



	Experiment title: Electronic and geometric structure of the Mn complex in Photosystem II – Combination of high-resolution XAS/XES and linear dichroism spectroscopy	Experiment number: SC2858
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Report: Detailed knowledge of the atomic structure of the photosystem II (PSII) Mn complex and its protein environment is a prerequisite for a complete mechanistic understanding of its water-oxidation chemistry. Due to the radiation damage problem in crystallographic data collection, the most important source for insights into the structure of the metal-oxo core of the $Mn_4Ca(\mu-O)_n$ complex presently is XAS data. On the basis of EXAFS studies it was proposed that the PSII Mn complex consists of two or three di- μ -oxo bridged manganese units and the distances between the Mn atoms were determined. As a step further the linear dichroism, LD-EXAFS spectra were collected and used to discriminate between the two short Mn-Mn vectors of 2.69 Å and 2.73 Å [1]. Now we wanted to use this information and the rationale for the simulation of the LD in XANES spectra described in [2] to get additional information on structure and orientation of the Mn complex in PSII. The present experiments were focused on LD XANES measurements at different energies at manganese $K\alpha$ emission lines in the S_1 state of the Mn complex in PSII. The goal was to study different electronic transitions upon resonant excitation. We also attempted site selective analysis exploiting the oxidation-state dependence of the $K\alpha_1$ line position [3]. Due to the fast radiation damage and the low count rate in the polarization-dependent detection of the $K\beta$ fluorescence (spin-selective studies) were not possible at this time.

Experimental: Samples with oriented PSII membrane particles were prepared as described elsewhere [4] by ultracentrifugation. After a preflash (given to synchronize the reaction centers in S_1 state and to oxidize Yz) the samples were partially dehydrated in dark and frozen in liquid nitrogen. The thickness of the PSII layer was $>300 \mu m$ which ensures an improved signal-to-noise ratio. X-ray measurements were carried out at room temperature. Before the measurements, the samples were thawed in a desiccator at room temperature at ~ 100 mbar for 15 min. Si311 monochromator crystals were used for the selection of excitation energy and $5\times Ge333$ crystals were used as emission analyzer. The angle, θ_E , between electric-field vector of the incoming, horizontally polarized X-rays and the normal of the PSII sample (membrane-normal direction of PSII) was set to 15° or 55° (the “magic” angle, where no dichroism effect is anticipated [2]). At these two angles Mn K-edge XANES spectra were recorded at three energies over the $K\alpha_1$ emission line. To avoid radiation damage, we performed rapid-scan measurements for ~ 1 s at different spots on the sample.

Results: (1) Mn $K\alpha$ and $K\beta$ line measurements on the Mn complex of PSII in the four S-states were performed after laser flash excitation of the protein to complete data sets from our previous experiments. (2) We chose three $K\alpha$ emission energies for XANES using information from the model compounds with Mn in oxidation states III and IV: at the maximum of the emission line, and at the two inflection points on both sides, aiming at site-selectivity on PSII (Fig. 1, left). The recorded XANES spectra showed a pronounced shift in the edge position, which is caused by the different location on the RIXS plane. The high energy resolution (~ 1 eV) allowed addressing different energy transitions upon resonant excitation (Fig. 1, middle). We are currently aiming at an interpretation of the underlying d-orbital energies and p-orbital mixing in the S_1 state. The averaged angle dependent XANES spectra as well as the calculated anisotropic contribution are shown in Fig. 1 (right).

The results expected from the data analysis will provide information about the angle to the membrane normal of the individual $(\mu-O)-(\mu-O)$ vectors of the $Mn-(\mu-O)_2-Mn$ units.

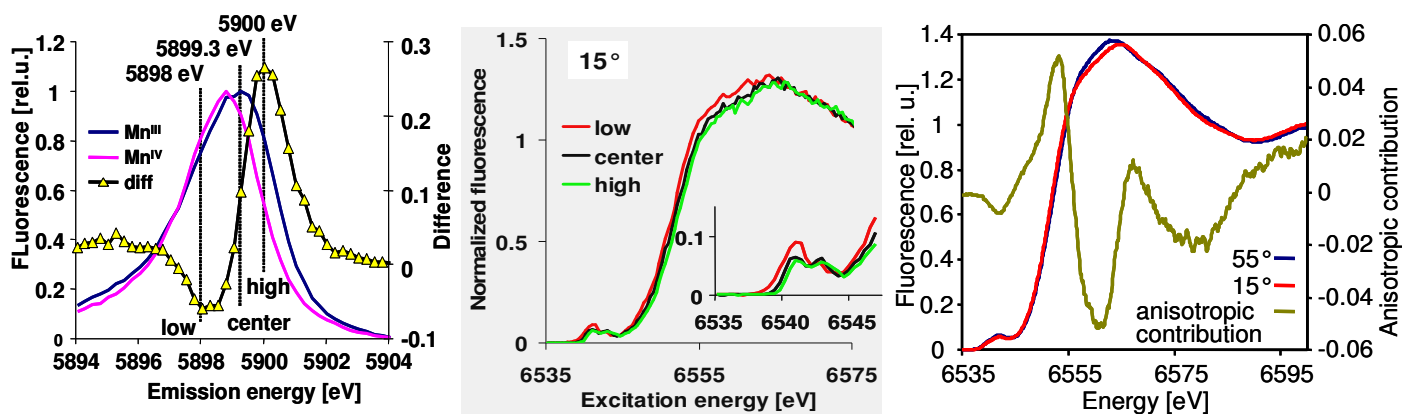


Fig. 1. Left: Selection of emission energies from measurements of $K\alpha_1$ emission lines of model complexes. Middle: XANES recorded on PSII at different emission energies. Right: The averaged linear dichroism in XANES.

