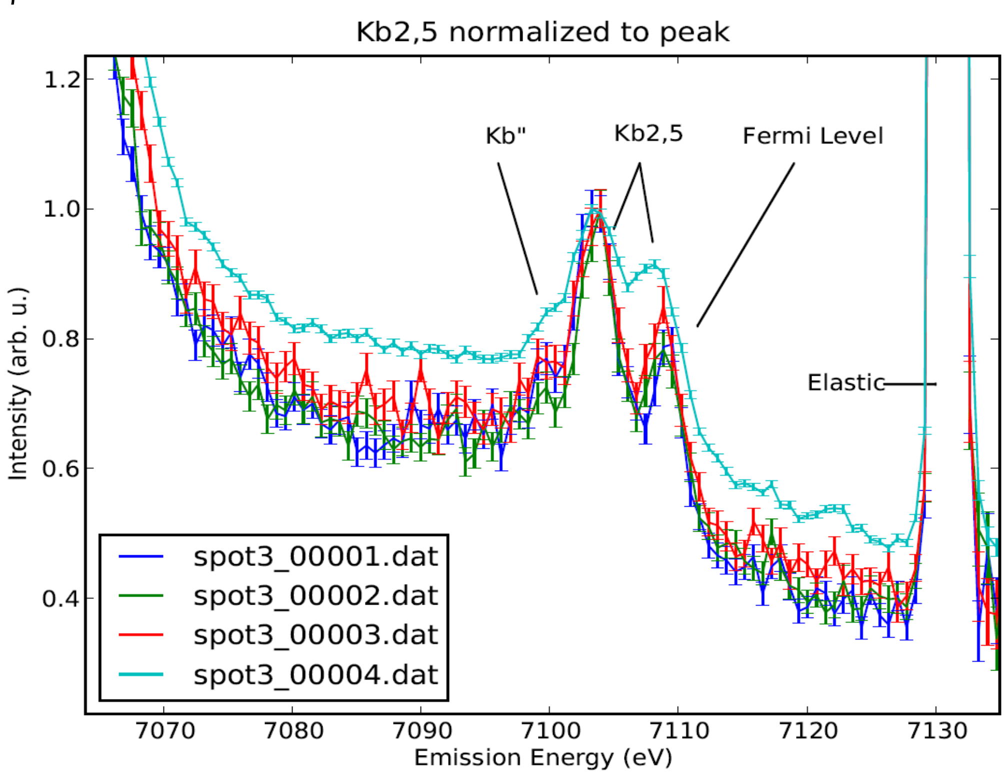


Fig. 9: Kβ Plot



Analysis

Fig. 8 shows that each subsequent exposure to the beam changes the Fe(IV)N spectrum. This is most evident in the Fe 3p→1s transition, where there is a large increase in Fe spin. This spin is likely explained by spin trapping (Vanko, et al, Angew. Chem. Int. Ed. 2007), where high energy excitations cause a cascade of ionization in the sample. As the cascades settle down, the valence electrons are captured in a high spin state.

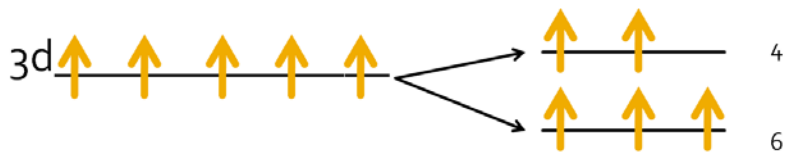


Fig. 10: Spin trapping

Fig. 9 provides additional detail on the effects of beam exposure on the sample, specifically, on the presence of the Kβ'' emission. The 10 minute exposure clearly shows the Kβ'', but not so much on the shorter exposures. The dramatic change in spectra indicates that beam damage is evident and should be taken into consideration during future experiments.

