ESRF	**Experiment title:** Structural changes of the tetra-manganese complex of oxygenic photosynthesis during the oxygen-evolving transition induced by Laser-flashes and studied by time-resolved BioXAS	**Experiment number**: SC1229
Beamline: ID26	**Date of experiment**: from: 30.04.2003 to: 12.05.2003	**Date of report**: 31.08.2003
Shifts: 33[&]	**Local contact(s)**: Dr. Thomas Neisius	*Received at ESRF:*

[&]Block allocation for SC1229 and SC1228 (see separate report for SC1228)

Names and affiliations of applicants (* indicates experimentalists):

[*]Dr. Michael Haumann, Freie Universität Berlin, Physik, Arnimallee 14, D-14195 Berlin, Germany,
[*]Marcos Barra, Freie Universität Berlin, Physik, Arnimallee 14, D-14195 Berlin, Germany,
[*]Peter Liebisch, Freie Universität Berlin, Physik, Arnimallee 14, D-14195 Berlin, Germany,
[*]Claudia Müller, Freie Universität Berlin, Physik, Arnimallee 14, D-14195 Berlin, Germany,
Prof. Dr. Holger Dau, Freie Universität Berlin, Physik, Arnimallee 14, D-14195 Berlin, Germany.

Report: Oxidation-state and/or structural changes at the metal center are the basis for the catalytic reactions of most metalloenzymes. Knowing the oxidation states and structures of reaction intermediates is a prerequisite to understand the catalytic function. To follow these changes in real time is a major challenge in structural biophysics. Time-resolved BioXAS represents a particularly promising tool to resolve such intermediates. The development of such techniques is one of the primary goals of our research; important progress has been obtained in May 2003.

The reaction cycle of oxygen production at the tetra-manganese complex of photosystem II (PSII) of photosynthesis involves 4 semistable intermediates (S_0, S_1, S_2, S_3) of the Mn complex which can be prepared by nanosecond Laser-flashes. Investigations on structural intermediates during the particularly interesting oxygen-producing step (occuring on the S_3-S_0 transition inducible by the 3rd Laser flash) are expected to elucidate details of the still unknown reaction mechanism of oxygen production.

PSII multilayer samples were prepared at the beamline by a novel drop-and-dry technique and positioned in the X-ray beam by a new computer-controlled sample-changer (20 samples per charge, µm accuracy). Illumination by trains of Laser flashes (5 ns, 532 nm, ~150 mJ) was carried out at room temperature. About 400 rapid-scan XAS measurements (synchronuous scan of monochromator and undulator gap) and ~700 timescans of X-ray fluorescence at fixed excitation energies for sampling-XAS measurements (see report SC-1013) were collected at the Mn K-edge. About 300 timescans with µs time-resolution were recorded by a personal computer equipped with an A/D card. Each scan was performed on a fresh sample. Specialized electronics provided synchronization of Laser, sample exchange, and data acquisition.

The following results were obtained:

(1) Rapid-scan XAS measurements (12 s per scan) on samples Laser-flash illuminated in the X-ray beam yielded EXAFS spectra of the Mn complex predominantly in states S_1 (0F, zero flash sample), S_2 (1F), S_3 (2F) and S_0 (3F) (see Fig. 1).

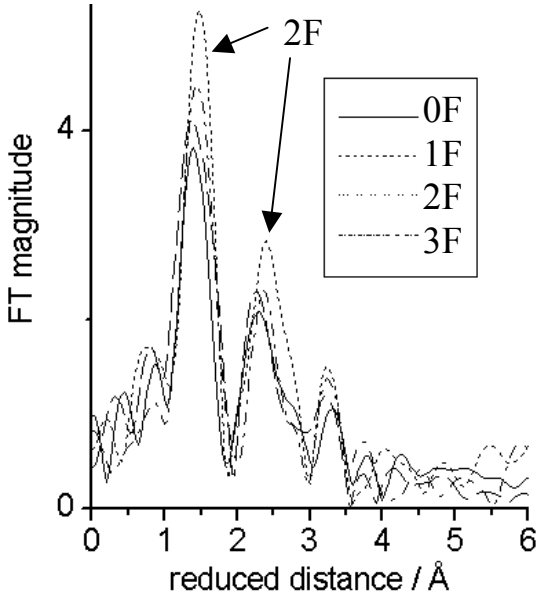


Fig. 1: Room temperature EXAFS spectra (data from SC1229 and SC1013 were averaged; ~80 scans each)

(2) The new technique of sampling-XAS in principle allows for the construction of complete XAS spectra spaced by only microseconds in time (see report SC1013). From timescans of X-ray fluorescence as shown in Fig. 2 recorded at 28 excitation energies, good-quality room temperature XANES spectra of the Mn complex were obtained (Fig. 3). The shown spectra have been measured within only 400 ms after the respective Laser flash. The upshifts of the K-edge energy in the 1F and 2F spectra indicate Mn oxidations whereas the downshift on the 3F spectrum is due to Mn reduction on the oxygen-producing step S_3-S_0 (arrows in Fig. 3 denote remarkable edge features).

