ESRF	**Experiment title:** The oxygen evolving Photosystem II manganese complex – time-resolved analysis of S-state formation and decay at room temperature	**Experiment number**: LS1582
Beamline: ID26	**Date of experiment**: from: 25.04.2001 to: 02.05.2001[§]	**Date of report**: 29.08.2001
Shifts: 10[§]	**Local contact(s)**: Dr. T. Neisius, Dr. L. Jacquamet	*Received at ESRF:*

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[§]block allocation for LS1582 + LS1583, separate report for LS1583

Report. Structural changes at the metal center are crucial for the catalytic function of many biological enzymes. In principle, these changes can be followed under functional conditions by time-resolved X-ray absorption spectroscopy. We studied the various oxidation states of the tetra-manganese complex of Photosystem II at room temperature using Laser-flash excitation of the samples exposed to the X-ray beam.

The following results and insights were obtained during our measurements at ID26 in March 2001.

(1) Methodical progress with respect to time-resolved XAS. For the first time, a Laser (Quantel Brilliant Nd-YAG supplied by the ESRF, frequency doubled, 532 nm, FWHM of 5 ns, ca. 150 mJ per flash) was used to excite Photosystem II samples exposed to the X-ray beam. The experimental setup is shown in Fig. 1. Ten Laser flashes spaced by 500 ms were applied and the changes in X-ray fluorescence were recorded (as function of time) at a fixed X-ray energy (half-height of the manganese K-edge. Figure 2 shows the observed oscillations of X-ray fluorescence.

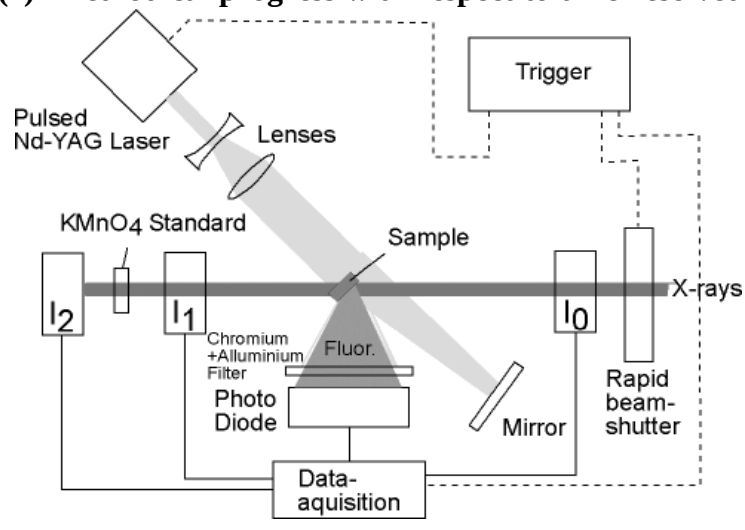

Figure 1: Experimental setup for Laser flash excitation of photosystem II samples at the beamline. The sample was placed in plain air.

Conclusion: Laser-flash excitation of samples at the beamline at room temperature is feasible. The results are much improved when compared to previous ones obtained with flash-lamp excitation. The pronounced oscillations of X-ray fluorescence intensity (Fig. 2) indicate that the progression to the next higher oxidation state of the manganese complex achieved by a Laser flash was ~85 %, sufficient but not optimal. Further improvements are expected by variation of the time period between flashes.

(2) EXAFS spectra of the manganese complex in three oxidation states. We applied 0, 1, and 2 Laser flashes to Photosystem II samples at room temperature. A full EXAFS spectrum (scan range: 6500-7100 eV) was recorded starting 1 s after the flash and within 10 s using the rapid-scan mode of ID26. The X-ray

exposure time of the sample was minimized by use of a rapid in-hutch beam shutter. The averaging of 20-30 scans yielded useful EXAFS spectra. They are ascribed to: 0 flashes, S_1-state; 1 flash, S_2-state; 2 flashes, S_3-state. The Fourier transforms of the EXAFS spectra are shown in Fig. 3 (Table 1, preliminary simulations).

