EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Insight into the Reduced States of Nitrogenase through High Resolution Fe and Mo K-edge XAS	**Experiment number**: CH-5305
Beamline: ID26	**Date of experiment**: from: 29.11.2017 to: 06.12.2017	**Date of report**: 26.02.2018
Shifts: 21	**Local contact(s)**: Blanka Detlefs	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Casey Van Stappen, Max Planck Institute for Chemical Energy Conversion

Serena DeBeer, Max Planck Institute for Chemical Energy Conversion

*Rebeca Gomez Castillo, Max Planck Institute for Chemical Energy Conversion

*Stefan Hugenbruch, Max Planck Institute for Chemical Energy Conversion

Report: High-Energy Resolution Fluorescence Detected (HERFD) XANES of a series of reduced forms of Mo nitrogenase (N2ase) were measured during the allotted beam time reported herein. Previous ^{57}Fe Mössbauer studies found that a distinctly different reduced state M^I was generated in Mo N2ase upon cryoreduction compared to that observed during native turnover (M^R). It was proposed this difference arises from reduction at the Fe-centers of the catalytic FeMoco cluster under cryoreducing conditions, whereas the Mo of FeMoco was reduced under native conditions. At the time of this study, the oxidation state of Mo in the FeMoco was believed to be Mo(IV), and the suggestion of reducing to Mo(IV) to Mo(III) in a biological system was quite reasonable. Since then, the oxidation state of Mo has been reassigned as Mo(III), and as the reduction of Mo(III) to Mo(II) is highly unfavorable under most biological conditions it is clear the question of oxidation state distribution in M^I and M^R must be revisited.

Activation of N_2 by Mo N2ase is accomplished through the subsequent transfer $8e^-$ and $8H^+$; to bind N_2, $4e^-$ and $4H^+$ must first be stored on the FeMoco cluster of the MoFe protein (**Figure 1**). At any step beyond E_2, H_2 may be produced, meaning that e^- and H^+ must be rapidly pushed onto the cluster at a rate greater than H_2 production from the E_2, E_3, and E_4 steps. This is accomplished through a crowding mechanism with the second component of the N2ase system, a reducing Fe protein (FeP). This also implies that under native turnover in the absence of N_2, there is always a distribution of $E_0 \rightarrow E_4$ states which is dependent upon the ratio of FeP:MoFe. At low ratios of FeP:MoFe, only the E_0, E_1, and E_2 can be accessed, and this can be tuned to generate favorable amounts of E_1. Cryoreduction allows a more selective reduction of the FeMoco cluster through controlled doses of radiation, allowing an even more restricted distribution i.e. only E_0 & E_1 to be generated. The amount of E_0 state has an S=3/2 ground state, allowing it to be quantified easily by electron paramagnetic resonance (EPR). Cryoreduced samples were generated at 40-50% E_1, and native samples of ~30% E_1 were produced.

We initially set out to employ Mo and Fe Kα-HERFD XANES to observe where oxidation state changes occur under cryoreducing and native turnover conditions, and compare these to both one another and to the resting state. During this time, the Kα-HERFD of Mo N2ase under reducing conditions was measured at both Fe and Mo for the first time. It was found that no significant changes occur at Mo, disproving the original hypothesis that a Mo-centered reduction occurs under native turnover (**Figure 2**). Additionally, an increase in the Fe white line was observed, which is consistent with some of our previous observations for reduction in iron-sulfur clusters. However, the observed changes in the white line and rising edge in are very, very minute (**Figures 3,4**). Comparing these results to previous data we have collected on model complexes (1), the differences are consistent with what one would expect after accounting for iron concentrations, but unfortunately are still statistically insignificant despite extensive data collection. Further investigations are underway to elucidate these changes at Fe through ^{57}Fe Mössbauer spectroscopy, and we look to continue these studies using ^{57}Fe NIS, Fe K-β XES, and Fe K-β HERFD XAS techniques.

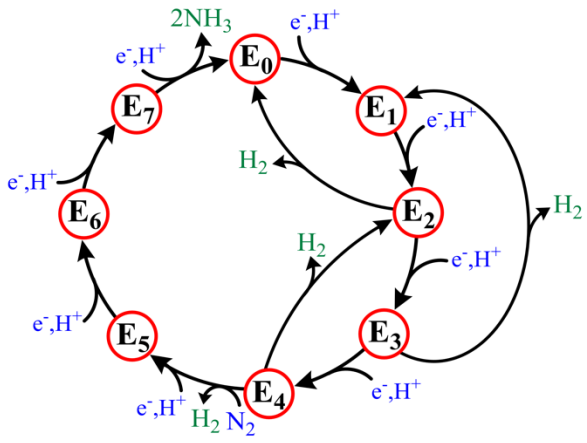

Figure 1. The catalytic cycle of FeMoco in Mo N2ase.