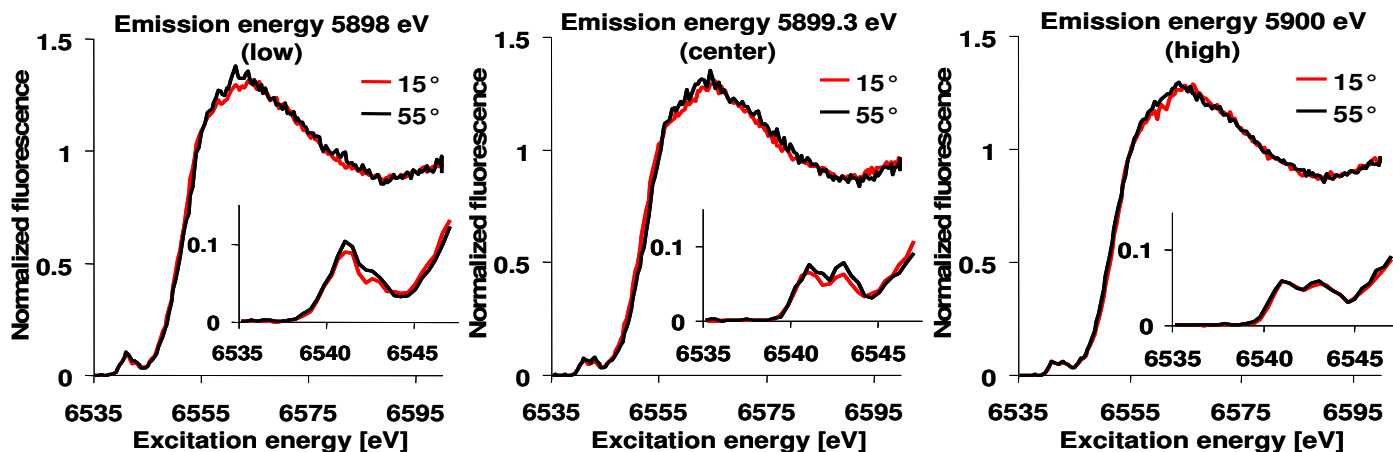


Fig. 2. XANES dichroism recorded at three different K-alpha emission energies.

(3) Study of PSII X-ray photoreduction with LD XANES (Fig. 3). In the pre-edge region the second transition (crystal field splitting) disappears upon X-ray induced reduction. It is expected that these measurements will provide information on the mechanism of photoreduction (for example if one of the $Mn-(\mu-O)_2-Mn$ units is reduced first) and therefore will provide further information on the structure of the Mn complex.

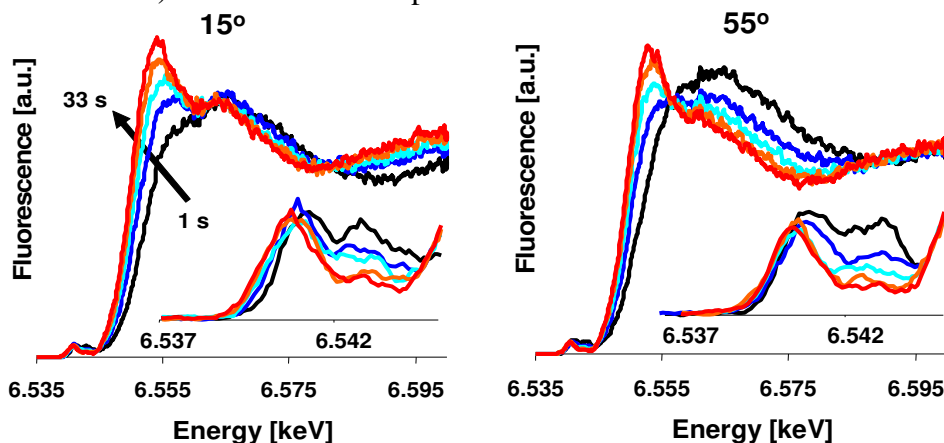


Fig. 3. XANES changes during X-ray induced photoreduction of the manganese complex in partially oriented PSII samples at two angles, providing information on linear dichroism due to Mn-backscatterer orientations. Spectra were recorded at the maximum of the $K\alpha_1$ emission line.

Conclusions: The run SC2858 was very successful. First experiments on the dichroism in the 1s-3d transitions region of K-alpha RIXS planes were performed on PSII. These experiments provided valuable information on necessary technical improvements for recording full K-alpha and K-beta RIXS planes in future experiments. Angle dependence in partially oriented PSII samples and photoreduction effects in the XANES pre-edge region were investigated. The results on the linear dichroism in the XANES spectra will be used to address the orientation of structural elements of the manganese complex of oxygenic photosynthesis.

References:

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