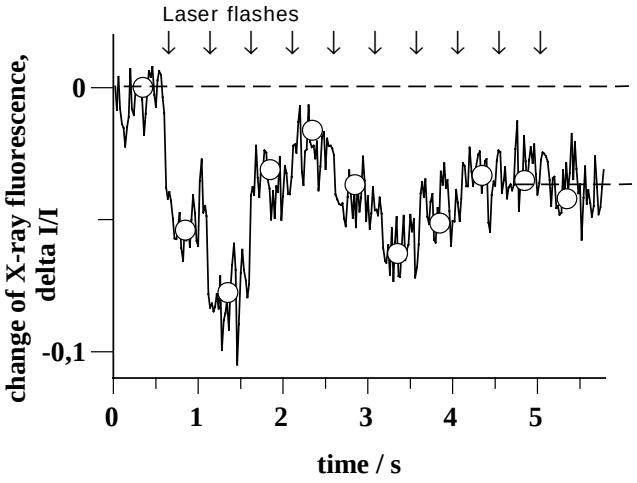

Figure 2: Oscillation of the intensity of the X-ray fluorescence (of the Photosystem-II Mn complex) excited at 6551.5 eV as function of time. Ten Laser flashes spaced by 500 ms were applied; 5 traces were averaged. Dots: simulation with 100% of centers initially in state S_1, 15 % 'miss probability' per flash.

Conclusion: The comparison of the room temperature spectra with previous ones obtained at 20 K reveals differences which are, probably, of functional relevance. The signal-to-noise ratio, however, needs to be further improved (by collection and averaging of 50-100 scans) to facilitate a more detailed analysis. Upon the S_2-S_3 transition, the second Fourier peak increases (Fig. 3). This feature is best simulated under the assumption of the formation of a third di-µ-oxo bridge between Mn atoms. These results strongly support our previous EXAFS results on the S_3-state obtained at 20 K. The formation of a third di-µ-oxo bridge on the $S_2 \rightarrow S_3$ redox transition is crucial for the function of water oxidation (Haumann et al., 2001a,b).

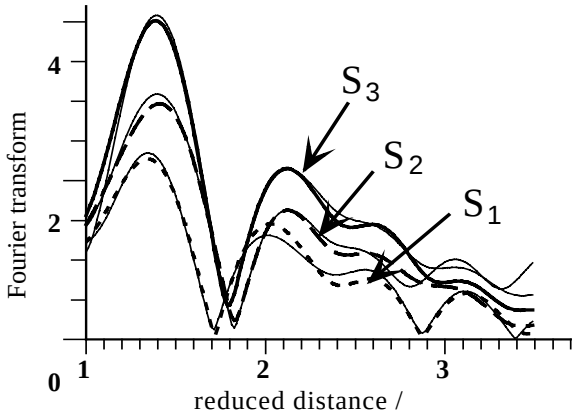

Figure 3: Fourier transforms (FTs) of k^3-weighted EXAFS spectra of the Mn complex of Photosystem II in S_1, S_2, and S_3 at room temperature. Thin lines, experimental data; thick, preliminary simulation parameters.

Shell, i	Vector	Coordination number, N_i	Distance, R_i, Å	Debye-Waller parameter, Å^2
I	Mn-O/N	5.0 / 5.0*	1.88 / 1.87*	0.037 / 0.025*
II	Mn-O/N	0.5 / 0.5*	2.27 / 2.23*	0.005 / 0.005*
III	Mn-Mn	1.0 / 1.5*	2.71 / 2.74*	0.018 / 0.016*
IV	Mn-Mn	0.5 / -	3.13 / -	0.010 / -
V	Mn-Ca	0.5 / 0.5*	3.38 /3.23*	0.010 / 0.010*
VI	Mn-O/N	3.0 / 3.0*	3.65 / 3.67*	0.011 / 0.008*

Table 1: Preliminary simulation parameters for the S_2- and S_3-state of the manganese complex of Photosystem II at room temperature. (The * labels the S_3 results.)

Summary. We consider the March run as highly successful. We have established Laser-flash excitation at the beamline. Already this first experimental period has resulted in (publishable) insights in the structure of the Mn complex of Photosystem II at room temperature. These experiments will help to pave the road for time-resolved XAS experiments on other metalloproteins.

Experimental improvements for future measurements. We intend to implement the following experimental/methodical improvements: use of a motorized device for automated sample exchange; cryo-streamer to control sample temperature and to slow redox-state decay by cooling to 4°C; optimized trigger devices for the synchronization of Laser flashes and data acquisition.

Publications on the basis of this experimental period.

Haumann, M., Grabolle, M., Werthammer, M., Iuzzolino, L., Dittmer, J., Meyer-Klaucke, W., Neisius, T., and Dau, H. The manganese complex of Photosystem II: A structural model for the S_1, S_2, and S_3 oxidation states derived from linear dichroism EXAFS spectroscopy at 20 K and at room temperature. Proc. 12th Int. Congress on Photosynthesis, CSIRO Publishing, Brisbane, Australia, in press, 2001.

Haumann, M., Grabolle, M., Neisius, T., and Dau, H. Structural changes during the redox transitions of the manganese complex of photosystem II studied by X-ray absorption spectroscopy at room temperature. J.Synch.Rad., in preparation, 2001.