



Beamline: ID26	Experiment title: BioXAS with sub-millisecond time resolution on structural and oxidation state changes at protein-bound metal centers – the manganese complex of photosynthesis	Experiment number: SC1474
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Report: Photosynthetic water oxidation at the tetramanganese complex of Photosystem II (PSII) is the source of atmospheric oxygen. Understanding its mechanism is of prime interest. Water oxidation proceeds in a five-stepped catalytic cycle involving five S-states, S_0 to S_4 . Upon each state-transition, an electron is abstracted from Mn by an oxidized tyrosine residue of PSII. State transitions are induced by nanosecond Laser flashes and thereby, PSII is stepped synchronously through its catalytic cycle [1-4]. For the first time, the oxidation/reduction of Mn during each state transition was directly monitored in time-resolved XAS experiments with 10 μ s resolution at room temperature. These experiments represent the first example of XAS experiments with microsecond resolution at room temperature on a biological metalloenzyme [5-7].

Experimental: Highly active PSII samples for XAS were prepared from stock solutions at the beamline by filling with an automatic multipipette of 10 μ l of PSII solution into holes (diameter 5 mm, 20 holes per sample holder) of black Teflon sample holders covered by Kapton foil (total of about 5000 PSII samples). XAS measurements at the Mn K-edge were performed at room temperature in fluorescence mode with samples placed in plain air. A new scintillation detector (11.3 cm² active area, 38BMI/2E1-YAP-Neg, Scionix) equipped with a 20 k Ω working resistor for C-V conversion and connected to a DC to 10 MHz preamplifier was used. The electrical bandwidth of the detector system was ~500 KHz. Signals were recorded on a PC equipped with a 20 MHz A/D card by a home-made transient-recorder program. Flash-and-rapid-scan [2,3,5] XANES spectra were collected by simultaneous scanning of a Si220 crystal monochromator (6500-7100 eV, scan duration 12 s, X-ray spot size about 1x1 mm) and the undulator gap. The energy axis was calibrated using a KMnO₄ standard measured simultaneously in absorption mode. Time-resolved XAS measurements rely on the monitoring of the time course of the X-ray fluorescence intensity at a fixed excitation energy [1-6]. About 1000 timescans (10 μ s per data point) were carried out at selected excitation energies employing a fresh sample for each scan. During the timescans, a series of 10 ns-Laser flashes (from a Q-switched, frequency-doubled Nd-YAG Laser, Continuum Minilite, λ = 532 nm, beam diameter ~4 mm, 20 mJ per 5 ns pulse, flash spacing 1 s) was fired. Between the flashes, the X-ray beam was blocked by a rapid photoshutter, which opened only for a time interval of \pm 50 ms around the point in time of each flash, to minimize X-ray photoreduction. Samples were positioned in the X-ray beam by a computer-controlled sample-changer [5]. Sample exchange, Laser flashes, and data acquisition were automated by computerized trigger electronics and allowed for measurements on 20 samples within ~5 min.

Results: Figure 1A shows room temperature rapid-scan XANES spectra of the Mn complex after 0 to 3 Laser flashes applied 200 ms prior to the XAS scans. The flashes induce K-edge shifts due to oxidation/reduction of Mn, which cause pronounced differences of spectra (Fig. 1B). K-edge shifts were time-resolved by recording X-ray fluorescence timescans at fixed excitation energies (arrows).

Figure 2 shows X-ray fluorescence changes (excitation energy 6552 eV) at a resolution of 10 μ s per data point observed on Laser flashes one to four. Negatively directed changes on flashes 1, 2, and 4 reflect oxidation of Mn; the uprising transient on flash 3 is due to Mn reduction on the oxygen-evolving transition $S_{3,4} \rightarrow S_0$ in the cycle. The half-rise times of S-state transitions range between ~20 μ s and ~1 ms. For the first time, redox reactions of Mn were directly monitored during all state transitions by time-resolved XAS.

