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:** Characterization of the Intermediates in Biological Methane to Methanol Conversion	**Experiment number**: CH - 4046
Beamline:	**Date of experiment**: from: 06.11.2013 to: 12.11.2013	**Date of report**:
Shifts: 18	**Local contact(s)**: Pieter Glatzel	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Soluble methane monooxygenase (MMO) enzymes are able to affect the conversion of methane to methanol under ambinet conditions. As such, this reaction is of great interst in the context of energy conversion. In recent years many details of the mechanism have been elucidated and a general schematic of the proposed mechanism is shown in Figure 1. The catalytic cycle begins with the active site in a diferric resting state, which is then reduced by two electrons to a diferrous state. The diferrous site then reacts with O_2 generating a (μ-peroxo)diferric species (called MMO-P) followed by a diiron(IV) species (MMO-Q), which is responsible for the hydroxylation of methane. The exact structure of Q, however, is hotly debated. Based on comparison to model studies, it is argued that MMO-Q may have either a closed diamond core conformation or an "open" core conformation, as shown in Figure 2. Until now, however, spectroscopic data to validate one model over the other has not been available.

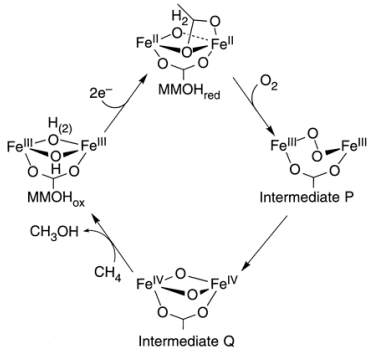

Figure 1: *A proposed catalytic cycle for MMO's conversion of CH_4 to CH_3OH.*

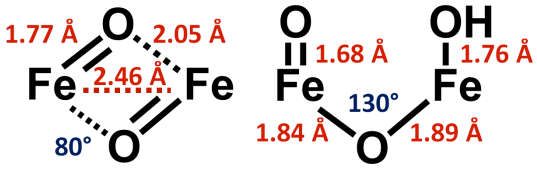

Figure 2: *Metrical parameters for the two competing MMO-Q structures.*

During our beam time, we were able to obtain high

quality HERFD XAS data on the diferrous, diferric and MMO-Q intermediate. The later was particularly challenging because the rate of beam damage was so fast that the sample had to moved to a new spot every ~10 seconds. This required a significant amount of protein sample in order to obtain good statistics. Further, analysis of the data were complicated by the fact that the intermediate sample could only be obtained in 30-40% yields. This required parallel Mössbauer analysis, in order to subtract the other components, and obtain "pure" intermediate spectra. As shown in Figure 3, the data we obtained after processing and subtraction based on Mössbauer analysis are consistent with an Fe(II)Fe(II), Fe(III)Fe(III) and Fe(IV)Fe(IV) series, in terms of both the pre-edge and rising edge.

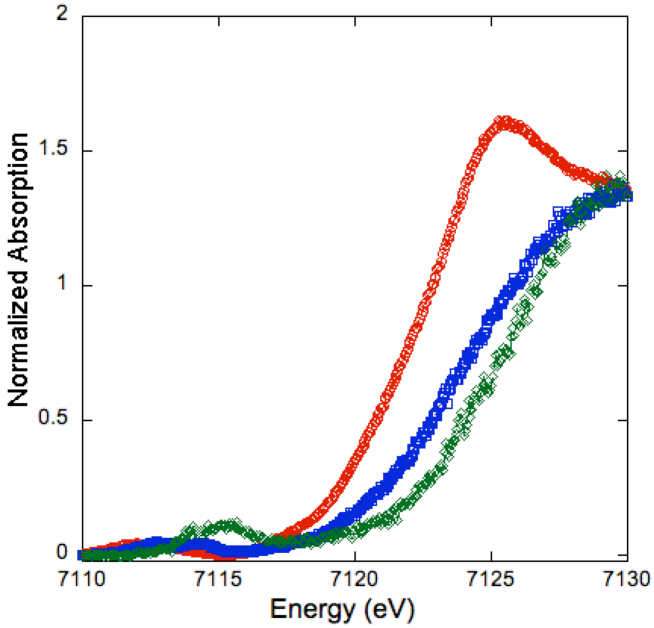

Figure 3. Compariosn of the Fe HERFD XAS data for the diferrous, diferric and MMO-Q intermediate.

In order to establish the spectral signatures for different oxygen binding modes in binuclear iron systems, we also obtained HERFD XAS data on a series of Fe dimers, provided by our collabroator Larry Que. These models provide essential data for establishing the exact structure of the MMO-Q core. As the synthesis and structure of these complexes are not yet published, we will provide furtehr details at a future date.

We are presently working on the correlation of these results to computations. Modeling the protein active site properly is a significant computational task and hence the analysis is taking sometime. Upon completeion of these correlations, we should be able to reliably assign the nature of the MMO-Q core. These data thus mark a very important step toward understanding the process of biological methane oxidation.