ESRF	**Experiment title:** Mo X-ray Magnetic Circular Dichroism Studies of FeMo cofactor of Nitrogenase	**Experiment number**: CH-4172
Beam line: ID12	**Date of experiment**: from: 26/08/2014 to: 29/08/2014	**Date of report**: 10/06/2016
Shifts: 9 in 16 bunches mode	**Local contact(s)**: Dr. Katharina Ollefs	*Received at ESRF:*

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Scientific Background:

The reduction of atmospheric nitrogen (N_2) to ammonia (NH_3) represents a fundamental biological, ecological and economical importance. In Nature, this reaction is realized via Mo-nitrogenase (Mo-N2ase) enzyme, which in contrast to the industrially known Haber-Bosch process operates at ambient pressure and temperatures. Thus, this motivates great interest in understanding how nature performs this conversion with optimal thermodynamics. The active site of Mo-N2ase is a MoFe7S9C cofactor (so called FeMoco, Figure 1). The understanding of the biological nitrogen fixation requires a detailed description of the electronic structure of N2ase, involving the oxidation states of all atoms and their magnetic coupling. The atomic and crystallographic structure of this cofactor is now complete: the central atom was revealed as carbon by X-ray Emission Spectroscopy (XES) (Lancaster et al. Science, 2011) and the Mo atom was assigned as Mo(III) via High Energy Resolution Fluorescence Detected (HERFD) X-ray Absorption Spectroscopy (XAS) supported with Quantum Mechanical Computations (Bjornsson et al. Chemical Science 2014). The above mentioned TDDFT (Time Dependent Density Functioncal Theory) calculations revelad an unusual non-Hund configuration at the Mo atom (2β and 1α spin electrons), with the electrons partially delocalized between the Mo and Fe atoms, pointing towards strong coupling between these two elements (proposals: CH-3556 and CH-3756).

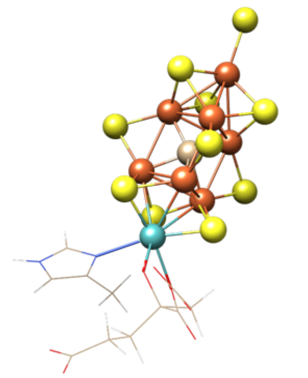

Figure 1. FeMo cofactor of nitrgenase

The goal of our study was to experimentally validate the unusual spin coupling proposal with XMCD at the Mo $L_{2,3}$ edges. This kind of spectroscopy has the ability to selectivly probe the local spin environment. Unravelling the spin coupling in this complex system may provide the key to understand the reactivity of this complex system.

Results:

Our ID12 beam time has employed pilot XMCD measurments at Mo $L_{2,3}$ edges on a $(Et_4N)[(Tp)Mo^{III}Fe_3S_4Cl_3]$ model (so called Holm cubane, Figure 2) that represents a structural model of the FeMoco, and also on Mo reference compounds: $Mo^{III}(acac)_3$ and $Mo^{IV}(tpy)_2$.

Since the proposed study is a unsual approach in studying bioinorganic system the allocated 3 days of beamtime were used as a pilot study for future, extended investigations.

This reports represent initial observations that will help us in the future to design properly the measurments and understand factors that wil influence the obtained signals.

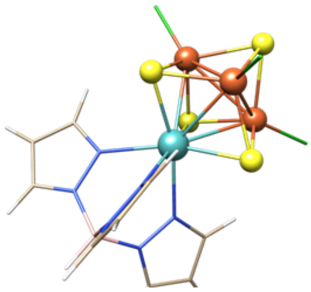

Figure 2. The structure of the Holm cubane

Because all of the systems are paramagnetic, the measurements were performed at a temperature of 2K and 10 T magnetic field. The L-edge X-ray absorption spectra were obtained at 0T magnetic field to get a background reference for further XMCD signals. Figure 3 (top) and Figure 4 (top) represents an average of about 10 spectra for both L-edges, obtained as seen with very good singal-to-noise ratio. Due to the small polarization rate (12% for L_3-edge and 6% for L_2-edge), we increased the number of scans for the measurements performed with applied magnetic field and optained 60 and 120 spectra for L_3- and L_2-edge spectra, respectively in order to get proper statistics for the XMCD signals.

