

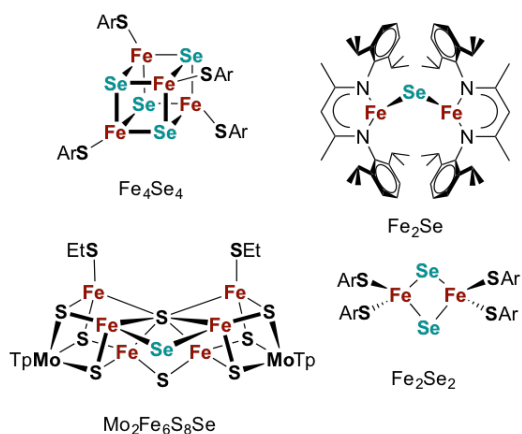


	<b>Experiment title:</b> X-ray Spectroscopic Studies of Catalytically Induced Selenium Incorporation in the Iron-Molybdenum Cofactor of Nitrogenases	<b>Experiment number:</b> CH-4964
<b>Beamline:</b> ID26	<b>Date of experiment:</b> from: 19.10.2016 to: 25.10.2016	<b>Date of report:</b> 23.02.2017
<b>Shifts:</b> 18	<b>Local contact(s):</b> Pieter Glatzel	<i>Received at ESRF:</i>
<b>Names and affiliations of applicants (* indicates experimentalists):</b> Prof. Dr. Serena DeBeer*, Max Planck Institute for Chemical Energy Conversion Dr. Justin Henthorn*, Max Planck Institute for Chemical Energy Conversion Dr. George Cutsail III*, Max Planck Institute for Chemical Energy Conversion Casey van Stappen*, Max Planck Institute for Chemical Energy Conversion Prof. Dr. Oliver Einsle, University of Freiburg		

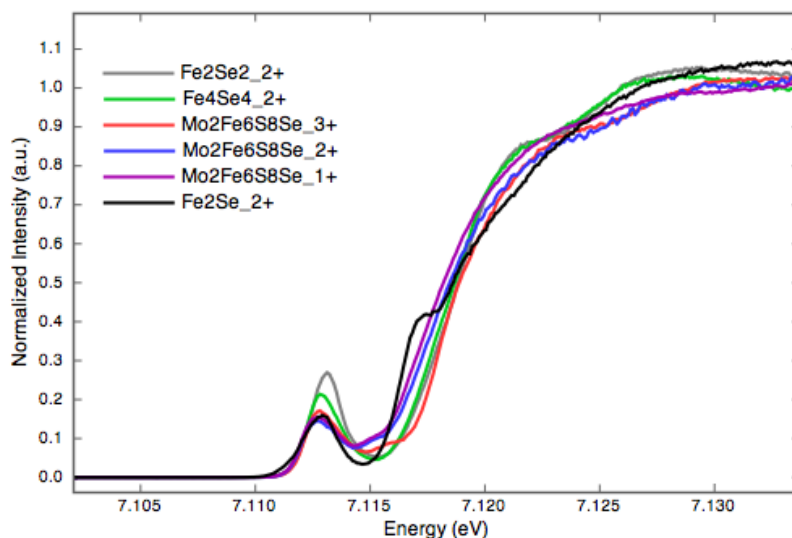
## Report:

The fixation of atmospheric nitrogen ( $N_2$ ) to bioavailable ammonia ( $NH_3$ ) is a critical process in sustaining life. In nature, this process is accomplished through the nitrogenase family of enzymes, of which there are three known types, distinguished by the identity of the heterometal in the active site. The most widely studied Mo-dependent nitrogenase utilizes the highly complex  $[MoFe_7S_9C]$  heterometallic iron-sulfur cofactor for catalysis, referred to as the iron-molybdenum cofactor, or FeMoco. Recent studies by Spatzal and Rees have shown that a sulfide bridge within the FeMoco active site can be replaced by Se and that the Se atom can migrate to the other belt sulfide positions only when the enzyme is turning over non-proton substrates.<sup>1</sup> Based on this discovery, we had proposed using Se HERFD XAS and EXAFS measurements as a selective probe of the electronic and geometric structure of the nitrogenase active site. Unfortunately, our collaborators were unable to provide selenated protein samples in time for the experiment due to purification issues, and we instead turned to Fe  $K\alpha$  HERFD XAS measurements of our FeSe model complexes as a complementary experiment to the proposed Se XAS experiments.

