



## 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://www.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.

**Experiment title:**

Resonant XES of Iron-Oxygen Intermediates

**Experiment****number:**

CH-3555

**Beamline:**

ID26

**Date of experiment:**

from: 27.06.2012 to: 03.07.2012

**Date of report:**

30.08.2012

**Shifts:**

18

**Local contact(s):**

Pieter Glatzel

*Received at ESRF:***Names and affiliations of applicants (\* indicates experimentalists):**

Dr. Pieter Glatzel - European Synchrotron Radiation Facility

Prof. Dr. Frank Neese - Max-Planck-Institut für Chemische Energiekonversion

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

Christopher Pollock\* - Max-Planck-Institut für Chemische Energiekonversion

Vlad Martin-Diaconescu\* - Max-Planck-Institut für Chemische Energiekonversion

Eleanor Hall\* - Max-Planck-Institut für Chemische Energiekonversion

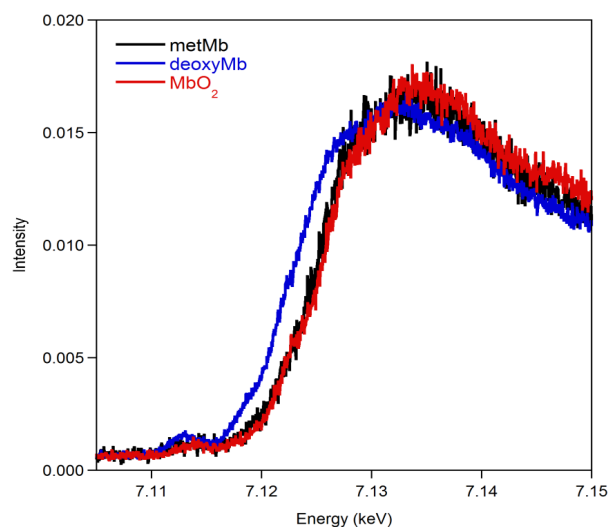
Frederico Alves Lima\* - Max-Planck-Institut für Chemische Energiekonversion

**Report:**

The experiments undertaken during the previous beamtime were aimed at understanding the electronic structure of oxy-myoglobin ( $\text{MbO}_2$ ), specifically regarding the nature of the bound  $\text{O}_2$ . The state of the  $\text{O}_2$ —oxy, superoxo, or peroxy—has been an outstanding question in bioinorganic chemistry for many decades and has important implications for the chemistry of oxygen activation. To attempt to understand this system, Fe  $\text{K}\beta$  x-ray emission spectra (XES) and  $\text{K}\beta$  high resolution fluorescence detected (HERFD) x-ray absorption data were taken on  $\text{MbO}_2$  as well as deoxy-Mb and oxidized met-Mb.