The Holm model contains a Mo(III) atom (1 unpaired electron) and has a total spin of S=3/2. However, the experimental Mo XMCD measurements do not clearly support the calculated prediction. The XMCD signal intensity was greatly diminished for this compound for both L_3 and L_2 edges (Figure 3 bottom and Figure 4 bottom).

This observation needs futher studies. It could indicate the presence of a delocalized electron density or strong covalent contribution from the neighboring atoms. Similar initial observation were made for the other measured compounds: Mo(III) and Mo(IV) references, although the later is predictable. In order to understand these results, we need to obtain data on models of increaing complexity, which incorporate both Mo and Fe atoms at differenet oxidation states and in different environment. Although we were not able to obtain an unambiguous assignment of the electronic structure in the Holm cubane, we were able to establish the proper experimental conditions for further measurements at this beam line, including: radiation damage rates and required signal to noise. In addition, our preliminary beam time established that the samples holders will need to be adapted in order to have a larger enough sample area to collect undamanged data.

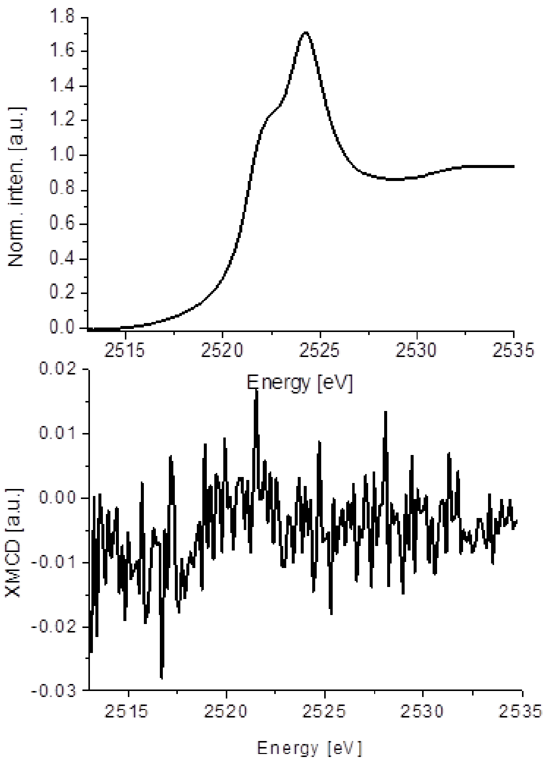

Figure 3. Mo L_3-edge (top) and XMCD (bottom) of $MoFe_3$ Holm cubane

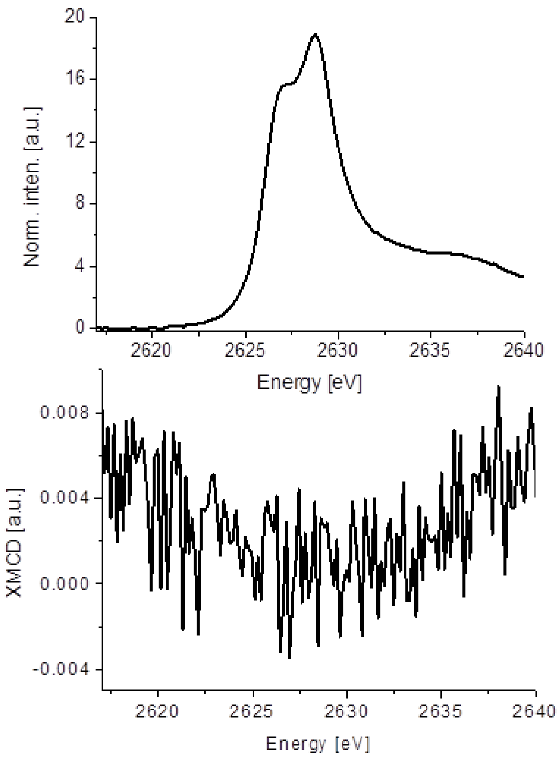

Figure 4. Mo L_2-edge (top) and XMCD (bottom) of $MoFe_3$ Holm cubane