The FeSe model complexes investigated included small di-iron complexes  $Fe_2Se$  and  $Fe_2Se_2$ , the cubane  $Fe_4Se_4$ , and the larger heterometallic fused double-cubane  $Mo_2Fe_6S_8Se$  complexes (**Figure 1**). In addition to the varying sizes and geometries, the model complexes represent average Fe oxidation states ranging from  $Fe^{2+}$  to  $Fe^{3+}$ . Through our measurements (**Figure 2**), we were able to observe anticipated trends in the pre-edge and rising edge features, with the pre-edge feature increasing in intensity and the rising edge shifting to higher energy as the complement of oxidized Fe increases in the complex. Additionally, for the heterometallic complexes a feature assigned to metal-metal charge transfer could be observed between the pre-edge and rising edge, indicative of the presence of Mo in the complex.



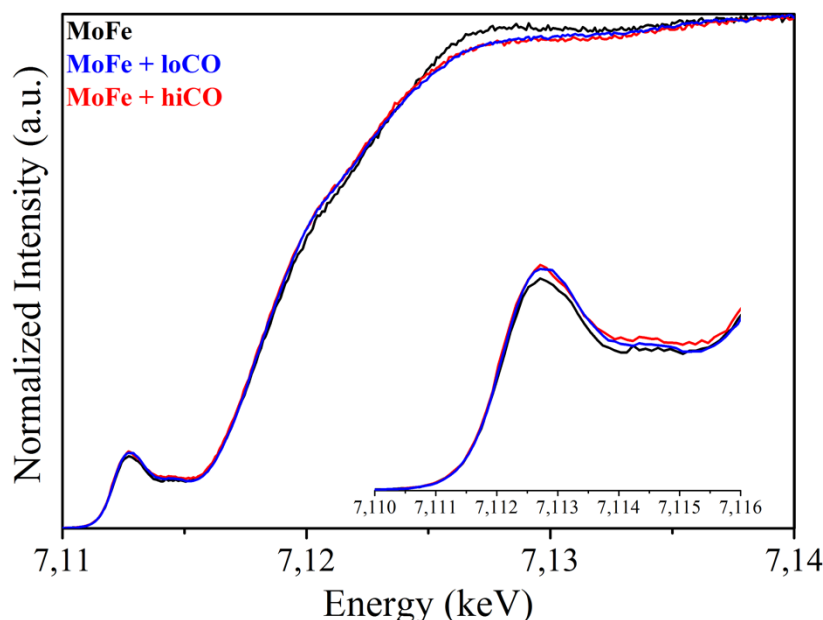
**Figure 1.** FeSe model complexes used in this study.



**Figure 2.** Fe K $\alpha$  HERFD XAS data for FeSe model complexes.

In addition to  $\text{N}_2$ , nitrogenases have recently been found to activate small molecules such as CO, CN, and  $\text{CO}_2$ .<sup>2</sup> At low concentrations CO acts as a potent inhibitor for  $\text{N}_2$  reduction, while at higher concentrations it may be reduced to form small energy rich hydrocarbons such as ethylene and ethane. Therefore, the nature of the interaction of CO with the catalytic iron-sulfur cluster is of high interest. Recent crystallographic studies have proposed a bridging binding motif of CO in the cluster, while other spectroscopic studies have favored alternative assignments.

In our measurements, we investigated whether the presence of CO induced significant perturbations in the electronic and geometric structure of FeMoco through the use of Fe K $\alpha$  HERFD XAS. With the addition of CO to the MoFe protein during turnover conditions, three notable changes were observed to occur (**Figure 3**). Firstly, the pre-edge region shows an increase in intensity. Secondly, a small shift occurs in the rising edge to lower energies.



**Figure 3.** Fe K $\alpha$  HERFD XAS data for MoFe protein compared to loCO and hiCO conditions.

Lastly, there is a significant decrease in the white line intensity. The second and third changes are particularly interesting together, since a shift to lower energy in the edge is typically indicative of a more reduced state; contrary to this, it has been shown that a decrease in white line intensity in iron-sulfur clusters points to a more oxidized state.<sup>3</sup> In addition to standard XAS measurements, the Fe K HERFD EXAFS was also measured on the MoFe and MoFe + hiCO samples. On average, it was seen that the Fe-S distances increased by 0.03 Å while the Fe-Fe distances decreased by 0.02 Å in the MoFe + hiCO sample relative to the resting state MoFe. These measurements also imply a slight expansion and distortion of the cluster upon CO binding.

- 1) T. Spatzal, K. A. Perez, J. B. Howard, D. C. Rees, *eLife* **2015**, 4, e11620.
- 2) a) Lee, C.C., et al. *Science* **2010**, 329, 642. b) Hu, T. et al. *Science* **2011**, 333, 753.
- 3) Rees, J.A., et al. *Dalton Trans.* **2017**, 46, 2445.