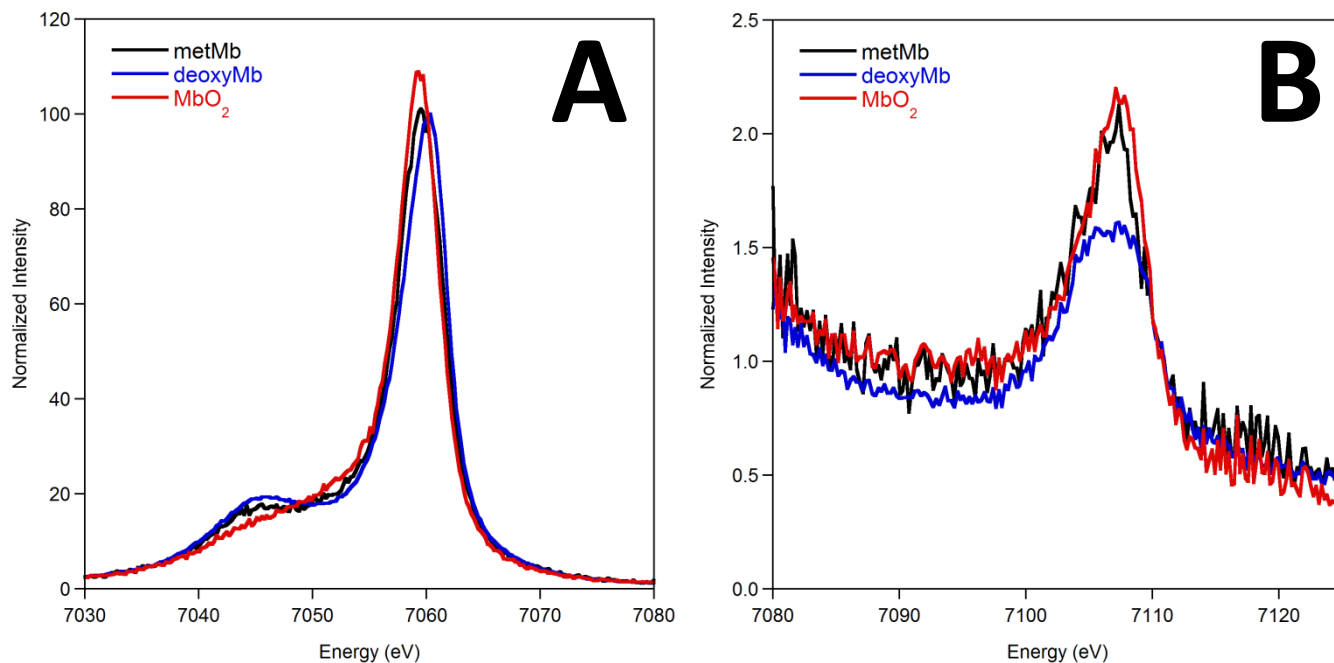
$\text{K}\beta$  HERFD spectra for the three protein states are shown in Figure 1. As expected, the edge for met-Mb appears slightly more than 1 eV higher in energy than that for deoxy-Mb, reflecting the presence of a more oxidized Fe in met-Mb ( $\text{Fe}^{\text{III}}$  versus  $\text{Fe}^{\text{II}}$  in deoxy-Mb). Interestingly, the edge for  $\text{MbO}_2$  lies very close to that of met-Mb, suggesting  $\text{MbO}_2$  possesses an  $\text{Fe}^{\text{III}}$ , as well. An  $\text{Fe}^{\text{III}}$  present in  $\text{MbO}_2$  would then indicate that the  $\text{O}_2$  must exist in its singly-reduced form, superoxide ( $\text{O}_2^-$ ). To further support this hypothesis, we next employed  $\text{K}\beta$  XES.

The XES mainlines and valence-to-core regions are shown in Figure 2a and b, respectively. The mainline region of the spectrum is known to be sensitive to both spin state at the metal center as



**Figure 1:**  $\text{K}\beta$  HERFD spectra for the myoglobin samples tested.

well as metal-ligand covalency, with the intensity of the  $K\beta'$  feature ( $\sim 7045$  eV) gaining intensity as the number of unpaired spins at the metal increases. Most straightforwardly, the mainline of deoxy-Mb clearly shows a high spin state, consistent with previous characterization of the Fe as high spin  $Fe^{II}$ . Similarly, with reference to the HERFD data, met-Mb is consistent with a relatively covalent high spin  $Fe^{III}$  complex. This high covalency can be explained by the significantly shorter bond lengths present in met-Mb compared to deoxy-Mb; similar effects have been seen in studies of other systems. The mainline for  $MbO_2$ , in contrast, indicates a low spin  $Fe^{III}$  center, also consistent with previous assignment.

