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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:** Employing Site Selective EXAFS to Structurally Characterize the Key Diiron Intermediate for Biological Methane Oxidation	**Experiment number**: CH4962
Beamline: ID26	**Date of experiment**: from: 09.11.2016 to: 15.11.2016	**Date of report**: 22/01/2017 *Received at ESRF:*
Shifts: 18	**Local contact(s)**: Lucia Amidani	

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Report:

During the allotted beam time reported herein, High-Energy Resolution Fluorescence Detected (HERFD) EXAFS of several catalytic states of soluble methane monooxygenase (sMMO). A previous EXAFS study of the key sMMO Q intermediate assigned an extremely short 2.46 Å Fe-Fe distance do the diiron active site. This distance has not been reproduced synthetically or computationally. The controversially short Fe-Fe distance has not been revisited in nearly 20 years, and has long been suspected to be metallic iron (α-Fe) as the published FT EXAFS spectrum has features that strongly resemble that of α-Fe.

Rapid-freeze quenched samples of sMMO are comprised of three states: the diiron(II) reduced state, the diiron(III) oxidized state and the diiron(IV) Q intermediate of interest, **Figure 1**. Quantification of each state within the trapped samples was performed prior to X-ray analysis by Mössbauer spectroscopy and samples contained an average of 30% Q. We initially set out to employ iron Kβ detected EXAFS for spin-state selectivity to inherit shifts in the Kβ mainline energies. During this beamtime, the Kβ mainline emissions of sMMO red, ox, and Q were collected for the first time. Both of the sMMO red and ox mainlines are fairly typically for Fe(II) and Fe(III) high-spin centers. It is known from Mössbauer spectroscopic studies that Q is an antiferromagnetically coupled diiron(IV) center, however, the local spin states (either $S_{loc} = 1$ or 2) have not been conclusively determined. The Kβ mainline of Q remarkably resembles the emission spectrum of a S = 1, Fe(IV) oxo model complex previously studied, particillary in the intensity of the Kβ` feature (**Figure 2**), an indication of intermediate spin.

For the case of sMMO, it was unfortunately observed that the shifts in the Kβ emission features were less than desirable and significantly less than the separation of spin-states exhibits in ‘proof of principle’ EXAFS experiments. Due to the decreased fluorescent count at the Kβ emission energy, we opted to forgo spin-state selectivity and ultilize HERFD detection of the EXAFS at the Kα emission energy. One frequently overlooked advantage of performing Kα HERFD XAS with a Johann spectrometer is high spatial resolution of the sample. Background floursecent events arising from metal within the experimental setup (i.e. cryostat walls, windows, shutters, etc) are eliminated as the fluorescence from these points in space will hit the analyzer crystals of the spectrometer at a different angle than fluorescence from the same and therefore will not be reflected and detected. Because of the HERFD detection employed here, the EXAFS data collected is thought to free of containment fluorescence.

XANES spectra of the various sMMO samples are consistent with previous findings, including the high-energy edge for the diiron(IV) Q intermediate and increased pre-edge area (See ESRF **CH-4570, 4417 and 3908**). Immediate examination of the Kα HERFD EXAFS of sMMO Q exhibits no 2.46 Å Fe-Fe scatterer. Fitting of sMMO red and ox based on their crystallographic structures is possible. The diiron distance fit in each is consistent with the crystal structures, and it FT EXAFS is fit more than satisfactory with only first shell O/N scatterers, the Fe-Fe scattering path and a necessary group second shell carbon scatterers (See **Figure 3** and **Table 1**). The EXAFS sample of Q is modeled as mixture of three diiron species, utilizing the scattering paths and fits for sMMO red and ox as standards within the fit at their predetermined amplitude. The component of the EXAFS corresponding to Q is best fit with a long diiron distance (3.4 Å), indicative of an open core structure. This finding reverses the previous core model proposed and corrects a potentially flawed EXAFS measurement.

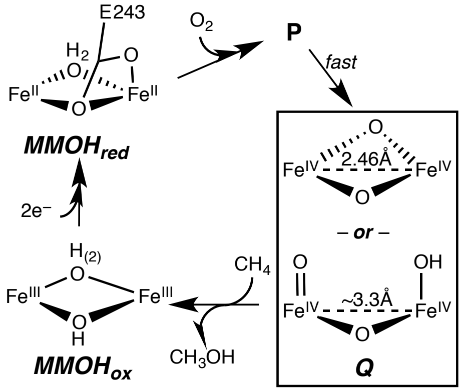

Fig 1. Proposed catalytic cycle of sMMO. The proposed structures of **Q** (in square brackets), which immediately precedes methane binding and oxidation. Closed and open (top and bottom, respectively) cores are shown.

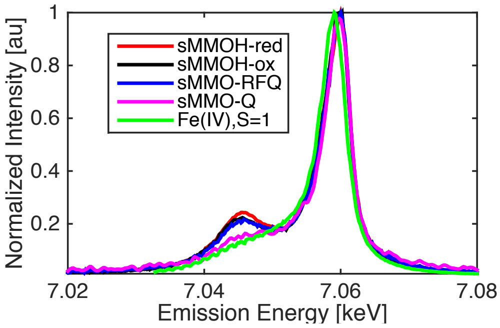

Figure 2. Kβ mainline emission spectra of sMMO intermediates and a Fe(IV), S = 1 model complex

Table 1. EXAFS fit parameters of Q

	N	Atom	R (Å)	+/–	σ^2 (Å^2)	+/–
Red 50%	5	O/N	2.0809	0.0165	0.0115	0.0014
	5	C	3.0313	0.0370	0.0060	0.0042
	1	**Fe**	**3.2505**	0.0311	0.0033	0.0028
	ΔE_0:	**-2.672**				
Ox 20%	1	O	1.8604	0.0509	0.0020	0.0040
	4	O/N	2.0415	0.0258	0.0049	0.0028
	5	C	2.9365	0.1023	0.0194	0.0108
	1	**Fe**	**2.9922**	0.0234	0.0025	0.0017
	ΔE_0:	**-3.314**				
Q 30%	5	O/N	2.0814	0.0572	0.0244	0.0072
	5	C	3.2505	0.1085	0.0121	0.0135
	1	**Fe**	**3.4121**	0.0500	0.0018	0.0042
	ΔE_0:	**8.004**			**R:**	**0.0343**

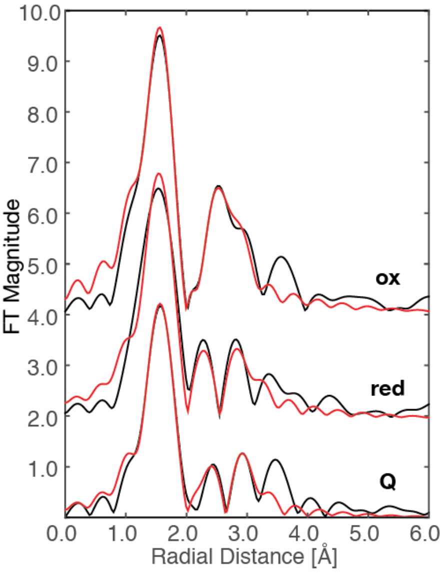

Figure 3. FT EXAFS of sMMO-ox, red and Q (black) and fits (red).