EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Characterization of the Intermediates in Biological Methane oxidation	**Experiment number:** **CH-4750**
Beamline: ID 26	**Date of experiment:** from: 29-06-2016 to:1-07-2016	**Date of report:**
Shifts: 9	**Local contact(s):** **Blanka Detlefs**	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

In previous beamtime at ESRF (Experiment CH-4417, CH-4748) we collected Kα HERFD data on Soluble Methane Monooxygenase (sMMO), sMMO-Q, MMO-ox, MMO-red and a serious of model complexes, including a diiron diamond core model and an "open" core model, which provided valuable insights for determining the controversial structure of the intermediate MMO-Q. MMO-Q is the species responsible for extracting a proton from inert methane in the oxidation reaction of methane to methanol by sMMO.

By performing Kα HERFD XAS measurements (proposals 4417 and 4417), we have provided the evidence for a possible "open core" conformation in MMO-Q, where we by comparing the Fe(IV)Fe(IV) bis–oxo diamond core model and the MMO-Q HERFD data strong differences were seen in the pre-edge area. During our most recent beamtime, CH-4750, we focused on expanding the available data for the characterization of different possible core confirmations. Our measurements focused on a dimeric Fe system reported by Kodera and coworkers **(Figure1)**, in wich a cis-μ-1,2-peroxo (**B**) converts to an open core high-spin μ -oxodioxodiiron(IV) (**C**) upon increasing the temperature from 25K to 294K.

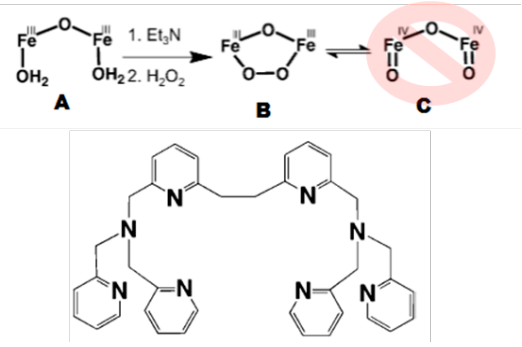

Figure 1 **Top.**Kodera complexes.

Bottom. *Ligand 6-hpa.*

Our Fe Kα HERFD XAS measurements were directly correlated to Mössbauer studies in order to determine the exact speciation. Unfortunately, we were unable to observe the temperature dependent interconversion of **B** and **C** (as reported by Kodera et al) in our measurements. Since measurements at different temperatures gave superimposable spectra, we arrived at the conclusion that the solution sample does not undergoes O-O bond scission. We are presently working to reporoduce the literature results in the solid state, as was originally reported.

In addition, by measuring individually the decay product and precursor, we were able to have clean Kα HERFD on the μ-Oxo-μ-peroxodiiron(III) **B** **(Figure2)** by subtracting the decay and unreacted precursor quantities present in the sample, as determined by Mössbauer. The pre-edge region of the "pure" peroxodiiron(III) shows lower intensity than the precursor μ-oxodiaquadi-iron(III) complex, with an anti-conformation. This parallels observations seen in previous experiments (report of CH4748 experiment) for the diiron (IV) complexes with diamond core and open core conformations.

During this beamtime, we were also able to demonstrate the reproducibility of the earlier mentioned open model ([(O=Fe^{IV}-O-Fe^{IV}-OH)$(L)_2$]), required for an upcoming publication **(Figure3, Top).** Following the Mössbauer analysis, as it was done for the Kodera sample, deconvolution of the open model was achieved and a comparison of the Kα HERFD of an "open core" diiron(IV) and a peroxodiiron(III) was possible for the first time (**Figure 3, bottom)**. Importantly these data provide essential fingerprints for interpreting the enzymatic P to Q conversion in sMMO, in which the O-O bond is cleaved.

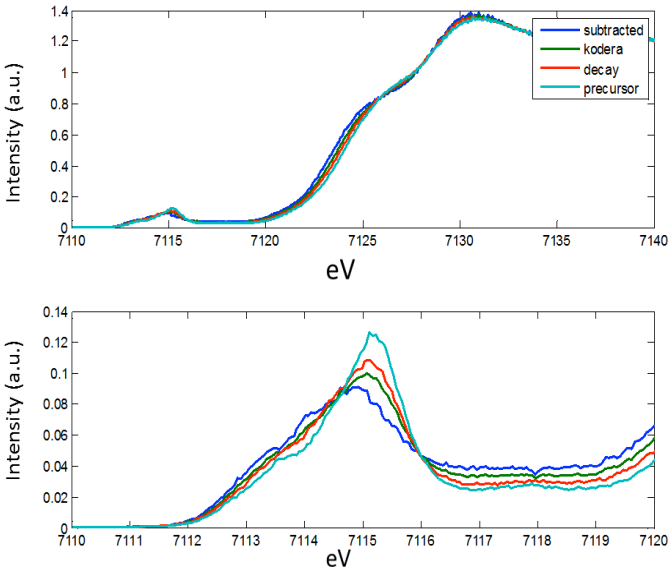

Figure 2. Top. *K-α HERFD XAS .*
Bottom*. K-α HERFD XAS pre-edge.*

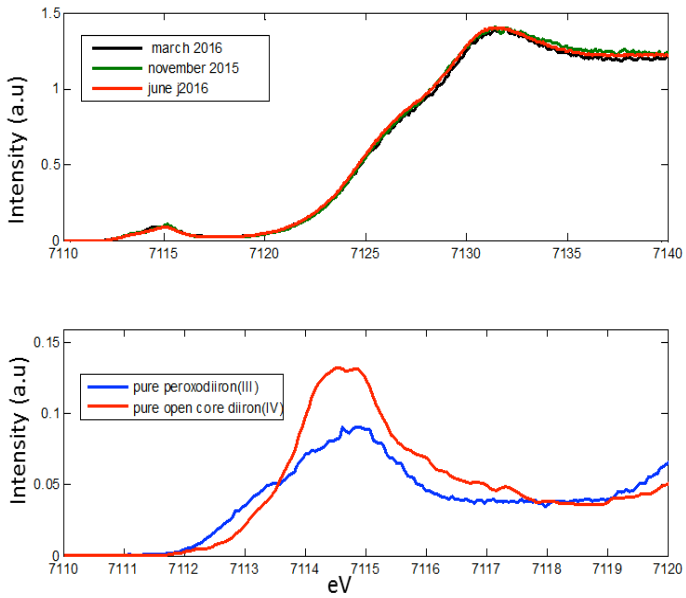

Figure 3. Top. *K-α HERFD XAS on open model FeIVFeIV.*
Bottom*. K-α HERFD XAS pre-edge open diiron vs peroxodiiron model.*