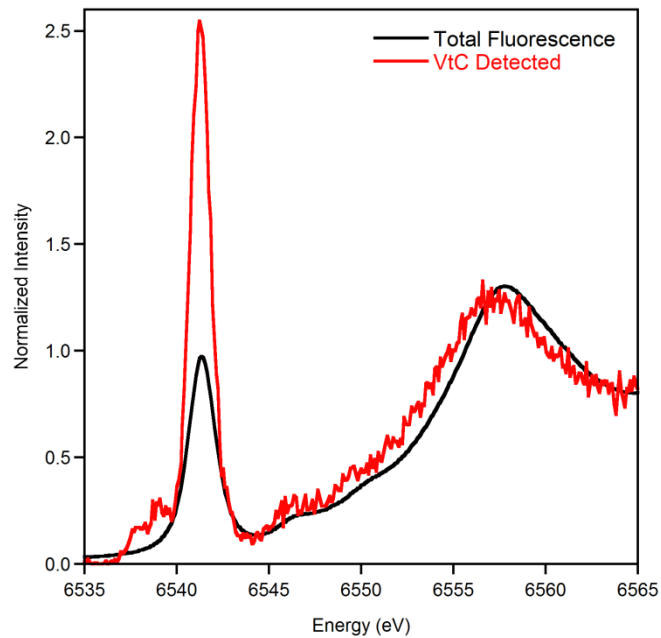
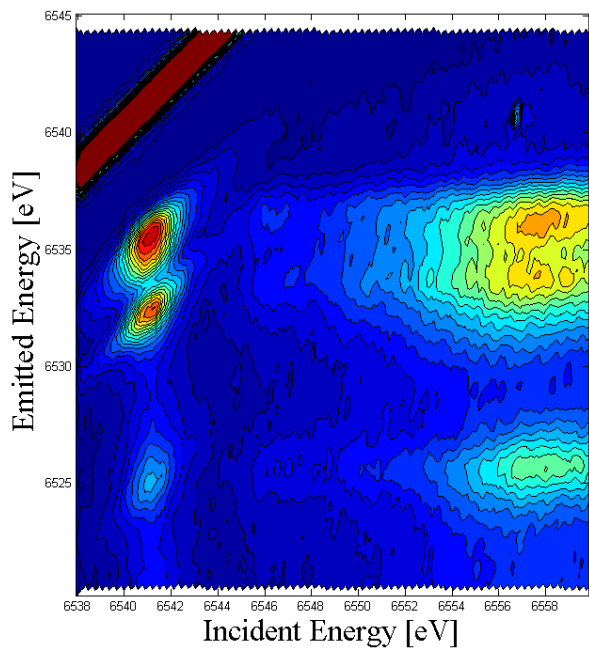


**Figure 2:** The  $K\beta$  mainlines for the three samples examined (A) along with an expanded view of the valence-to-core region (B).

The valence-to-core region (Figure 2b) consists of transitions that result from dominantly ligand localized valence orbitals and thus acts as a probe of ligand identity and electronic structure. Of greatest importance for the current study are the so-called “satellite” features occurring between 7085 and 7100 eV that originate from predominantly ligand 2s-based orbitals and that can be used to assess the O-O bond length. Broadly, the valence-to-core data are consistent with what has already been seen: deoxy-Mb has the lowest intensity  $K\beta_{2,5}$  feature ( $\sim 7107$  eV), consistent with a high spin state and long metal-ligand bonds, while met-Mb and  $MbO_2$  have a higher intensity, sharper  $K\beta_{2,5}$  that is typically associated with a lower spin state and/or shorter bond lengths. Unfortunately, the satellite features we were hoping to observe were of very low intensity. From attempts to deconvolute the spectra, it is known that peaks exist in this region, but the intensities are too low to assign precise energies.

One idea to remedy this situation was to employ resonant excitation via a RIXS experiment to enhance certain valence-to-core features, similar to what is done in resonance Raman. By taking advantage of the ligand localized transitions that dominate the valence-to-core and pre-edge regions of XES and XAS, it was hoped that valence band resonant RIXS could be employed to enhance the weak satellite features we hoped to see.

To test the feasibility of this experiment, we examined a selection of manganese model compounds known to have intense pre-edge and valence-to-core features. A representative valence band RIXS plane as well as a comparison between standard total fluorescence detected XAS and a valence-to-core detected XAS spectrum are shown in Figure 3a and b, respectively, for the compound  $(NH_4)_2[Mn(CN)_4N]$ . It is very easy to see that dramatic enhancements of pre-edge intensity are attainable by detecting XAS from certain valence-to-core region features (over 200% enhanced intensity). This observation suggests that applying RIXS to the myoglobin system will enable much more definitive conclusions to be drawn that could be done from the standard, non-resonant techniques.



**Figure 3:** The valence band RIXS plane for  $\text{Mn}(\text{CN})_4\text{N}_2^-$  (A) and an overlay of total fluorescence detected XAS compared to a slice through the plane showing the enhancement possible via RIXS (B).