ESRF	**Experiment title:** XAS and XES Investigation of VFe Nitrogenase	**Experiment number**: CH-4356
Beam line: BM23	**Date of experiment**: from: 31/03/2015 to: 07/04/2015	**Date of report**: 01/09/2015
Shifts: 18	**Local contact(s)**: Dr. Pieter Glatzel	*Received at ESRF:*

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Nature's enzymatic catalyst for the reduction of atmospheric dinitrogen (N_2) to ammonia is the nitrogenase enzyme, a complex, multicomponent metalloprotein. The complete atomic structure of the active site cofactor of the Mo nitrogenase, a [Mo-7Fe-9S-C] cluster (FeMoco), was only discovered in 2011, through the use of Kβ valence-to-core (VtC) X-ray emission spectroscopy. In addition to the Mo-dependent nitrogenase, there is also a vanadium-dependent variant which is also capable of reducing N_2, however with less efficiency. Intriguingly, the V nitrogenase is also capable of reducing CO, and can perform reductive C-C bond coupling to form two and three carbon chains in a Fischer-Tropsch fashion. The subtle differences in geometric and electronic structure between these two variants that is responsible for regulating this functionality are not understood, and in fact the atomic structure of the V nitrogenase is not yet known. Considerable progress towards this latter point has been made using data from this experiment however, as the presence of a central carbon in the Fe-V cofactor (FeVco) has been experimentally determined for the first time.

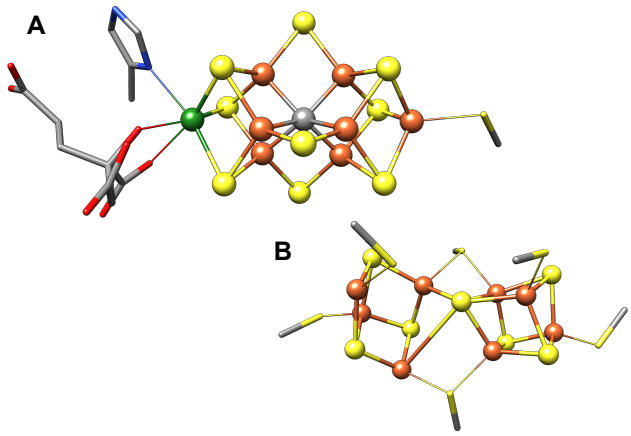

Figure 1. Structural models of FeMoco (A) and the [8Fe-7S] P-cluster (B) from the X-ray structure of *Azotobacter vinelandii* Mo nitrogenase MoFe protein (PDB 3U7Q).

Figure 2 shows the VtC regions of the Fe Kβ XES spectra of the MoFe and VFe proteins of *Azotobacter vinelandii*, and the indicated Kβ" feature at ~7100 eV has been assigned as fluorescent emission from the central carbide.

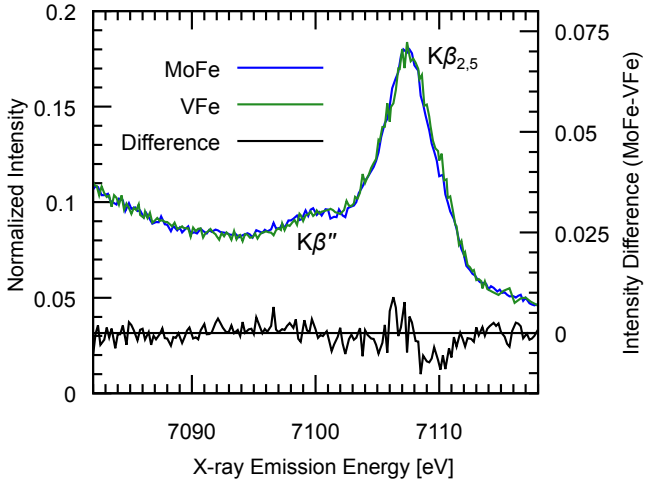

Figure 2. Fe Kβ VtC XES spectra of MoFe and VFe proteins of *A. vinelandii*

This work has been accepted for publication in Angewandte Chemie Int. Ed. and is currently in press
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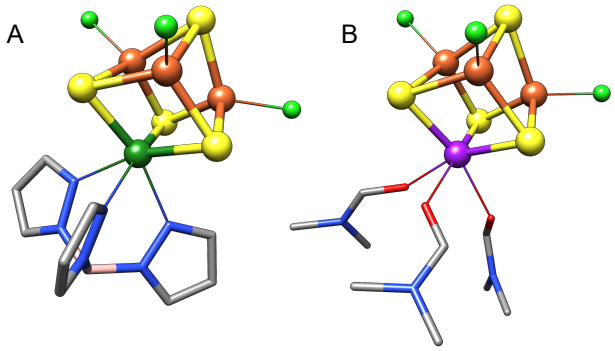
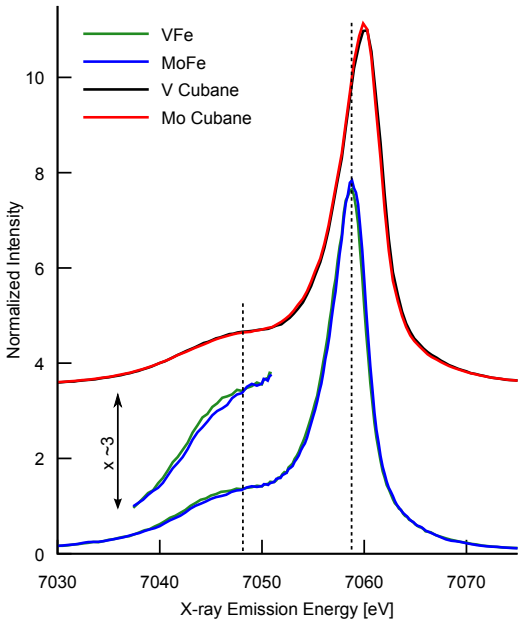


Figure 3. X-ray structural representations of the $MoFe_3S_4$ (A) and VFe_3S_4 (B) models used in this study.

Additionally two synthetic metallocubane models for the Mo and V nitrogenases were studied. Using Kβ XES and Kβ-detected XAS (HERFD), the interaction of the heteroatom with the remainder of the cluster has been characterized, with the goal of determining a) the manner and extent of direct metal-to-metal bonding b) the oxidation and spin states of the Fe atoms and c) the relative covalencies of the clusters possessing different heterometals. The Kβ XES spectra were recorded for both cubane models and proteins, and are shown in Figure 4. The decreased splitting of the $K\beta_{1,3}$ and Kβ' features (vertical dashed lines) indicate that the Fe atoms in the protein clusters are more covalent, consistent with the expected effect of a central carbon atom. Additionally, the increased Kβ' feature of VFe (inset) shows that the Fe atoms in the V nitrogenase are less covalent than in the Mo nitrogenase. In addition to XES data, the XAS spectrum at both the $K\beta_{1,3}$ and Kβ' features were recorded for all complexes.



A manuscript detailing this work is in preparation for publication.