Conclusions

The study of nitrogenase has been greatly aided by experimenting on. Fe(VI)N. Most important is the indication of beam damage on the Fe(IV)N. Additionally, it was possible to observe the faint emission of Kβ'', giving further insight as to what might be contained in nitrogenase's MoFe cofactor. For future experiments, one must take beam damage countermeasures. Such countermeasures could include cooling the sample to counter act small damages and possibly sweeping the beam over the sample to prevent large scale damage from prolonged exposure.

Table 1: Emission line energy and corresponding crystals

Element	Emission Line	Emission line Energy	Low E	High E	Crystal	Low θ	High θ	Average angle
Sc	Kα	4090.600	4065.600	4105.600	Ge(311)	62.2765	63.3691	62.8228
Sc	Kα	4090.600	4065.600	4105.600	Si(311)	67.237	68.6204	67.9287
Sc	Kβ	4460.500	4415.500	4475.500	Ge (400)	78.3384	83.0569	80.69765
Ti	Kα	4510.800	4485.800	4525.800	Ge(400)	75.5749	77.7173	76.6461
Ti	Kβ	4931.810	4886.810	4946.810	Ge(311)	74.9171	77.7971	76.3571
V	Kα	4952.200	4927.200	4967.200	Ge(331)	74.0677	75.7878	74.92775
V	Kβ	5427.290	5382.290	5442.290	Ge(422)	80.5352	85.8537	83.19445
Cr	Kα	5414.700	5389.700	5429.700	Ge(422)	81.3685	84.8809	83.1247
Cr	Kβ	5946.710	5901.710	5961.710	Ge(511),(333)	72.7593	74.7479	73.7536
Cr	Kβ	5946.710	5901.710	5961.710	Si(422)	69.7171	71.357	70.53705
Cr	Kβ	5946.710	5901.710	5961.710	Si(511),(333)	84.2059	90	87.10295
Mn	Kα	5898.750	5873.750	5923.750	Ge(511),(333)	74.3258	75.7825	75.05415
Mn	Kα	5898.750	5873.750	5923.750	Si(422)	71.0142	72.1831	71.59865
Mn	Kβ	6490.450	6458.450	6505.450	Si(440)	83.0123	88.8416	85.92695
Mn	Kβ	6490.450	6458.450	6505.450	Ge(531)	85.2091	90	87.60455
Fe	Kα	6403.800	6378.800	6418.800	Ge(440)	74.9512	76.3513	75.65125
Fe	Kβ	7057.980	7012.980	7072.980	Ge(620)	78.4729	81.1944	79.83365
Co	Kα	6930.300	6905.300	6945.300	Si(531)	76.4868	77.9453	77.21605
Co	Kβ	7649.430	7604.430	7664.430	Ge(444)	82.1052	86.6954	84.4003
Co	Kβ	7649.430	7604.430	7664.430	Si(533)	77.582	79.8372	78.7096
Ni	Kα	7478.150	7453.150	7493.150	Ge(533)	73.525	74.5989	74.06195
Ni	Kα	7478.150	7453.150	7493.150	Si(620)	74.4618	75.6091	75.03545
Ni	Kα	7478.150	7453.150	7493.150	Si(533)	87.3472	90	88.6736
Ni	Kβ	8264.660	8219.660	8279.660	Ge(642)	82.049	86.043	84.046
Ni	Kβ	8264.660	8219.660	8279.660	Si(711),(551)	79.9148	82.6286	81.2717
Cu	Kα	8047.800	8022.800	8062.800	Ge(711),(551)	76.0628	77.2648	76.6638
Cu	Kα	8047.800	8022.800	8062.800	Si(444)	78.7668	80.31	79.5384
Cu	Kβ	8905.290	8860.290	8920.290	Ge(800)	79.3363	81.6443	80.4903
Cu	Kβ	8905.290	8860.290	8920.290	Si(731),(553)	79.3906	81.714	80.5523
Zn	Kα	8638.900	8613.900	8653.900	Ge(731),(553)	76.5583	77.721	77.13965
Zn	Kα	8638.900	8613.900	8653.900	Si(642)	80.7753	82.5909	81.6831
Zn	Kβ	9572.000	9527.000	9587.000	Ge(822),(660)	75.896	77.4122	76.6541
Zn	Kβ	9572.000	9527.000	9587.000	Ge(751),(555)	81.8314	84.9304	83.3809
Zn	Kβ	9572.000	9527.000	9587.000	SI(733)	77.0546	78.7313	77.89295

