



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: Mn valence to core spectroscopy of the oxygen evolving complex (Mn ₄ CaO ₅ cluster) in Photosystem II	Experiment number: SC-3719
Beamline: ID26	Date of experiment: from: 27/8/2013 to: 10/9/2013	Date of report: 22/9/2013
Shifts: 36 in 16 bunch mode	Local contact(s): Pieter Glatzel	<i>Received at ESRF:</i>

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Report:

Scientific background :

The oxygen-evolving complex (OEC) located in the Photosystem II (PSII) membrane-bound protein in plant, algae, and cyanobacteria catalyses the water-oxidation reaction. The OEC, with four Mn and one Ca, couples the 4-electron chemistry of water oxidation with the one-electron photochemistry of the reaction center by sequentially storing oxidizing equivalents through five intermediate S-states (S_i , $i = 0$ to 4), before one molecule of dioxygen is evolved (**Fig. 1**).¹ The Mn_4CaO_5 cluster provides a high degree of redox and chemical flexibility so that several oxidizing equivalents can be stored during the S-state cycle. To understand the mechanism of water oxidation in detail, it is crucial to know how the electronic and geometric structure of Mn_4CaO_5 changes during the catalysis and their effect on the nature of the Mn-oxo bridges.²

Previously, we investigated the application of valence-to-core (vtc) $K\beta$ emission spectroscopy to probe the ligands of Mn in PS II.³ We reported that the vtc spectroscopy is possible even in dilute samples like PSII (~1mM Mn), and showed the S_1 state spectrum of the $K\beta''$ region.³ XES at the Mn K-edge complements The emission from the valence levels (the spectral features have been named $K\beta''$ and $K\beta_{2,5}$) is sensitive to the ligand environment. Mn $K\beta_{2,5}$ and $K\beta''$ arise from emission from the ligand valence 2s and 2p shells of the metal complexes to the Mn core 1s level. We have used this spectroscopic technique to study the O ligands of the Mn_4CaO_5 cluster and have been able to directly detect for the first time the bridging oxo groups of Mn.³

Results :

In our first beamtime at ESRF ID26 within this project we measured the 1s2p RIXS planes of a series of highly relevant model systems, i.e. coordination complexes that are thought to be good models of the Mn cluster in the OEC, and of Mn in the OEC (see experimental report SC3580). During the last beamtime (August 2013) at ID26 we continued the measurements on the model systems that we have received from our collaborators at Caltech. We have identified by visual comparison of the RIXS planes the structural motif that best models the Mn cluster in the OEC. We have established a collaboration with the Molecular Foundry

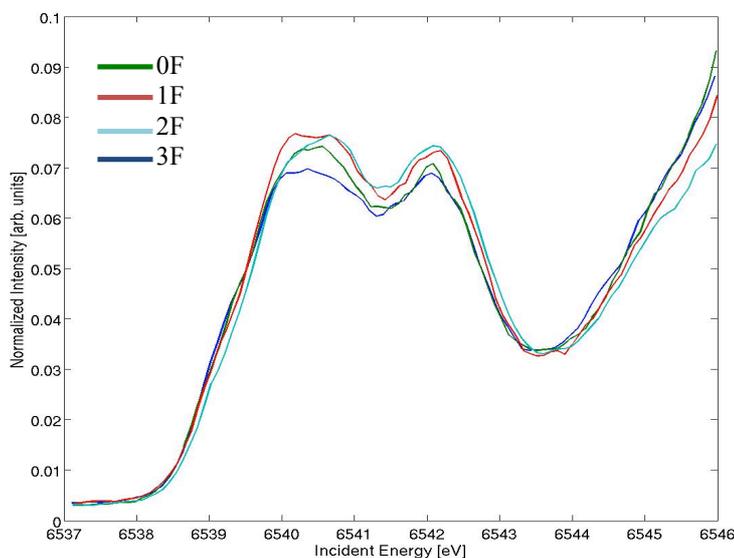


Fig. 2: HERFD spectra of the S-state cycle of PSII.

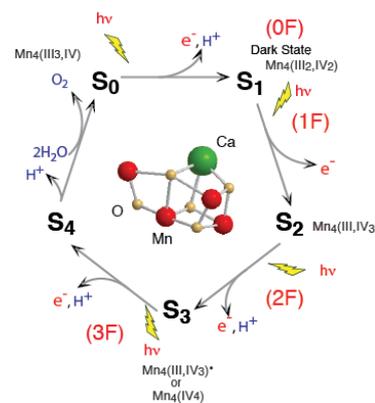


Fig. 1: Kok Cycle for water splitting reaction in PSII.

at the Berkeley Lab for quantum chemical calculations and a manuscript is in preparation.

