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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** XAS and RIXS Studies of Nitrogenases	**Experiment number**: CH-3756
Beamline: ID26	**Date of experiment**: from: 26.06.2013 to: 02.06.2013	**Date of report**: 21.08.2013
Shifts: 18	**Local contact(s)**: Pieter Glatzel	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Scientific Background: Nitrogenase is a complex enzyme containing a $MoFe_7S_9C$ active site, the so called FeMoco cluster (Figure 1), which enable the conversion of nitrogen to ammonia under ambient conditions. In contrast the industrial process using heterogeneous catalysts requires high temperatures and pressures. This has thus motivated great interest in how the biological system enables this remarkable conversion. It was relatively recently (~2 years ago) that XES spectroscopy, by our group revealed the presence of a central carbon in this cluster. With the atomic composition of the cluster now understood, we have turned our focus to understanding the electronic structure of this complex cluster. Namely, the important questions to address are the oxidation states of the Mo and of the Fe.

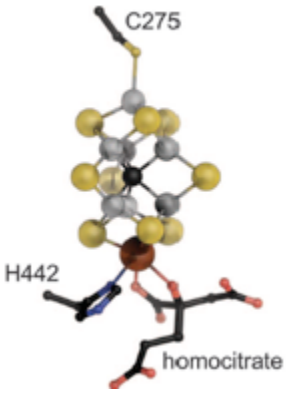

Figure 1. The FeMoco active site of nitrogenase

Results:

Our ID-26 beam time has employed a combination of Mo HERFD and Fe K-α RIXS measurements in order to obtain insight into the FeMoco cluster. Previously (under proposal 3556) we obtained Mo HERFD data on a series of monomeric and dimeric Mo model complexes. These data were calculated using a TDDFT approach, and served as an essential calibration for understaning the Mo HERFD spectra. These results were recently submitted for publication.

Comparison of the Mo models to the nitrogenase protein data, however, required the synthesis and characterization of more complex model complexes. Hence during the present experimental run, we obtained

data on models of increasing complexity, which incorporated both Mo and Fe. These models are depcited in Figure 1 below.

