

**Experiment title:**

Structural changes of the photosynthetic tetra-manganese complex induced by Laser flashes and monitored by time-resolved BioXAS at room temperature

Experiment number:

SC1013

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Report. X-ray absorption spectroscopy under conditions where metalloenzymes are functional (e.g. at room temperature, non-crystalline samples) is a new method (BioXAS) to investigate the catalytic reaction by monitoring structural and oxidation state changes of the biological metal center. Such changes are mostly inaccessible in crystallographic studies. One prerequisite for the time-resolution of these changes is the use of high-brilliance beamlines with rapid-scan capability at third-generation synchrotrons.

(1) collection of point-by-point EXAFS spectra (within 200 ms): We investigated structural and oxidation state changes at the tetra-manganese complex of photosystem II, the enzyme which produces the oxygen in the atmosphere. The oxidation states S_2 , S_3 , S_0 of the Mn complex were populated (starting with the dark-stable S_1 -state) by 1 to 3 single-turnover ns-Laser flashes in samples exposed to the X-ray beam. For the first time, the new technique of recording point-by-point EXAFS spectra was explored (Figure 1, A-C). This technique allows for an almost unlimited time resolution [1]. Here, the time resolution was limited to 1 ms per point. We succeeded in following the formation of $Mn(II)_4$ from the $Mn(III)_2Mn(IV)_2$ complex during X-ray exposure (Fig. 1F). Using timescans at fixed X-ray energy and rapid-scan reference spectra (Fig. 1 A,C), point-by-point EXAFS spectra before and after 1 to 3 Laser flashes were obtained (Fig. 1G). The XANES region and the Fts of the EXAFS oscillations (Fig 1, G,H) revealed oxidation state changes (as apparent from shifts of the Mn K-edge) and structural changes (as apparent from changes in the peak amplitudes of the Fts) in the four S-states which resembled those previously observed by continuous rapid-scan XAS [2].

(2) time resolution of oxidation state changes (1 ms per point): The oxidation/reduction of the Mn complex occurs with half-rise-times of $\sim 100 \mu s$ and $\sim 1-2$ ms upon transitions $S_1 \rightarrow S_2$ and $S_3 \rightarrow S_0$, induced by Laser flashes 1 and 3. These reactions were followed by recording timescans at 6551.5 eV (1 ms per point) (Fig. 1D). For the first time, on the third flash the reduction of the Mn complex was monitored in a time-resolved BioXAS experiment.

(3) technical improvement for higher sample throughput: A new computer-controlled sample-changer was tested which is needed to increase the sample throughput for the optimization of the signal-to-noise ratio in time-resolved BioXAS. On basis of the obtained results, the device is currently being optimized.

Conclusions: We consider the May run to be particularly successful. For the first time, the ability to record complete point-by-point EXAFS spectra on a metalloenzyme within only 200 ms after a trigger event (Laser flash) was demonstrated and the oxygen-evolving transition of photosynthesis was monitored by time-resolved BioXAS. A manuscript on the obtained results is in preparation. These experiments open the road for the investigation of structural changes at biological metal centers which occur during a catalytic reaction, e.g., in the case of photosystem II, directly (within μs to ms) after the initiating Laser flash.

- 1 Haumann, M., Pospisil, P., Grabolle, M., Müller, C., Liebisch, P., Sole, A., Neisius, T., Dittmer, J., Iuzzolino, L., & Dau, H. (2002) *J. Synchrotron Rad.*, in press.
- 2 Haumann, M., Grabolle, M., Neisius, T., & Dau, H. (2002) *FEBS Lett.* 512,116-120.