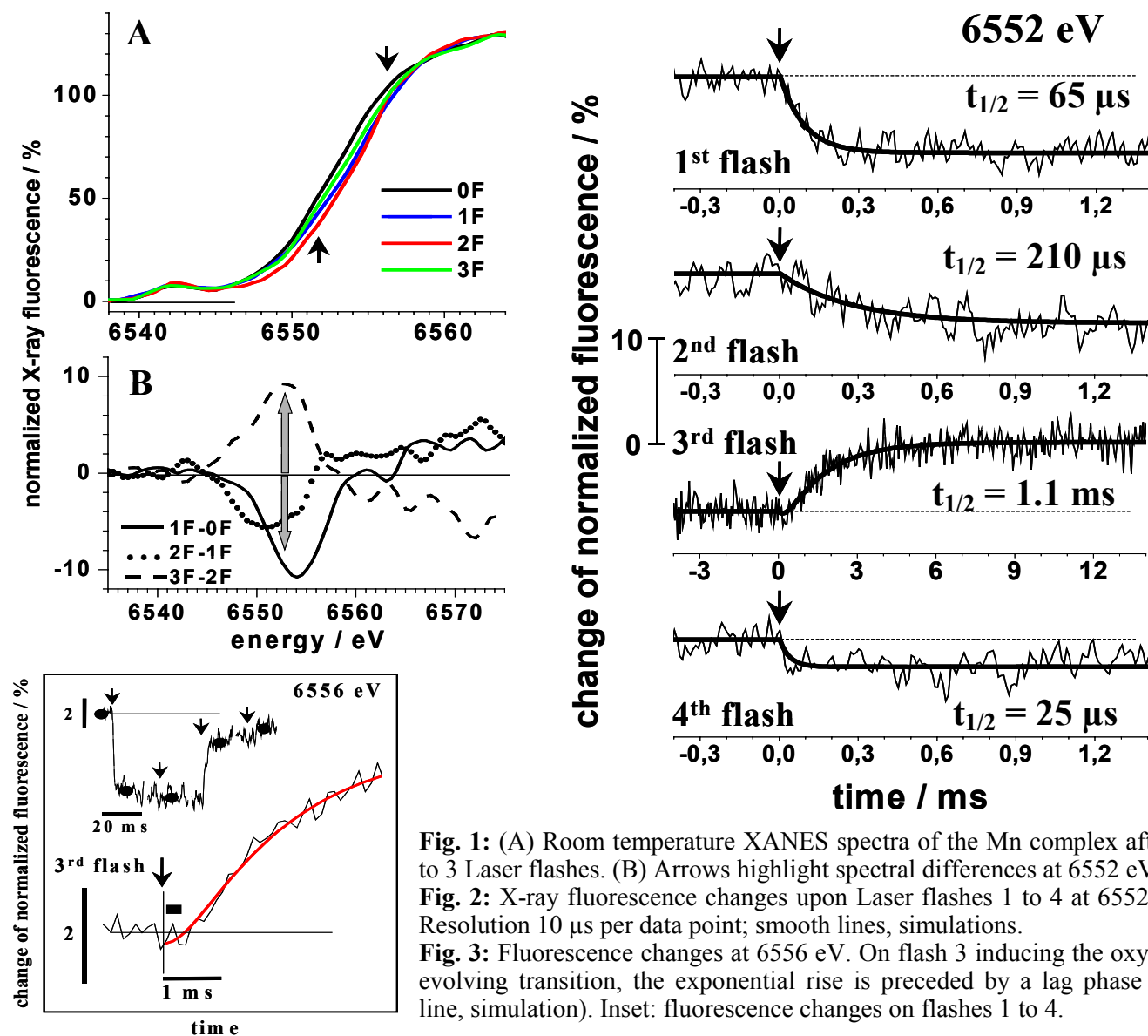


Fig. 1: (A) Room temperature XANES spectra of the Mn complex after 0 to 3 Laser flashes. (B) Arrows highlight spectral differences at 6552 eV.
Fig. 2: X-ray fluorescence changes upon Laser flashes 1 to 4 at 6552 eV. Resolution 10 μs per data point; smooth lines, simulations.
Fig. 3: Fluorescence changes at 6556 eV. On flash 3 inducing the oxygen-evolving transition, the exponential rise is preceded by a lag phase (red line, simulation). Inset: fluorescence changes on flashes 1 to 4.

Of specific interest is, whether intermediates are formed during the O_2 -evolving transition occurring within ~ 1 ms. At an excitation energy of 6556 eV, contributions from lower S-transitions are negligible. Fluorescence changes on flash 3 (Fig. 3), inducing the O_2 -evolving step, reveal that the exponential rise due to Mn reduction is preceded by a lag-phase (bar) where the oxidation state of Mn remains constant. This result provides first evidence that the unstable S_4 -state corresponds to a real intermediate with $\sim 200 \mu s$ lifetime.

Summary: (1) For the first time, time-resolved XAS measurements of oxidation state changes of Mn in PSII during its catalytic cycle provided unambiguous evidence for Mn oxidation during three S-transitions [7]. **(2)** First evidence for a short-lived intermediate formed during the oxygen-evolving step, which may only be visible in XAS experiments, was obtained [7]. **(3)** Technical and experimental progress achieved during the particularly successful Run SC1474 opens the road for meaningful XAS experiments with μs -resolution to track oxidation-state changes also on other biological metal centers and model compounds.

References:

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