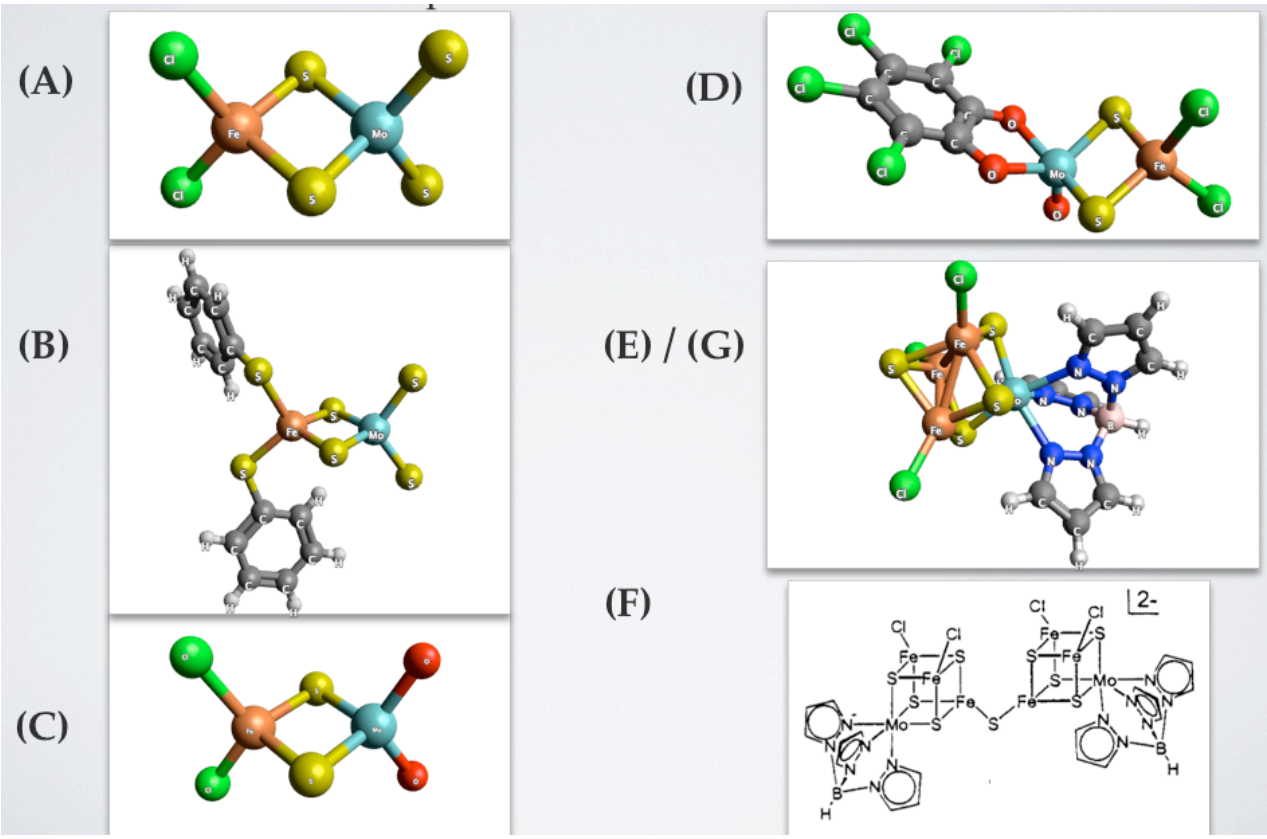

Figure 1. Model complexes examined during the present beam time.

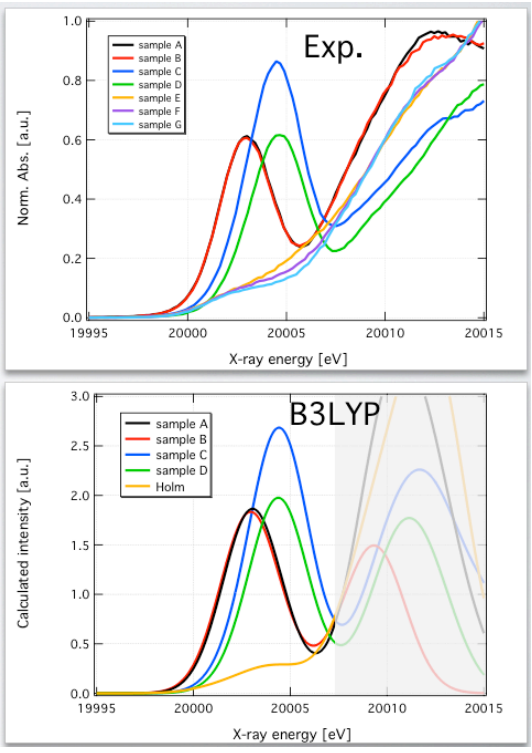

Figure 2. Comparison the experimental and calculated Mo HERFD spectra for a series of FeMo model complexes

These results were correlated to TDDFT calculations, as shown in Figure 2. The results show an excellent correlation between experiment and theory. Importantly, with these data, we were able to show that the commonly accepted assumption of a Mo(IV) in the active site of nitrogenase is incorrect. Our results thus provide evidence for a ***more reduced*** Mo site and indicate an essential role of the Mo in the magnetic coupling within the cluster. The results thus contrast what is commonly accepted in the literature and will likley have a great impact on our understanding of the electronic structure and its contribution to function. A manuscript describing these results is currently being prepared for submission.

In addition to the Mo HERFD data, Fe 1s2p RIXS and Fe HERFD data were obtained for both the nitrogenase protein and the model complexes. Preliminary RIXS planes for the protein are shown below (Figure 3). The protein data is complicated by the fact that the MoFe protein contains both the FeMo cofactor (the active site of interest) and a second eight iron site (the so called P-clusters). Our collaborators (O. einsle) can prepare a gene deletion mutation of the protein which loads only the p-clusters. By obtaining data on this form, we should be able to isolate the FeMo cofactor contribution to the Fe RIXS.

Importantly, our preliminary data indicate that 1s2p RIXS on the dilute protein is feasible, though a large number of protein samples

will be required to obtain damage free data with sufficient signal to noise.

In addition we will collect data on the isolated cofactor (in NMF). We hope to complete the required Fe RIXS measurements in a future ID26 run. By obtaining these data a deatiled picture of the resting state of nitrogenase will be in hand and we will be well positioned to begin more detailed studies of later states in the catalytic cycle.

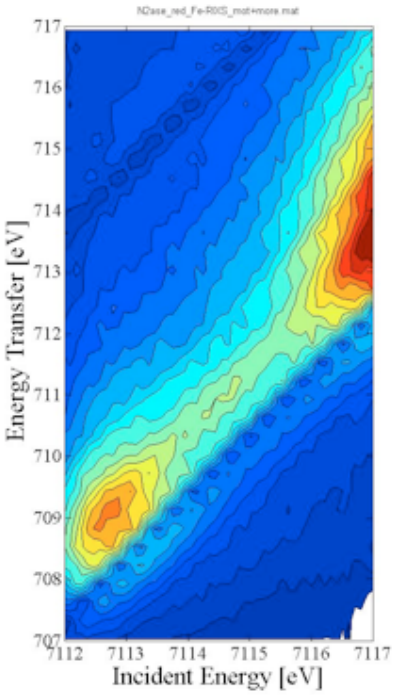

Figure 3. Fe 1s2p RIXS plane of the MoFe protein of nitrogenase.