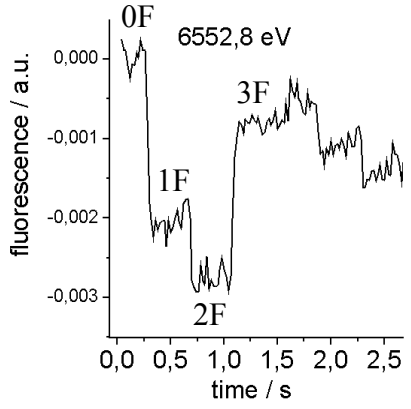
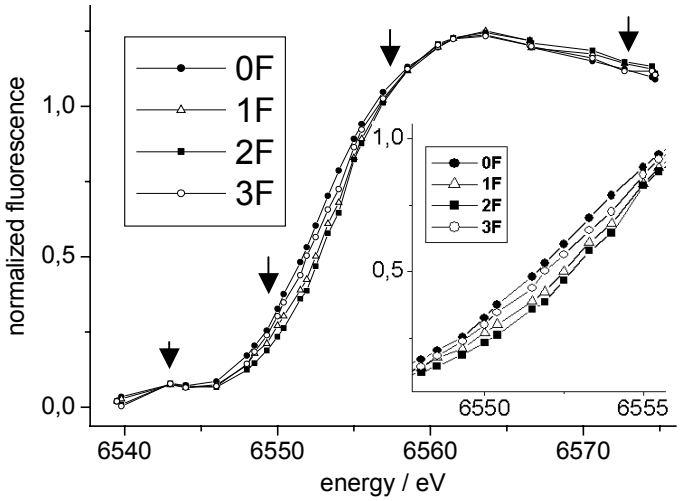


Fig. 2: Timescan of fluorescence during Laser-flash illumination (20 ms per point, 18 scans averaged).

Fig. 3: Sampling-XANES spectra at room temperature. Spectra were constructed from the fluorescence levels during 400 ms before flash 1 and after flashes 1, 2, and 3, respectively (see Fig. 2).



Sampling-XAS experiments have also been performed at ~60 energies in the EXAFS region. The analysis of data is expected to provide room temperature EXAFS spectra of the Mn complex obtained within only 400 ms in the S_1 state and after predominant population of the S_2, S_3, and S_0-states by Laser flash illumination.

(3) To follow the reduction of Mn on the oxygen-producing transition S_3-S_0 (on the 3rd Laser flash) in real time, timescans of fluorescence were recorded, for the first time, at 200 µs per point. At 6552.8 eV a rise-time of 1.2 ms is well resolved (Fig. 4, left). This rise-time is typical for most intact and active PSII preparations.

Time-resolved measurements were performed at 5 excitation energies and sampling-XANES spectra were constructed before and after the flash 3 which induces the oxygen-producing transition S_3-S_0 (Fig. 4, right). Spectra are spaced by only 500 µs in time. To our knowledge, the data in Fig. 4 (right) represent the first example of XANES spectra that have been obtained at sub-ms time resolution and at room temperature *during* an oxidation state change at the metal center of a metalloenzyme.

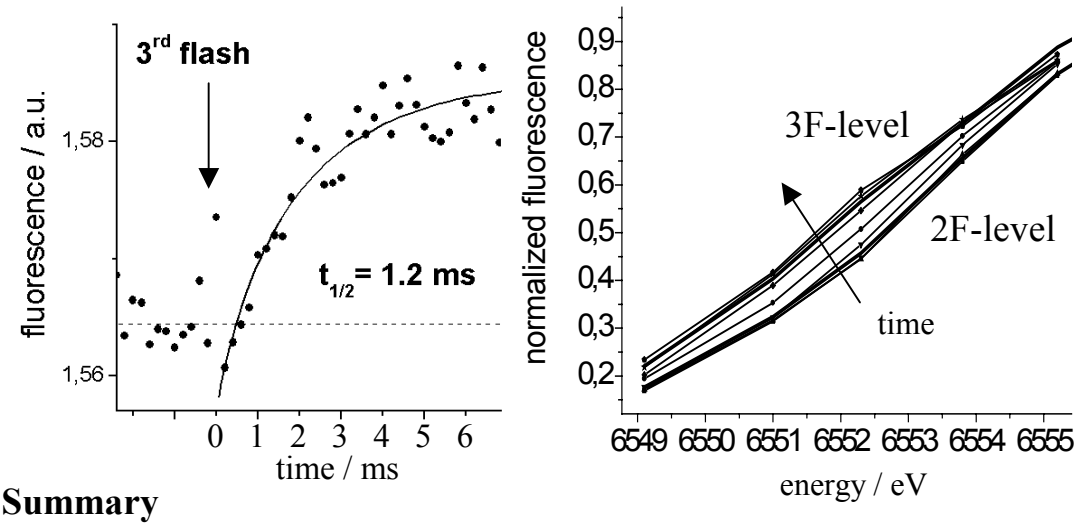


Fig. 4: Left: Fluorescence transient (dots) at 6552.8 eV induced by Laser flash no. 3 (36 scans averaged, 200 µs per point, line = simulation). Right: Room temperature sampling-XANES spectra (symbols and thin lines) obtained from timescans at 5 excitation energies at 500 µs before flash no. 3 and at 500 µs, 1000 µs, 1500 µs, 2000 µs, 2500 µs, and 3000 µs after flash no. 3. The thick lines represent the 2F and 3F spectra obtained within 400 ms from Fig. 3.

Summary

We consider the run SC1229 as particularly successful because important progress was made with respect to the development of time-resolved BioXAS techniques which allow for sub-ms resolution of structural and oxidation state changes at biological metal centers. For the first time, by using new methods for sample preparation and positioning and for data acquisition, we succeded in following the reduction of the Mn complex on the most important oxygen-producing transition with microsecond time resolution under physiological conditions (room temperature). First transient XANES spectra during this step were obtained. The quantitative evaluation of data is in progress. We intend to submit a paper on (some of) the obtained results already in October/November 2003.