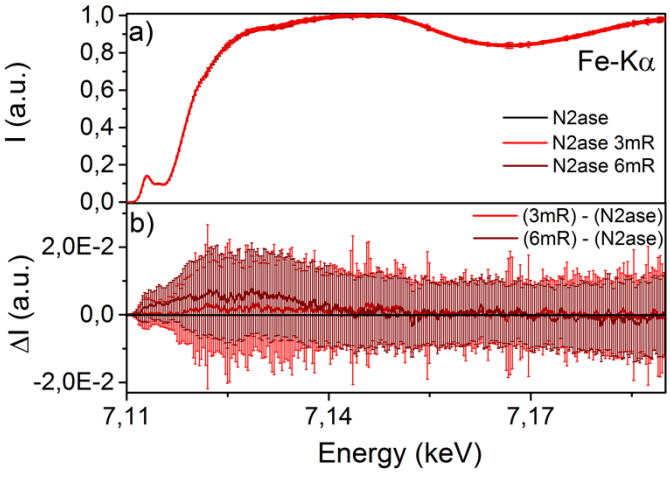

Figure 3. Comparison of resting, 3mR, and 6mR Fe Kα-HERFD spectra as both a) absorption, and b) difference spectra. Standard deviations are included in both a) and b).

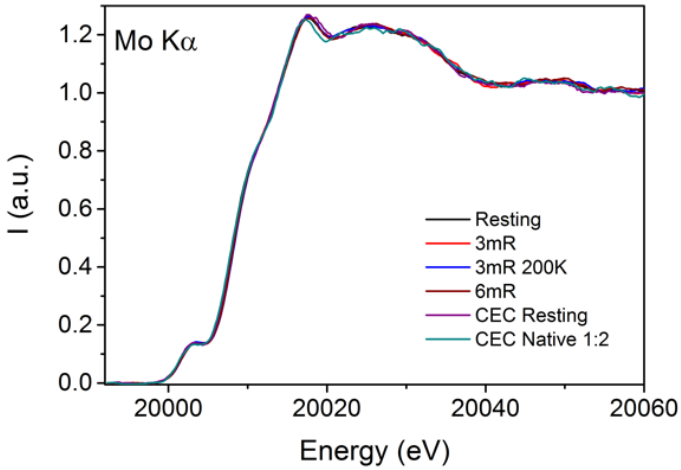

Figure 2. Mo K-α HERFD XAS of a series of Mo N2ase samples. CEC Resting and Resting are resting state MoFe from two separate sources. Native 1:2 corresponds to a 1:2 FeP:MoFe protein ratio. Cryoreduced samples were produced at 3 mega rad (mR) and 6 mR, and the 3 mR sample annealed at 200 K to allow for proton transfer.

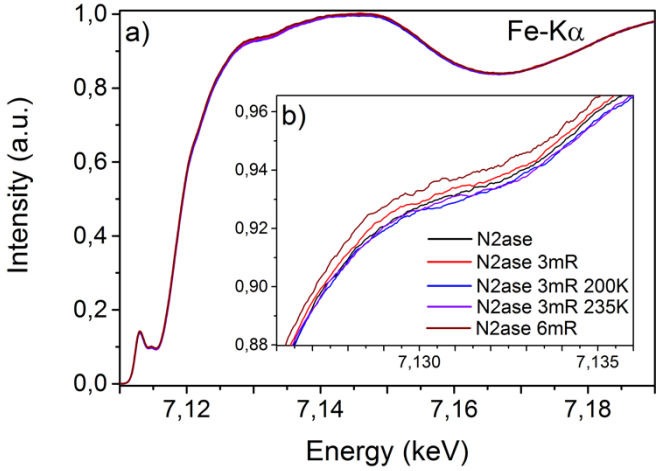

Figure 4. Comparison of Fe Kα-HERFD spectra for resting, 3 mR, 6 mR, and annealed 3mR samples at 200 and 235 K of Mo N2ase. mR = mega rad.

(1) Rees, J.A. & DeBeer, S., et. al. *Dalton Trans.* **2017**,*46*, 2445-2455.