We measured High-Energy Resolution Fluorescence Detected (HERFD) XAS experiments on all S-states of PSII (**Fig. 2**) that are accessible using the flash (F)-freeze technique (0F (dark state), 1F, 2F, and 3F; cf. **Fig. 1**) to gain a comprehensive picture of the Mn electronic structure and its changes during the catalytic cycle in PSII. Great care was taken to monitor the changes of the electronic structure induced by the X-ray beam. The maximum acceptable X-ray exposure time was determined to 5 seconds per spot. Therefore, a large number of PSII samples was measured to obtain sufficient statistics (Mn concentration ~1mM in PSII with 0.5mm sample

thickness). A set of macros was written by the BL staff and ESRF software groups to deal with this problem. The changes in the HERFD spectra are detected, confirming the electronic structural changes at the Mn sites during the catalytic cycle. We plan to further analyse this data with a theory group.^{4,5}

We have also collected $K\beta$ X-ray emission spectra of PSII extracted from *T. elongatus* during this beamtime. At first, we focused on the $K\beta_{1,3}$ region, in order to establish a protocol to collect intact PSII XES data below the threshold of radiation damage within a reasonable amount of time (**Fig. 3**). We measured every data point on a new sample spot and we acquired 1 second per spot. In order to obtain XES spectra with correct normalization, the Mn concentration had to be determined for each spot. This was done in two ways: a) a 13-element Ge detector monitored the intensity of the Mn $K\alpha$ lines during the XES scan thus giving a signal proportional to the incoming flux and the Mn concentration in the sample; b) after the XES scan of a damage free sample each point was revisited and the XES signal on the maximum of the $K\beta$ line was recorded for 5s to obtain a map of the Mn concentration. While a) is considerably more efficient, b) gives better results because the identical solid angle is covered.

From these measurements, we estimate that the data collection of the valence to core XES spectra for each S- state require 8-12 shifts in order to improve the current statistics, and we plan to complete this in the next beamtime.

The XES measurements during the recent beamtime were considerably hampered by a broken translation stage inside the cryostat. This motion is crucial in order to change the beam position on the sample. Despite heroic efforts of the BL staff to find alternative ways of changing the beam position, the data quality was critically compromised and only a fraction of the sample surface could be used. Due to this technical problem, the recent beamtime was mainly used to confirm and complete the previous measurements.

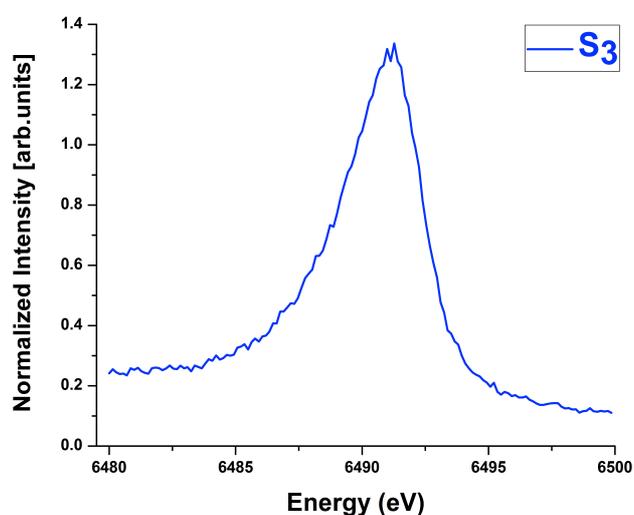


Fig. 3: $K\beta_{1,3}$ XES spectra of the S_3 state of PSII. The graph shows the sum of 5 samples, i.e. a total counting time of 5 seconds.

References:

- (1) Wydrzynski, T. & Satoh, S. (eds). (2005) *Photosystem II* Springer, Dordrecht.
- (2) Messinger, J. (2004) *Phys Chem Chem Phys* **6**, 4764.
- (3) Pushkar, Y. et al. (2010) *Angew. Chem. Int. ed.* **49**, 800.
- (4) Glatzel, P.; Bergmann, U.; Yano, J.; Visser, H.; Robblee, J. H.; Gu, W. W.; de Groot, F. M. F.; Christou, G.; Pecoraro, V. L.; Cramer, S. P.; Yachandra, V. K. (2004) *J. Am. Chem. Soc.* **126**, 9946.
- (5) Glatzel, P., Yano, J., Bergmann, U., Visser, H., Robblee, J. H., Gu, W., de Groot, F. M. F., Cramer, S. P., and Yachandra, V. K. (2005) *J. Phys. Chem. Solid.* **66**, 2163.