

Experiment Report Form

	Experiment title: Characterization of the Intermediates in Biological Methane oxidation	Experiment number: CH-4417
	Beamline: ID 26	Date of experiment: from: 13/05/2015 to: 18/05/2015
Shifts: 18	Local contact(s): Pieter Glatzel	

Names and affiliations of applicants (* indicates experimentalists):

Prof. Dr. Serena DeBeer - Max-Planck-Institut für Chemische Energiekonversion

Dr. Pieter Glatzel - European Synchrotron Radiation Facility

Dr. Benjamin Van Kuiken* – Max-Planck-Institut für chemische Energiekonversion

Stefan Hugenbruch* – Max-Planck-Institut für chemische Energiekonversion

Julian Rees* – Max-Planck-Institut für chemische Energiekonversion

Rebeca Gomez Castillo* – Max-Planck-Institut für chemische Energiekonversion

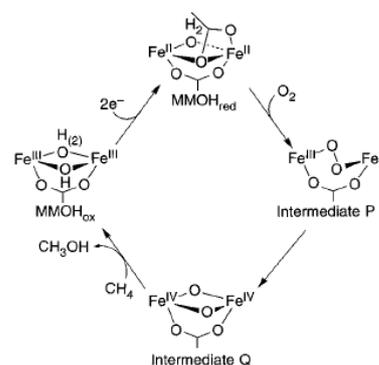
We note that our Mn-O2 intermediates were delayed in Customs and did not arrive in time for the proposed measurements. We thus focused on Fe-O2 intermediates as described below.

Report:

The conversion of methane to a transportable fuel such as methanol by a single step process is a chemical transformation with great value in synthetic and biological chemistry. However, the oxidation of the methane requires a large activation to break the strong C-H bond (104 Kcal/mol) of methane and, the current strategies to achieve this reaction are still inefficient.

In biological systems, the bacterial enzyme MMO is the able to perform the conversion of methane to methanol at ambient temperatures and pressures.

Therefore, understanding the oxidative chemistry performed by this protein will assist in the development of small-molecule catalysts capable of generating liquid fuels from methane in a sustainable manner.



The catalytic cycle in sMMO is shown in **Figure 1**. First molecular oxygen reacts with the reduced diiron centers leading to a peroxo intermediate (MMO-P). Subsequently, a diiron (IV) species called intermediate Q forms. This is the active species for the hydroxylation of methane. Finally, the diferric enzyme (MMOHox) is formed, and it is reduced to complete the catalytic cycle. The structure of Q has been described as high valence iron oxygen intermediate with a diamagnetic ground state as a result of two antiferromagnetically coupled Fe(IV) (S=2) centers. The exact structure of Q, however, is still under discussion. Based on reactivity studies on model complexes and also the recent resonance raman study on MMO-Q, the debate is mainly divided in two conformation options: a “diamond core” conformation or an “open core”.

Figure 1. Catalytic cycle MMO

Last beamtime allowed us to collect consistent Fe K_{α} HERFD XAS data on sMMO, MMO-Ox and a series of Fe models provided by our collaborator, Larry Que: $[\text{Fe(IV)}_2(\text{SRTPA})_2(\mu\text{-O})_2]^{4+}$, $[\text{Fe(IV)Fe(III)(SRTPA)}_2(\mu\text{-O})_2]^{3+}$, $[\text{Fe(IV)(O)(SRTPA)}]^{2+}$ and $[\text{FeIV(O)(TMG3tren)}]^{2+}$. With this technique we fix the analyzer crystal to the maximum of the Fe K_{α} emission while scanning the incident energy of the monochromator. Measurements using HERFD are more challenging than conventional X-ray absorption since the high resolution of the fluorescence monochromators results in a much weaker signal. This requires an intense X-ray beam, which results in time-dependent damage processes for biological samples. Therefore detailed damage studies needed to be performed to assess the dwell time per spot. It was determined that 30 seconds per position on the sample was appropriate to avoid photoreduction. Since we worked with low concentration samples approximately 200 scans were required to have sufficient signal to noise in the spectra. MMO-Q can not be isolated in a pure form, therefore an appropriate subtraction on MMO according to Mössbauer measurements (MMO sample contained 24% of MMO-Q) was needed. A posterior data analysis revealed we successfully collected reproducible HERFD data on MMO-Q. (**Figure 2**).