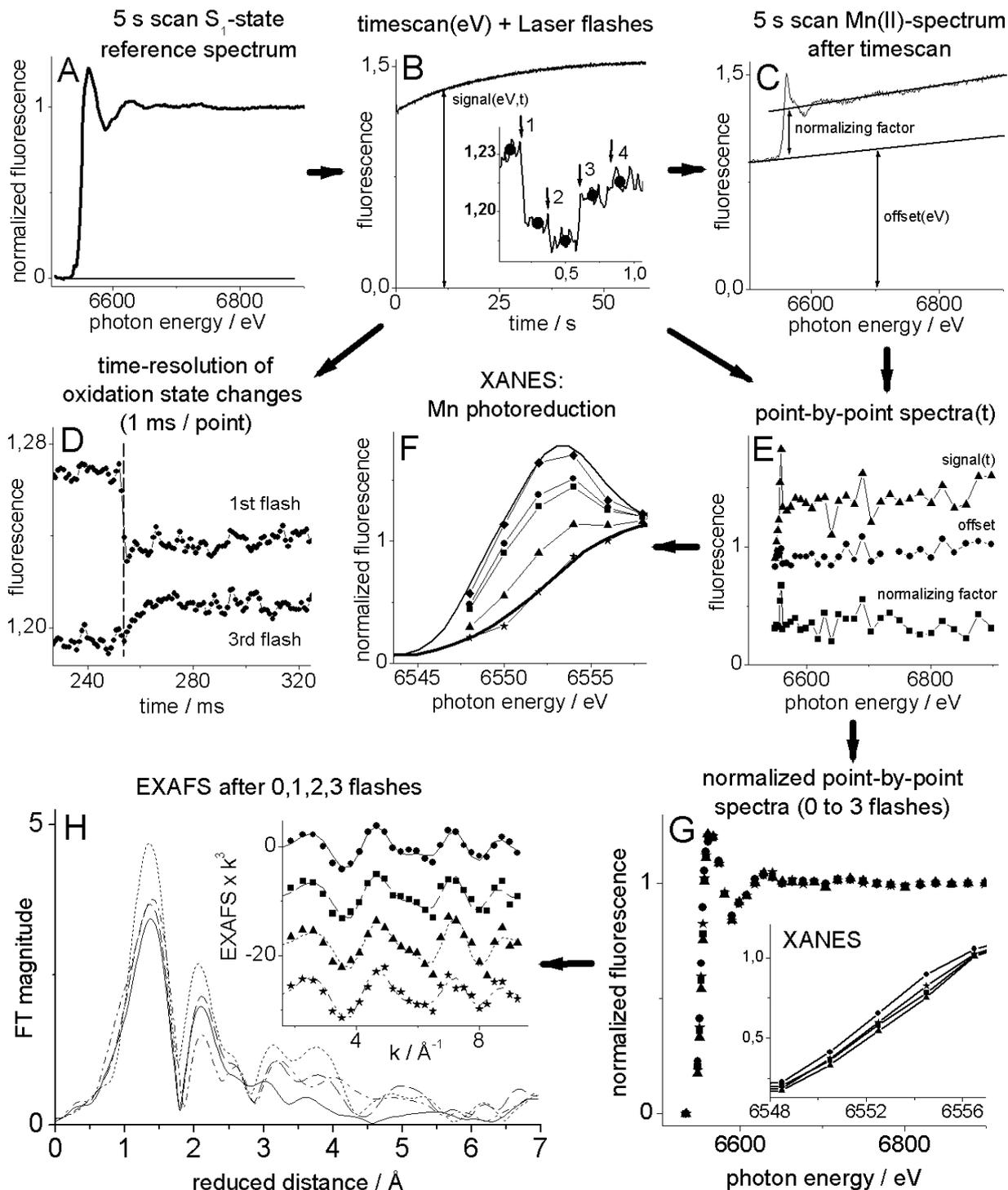


Figure 1: Time-resolved BioXAS on the Mn complex of photosystem II at room temperature. Experimental procedure: (A) On each sample, a rapid EXAFS scan was performed within 5 s yielding the S_1 -state reference spectrum. Then, (B) a timescan at fixed energy of 1 min duration (10 ms per point) was performed on a different sample spot (the inset shows the first second where four Laser flashes (arrows) were fired), followed by (C) a rapid scan (5 s) on the same spot. Timescans were recorded at 32 selected X-ray energies; at each energy, a fresh sample was used. (D) Timescans at 6551.5 eV with a resolution of 1 ms per point were recorded to follow fluorescence intensity changes upon Laser flashes 1 to 4. Data as shown in (B) and (C) yielded (E) point-by-point spectra at increasing time points. From data as shown in (E), point-by-point XANES spectra (F) were constructed according to: $fluorescence(eV, t) = (signal(t) - offset) / normalizing\ factor$ for $t = 0$ s (\star), 10 s (\blacktriangle), 25 s (\blacksquare), 30 s (\bullet), and 60 s (\blacklozenge) (thick and thin solid lines: S_1 -state (A) and Mn(II) (C) spectra collected before and after the timescans). To yield the point-by-point EXAFS spectrum of the S_1 -state, the offset values (E) were fine-adjusted using (small) factors derived from the S_1 -state reference spectrum in (A). Then, the point-by-point spectra (G) after 0 to 3 flashes (\bullet , \blacksquare , \blacktriangle , \star) were calculated according to: $fluorescence(eV, 0-3\ flashes) = (signal(0-3\ flashes) - (offset * S_1-factor)) / normalizing\ factor$. The respective signal values were obtained from the averaging of 18 time points before and between the Laser flashes (see inset in (B)); point-by-point spectra were thus recorded within 200 ms. The inset in (G) shows the XANES region; shifts of the Mn K-edge after 0 to 3 Laser flashes due to oxidation/reduction of the Mn complex are obvious. From the spectra in (G), the EXAFS region was extracted (inset in (H)), and the respective Fourier transforms (H) of spectra after 0 to 3 flashes (solid, dashed, dotted, and dashed-dotted lines) were calculated.