

Where Water is Oxidized to Dioxygen: Structure of the Photosynthetic Mn₄Ca Cluster

Oxygen, that supports all aerobic life, is abundant in the atmosphere because of its constant regeneration by photosynthetic water oxidation by green plants and cyanobacteria. The metal catalyst responsible for this reaction resides in a large protein complex, called photosystem II (PS II). The structure of the catalytic Mn₄Ca complex has been the subject of intense study ever since Mn was identified as an essential element, using X-ray absorption, EPR, and FTIR spectroscopies.^{1,2} In addition, the four recent X-ray crystallography studies of PSII promise to add valuable information to our knowledge about the structure of PS II and the Mn₄Ca complex.³⁻⁶ But until now, the precise structure of the catalyst has eluded all attempts of determination by these techniques, partly because of the susceptibility of the Mn₄Ca complex to X-ray radiation damage.⁷

Polarized extended X-ray absorption fine structure (EXAFS) measurements of oriented PS II single crystals were collected by aligning the crystals in situ using the X-ray diffraction (XRD) pattern. The set up used for this study is shown in **Figure 1**. EXAFS has the advantage of obtaining Mn-neighboring atom distances with high accuracy and at a low X-ray dose. Moreover, the polarized EXAFS spectra, collected using the pre-oriented single crystals using XRD, provide a powerful filter for choosing among many proposed structural models on the basis of the dichroism of the spectra from the single crystals. These studies resulted in deriving a set of three similar high-resolution structures for the Mn₄Ca cluster (**Figure 2**). This study, led by scientists at the Physical Biosciences Division of Lawrence Berkeley National Laboratory, involves an international collaborative effort between groups at the Technische Universität and the Freie Universität, Berlin, and the Max-Planck-Institut für Bioanorganische Chemie in Mülheim. The methodology and the set up for collecting single crystal XAS data from PS II was developed in collaboration with the Structural Biology group at SSRL.

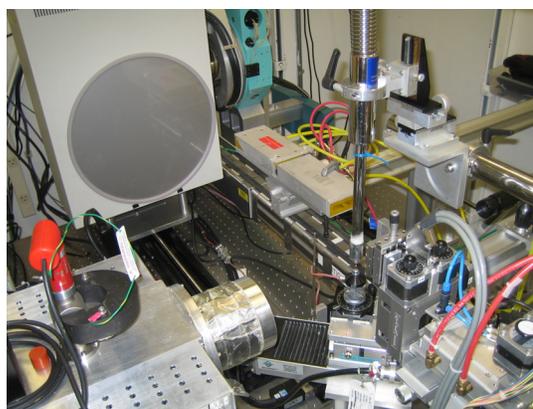


Figure 1. Single crystal experimental setup on BL 9-3 at SSRL. Polarized XAS of PS II single crystals were collected at 10 K using either a liquid He cryostat or a liquid He cryostream (shown above). X-ray diffraction pattern was collected using the imaging plate placed down stream of the crystal and was used for orienting each crystal.

Combining polarized EXAFS and the electron density obtained from X-ray crystallography data, the cluster was placed within PS II taking into account the overall trend of the electron density of the metal site and the putative ligands (**Figure 3**). This process successfully eliminates the symmetry-related orientations that arise from the $\sim \cos^2\theta$ dependence of the EXAFS signal and the non-crystallographic C₂ symmetry of the monomers in the PS II dimer. Thus, the best-fit ligand environment was obtained for all the models. The structure of Mn₄Ca cluster favoured in the present study contains structural features that are unique and are likely to be important in mechanistically facilitating water-oxidation. These models are unlike either the 3.0 or 3.5 Å resolution X-ray structures, and other previously proposed models.