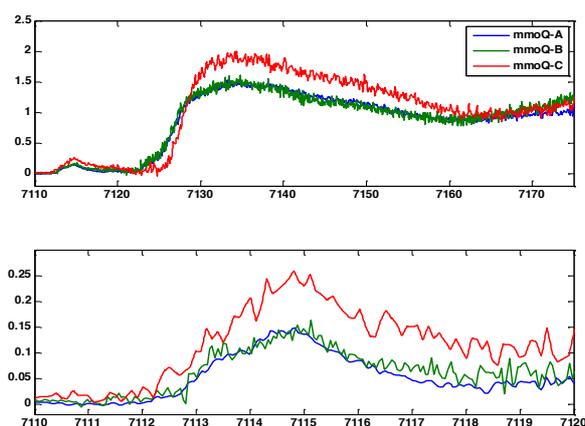


Figure 2. Iron HERFD data on MMOQ. Note quantitative Mössbauer data were not available for the red spectrum, leading to greater uncertainty in the subtractions than for the blue and green spectra.

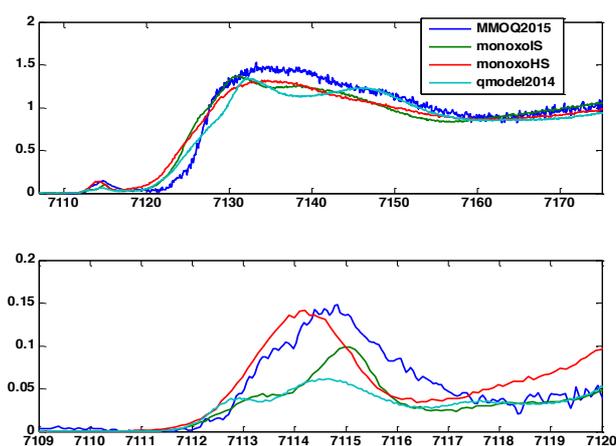


Figure 3. MMOQ (blue) vs model complexes. Dimer $[\text{Fe(IV)}_2(\text{SRTPA})_2(\mu\text{-O})_2]^{4+}$ (LS) (light blue), monomer $[\text{Fe(IV)(O)(SRTPA)}]^{2+}$ (LS) (Green), $[\text{FeIV(O)(TMG3tren)}]^{2+}$ (HS) (Reed)

This data is an important step for clarifying the question about the geometry of the enzyme. The pre-edge area is intense in comparison to the measured Fe(IV) dimeric model complex, which has a diamond core conformation. In the series of Fe complexes provided by Larry Que, a monomeric Fe(IV)(O) S=1 model and a monomeric $[\text{Fe(IV)(O)(TMG3tren)}]^{2+}$ S=2 trigonal bipyramidal complex were also included.

These spectra allowed us to understand better changes in the pre-edge due to different spin states and also to make a comparison between the MMO-Q and the monomers in order to study the possible presence of an iron-oxo unit in the intermediate (**Figure 3**). Although we still need to repeat the measurement for the monomer $[\text{FeIV(O)(TMG3tren)}]^{2+}$, since we found some inconsistencies in the pre edge shape comparing with previous data, the intensity in the pre-edge shows some similarities with the pre edge showed in MMOQ. Therefore, this information provides spectroscopic evidence suggesting that the MMO-Q active site may have an open dimer conformation. Computational studies including a series of “open models” are currently in development in order to study, spin states effects, the presence of Fe terminal-oxo in the core

and, hence, the relation between (o)p-(Fe)d mixing effects and intensity in the pre-edge. Future studies will involve measurements of additional models providing a more detailed library for spectroscopic interpretations.

In summary, we have collected Fe Kalpha HERFD data on the protein MMO and a series of molecular samples, this data provides unique insight into the mechanism of biological methanol production, however experimental data on open models will be needed to complete this study.