Ce	Kα	4650.970	4619.230	4665.970	Si(400)	78.1111	81.2836	79.69735
Pr	Kα	5033.700	4998.500	5048.700	Si(331)	80.235	84.5078	82.3714
Nd	Kα	5230.400	5192.700	5245.400	Si(331)	71.5419	73.3719	72.4569
Pm	Kα	5432.500	5392.800	5447.500	Ge(422)	80.212	84.5256	82.3688
Sm	Kα	5636.100	5594.000	5651.100	Ge(422)	71.794	73.6654	72.7297
Sm	Kα	5636.100	5594.000	5651.100	Si(422)	81.7091	88.4827	85.0959
Eu	Kα	5845.700	5801.600	5860.700	Ge(511),(333)	76.2951	78.9396	77.61735
Eu	Kα	5845.700	5801.600	5860.700	Si(422)	72.5844	74.5533	73.56885
Gd	Kα	6057.200	6010.000	6072.200	Ge(511),(333)	69.6671	71.3329	70.5
Gd	Kα	6057.200	6010.000	6072.200	Si(511),(333)	77.6309	80.7147	79.1728
Tb	Kα	6272.800	6223.000	6287.800	Ge(440)	80.3412	84.9315	82.63635
Tb	Kα	6272.800	6223.000	6287.800	Si(511),(333)	70.6125	72.3862	71.49935
Dy	Kα	6495.200	6442.700	6510.200	Ge(440)	72.2033	74.1799	73.1916
Dy	Kα	6495.200	6442.700	6510.200	Ge(531)	84.7338	90	87.3669
Dy	Kα	6495.200	6442.700	6510.200	Si(440)	82.6792	90	86.3396
Ho	Kα	6719.800	6664.500	6734.800	Ge(531)	74.2744	76.5872	75.4308
Ho	Kα	6719.800	6664.500	6734.800	Si(440)	73.4901	75.6696	74.57985
Er	Kα	6948.700	6890.000	6963.700	Ge(620)	84.3878	90	87.1939
Er	Kα	6948.700	6890.000	6963.700	Ge(531)	68.5803	70.2013	69.3908
Er	Kα	6948.700	6890.000	6963.700	Si(440)	68.011	69.5823	68.79665
Er	Kα	6948.700	6890.000	6963.700	Si(531)	75.8707	78.5563	77.2135
Tm	Kα	7179.900	7118.100	7194.900	Ge(620)	74.4137	76.8102	75.61195
Tm	Kα	7179.900	7118.100	7194.900	Ge(533)	87.0723	90	88.53615
Tm	Kα	7179.900	7118.100	7194.900	Si(531)	69.8153	71.5703	70.6928
Yb	Kα	7415.600	7352.300	7430.600	Ge(620)	68.8554	70.4939	69.67465
Yb	Kα	7415.600	7352.300	7430.600	Ge(533)	75.2433	77.7725	76.5079
Yb	Kα	7415.600	7352.300	7430.600	Si(620)	76.3032	79.085	77.6941
Lu	Kα	7655.500	7589.900	7670.500	Ge(533)	69.5163	71.2126	70.36445
Lu	Kα	7655.500	7589.900	7670.500	Ge(444)	81.7847	90	85.89235
Lu	Kα	7655.500	7589.900	7670.500	Si(620)	70.25	72.0212	71.1356
Lu	Kα	7655.500	7589.900	7670.500	Si(533)	77.3778	80.4685	78.92315

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