The current study demonstrates that the combination of XRD and polarized EXAFS on single crystals has several advantages for unraveling structures of X-ray damage-prone, redox-active metal sites in proteins. XRD structures at medium resolution are sufficient to determine the overall shape and placement of the metal site within the ligand sphere, and refinement using polarized EXAFS can provide accurate metal-metal/ligand vectors. In addition, different intermediate states of the active site (including different metal oxidation

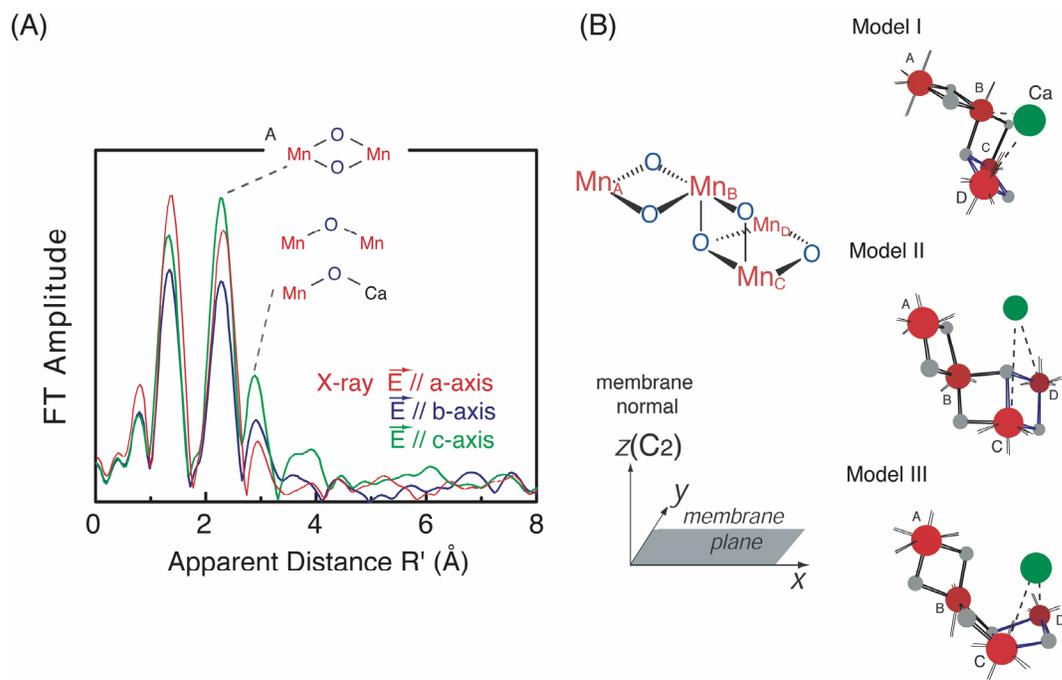


Figure 2. (A) FTs of polarized Mn EXAFS spectra from single crystals of PS II in the S_1 state. The FTs are from EXAFS spectra with the X-ray \mathbf{e} -field vector aligned parallel to the crystal unit cell axes of PSII [a (red curve), b (blue curve), and c (green curve)]. Each of the three FT peaks characteristic of Mn EXAFS from PSII is dichroic. FT peak I is from Mn-ligand backscattering; FT peak II is from three Mn-Mn distances at 2.7 to 2.8 Å; and FT peak III is from one Mn-Mn and two Mn-Ca distances at 3.3 and 3.4 Å, respectively. All Fourier peaks appear at an apparent distance R' that is shorter than the actual distance R by ~ 0.5 Å due to a phase shift. The dichroism of the metal-to-metal distances reflects the geometry of the Mn_4Ca cluster.

(B) Structural models for the Mn_4Ca cluster in PS II from polarized EXAFS. Each model is compatible with the polarized Mn EXAFS spectra of single crystals of PS II. The Mn_4 motif common to the three structures is shown in the middle (top). The models are shown in the orientation in which they should be placed in the PS II membrane according to the axis system shown in the middle (bottom). Among the symmetry-related orientation of each model, the particular orientations shown above were chosen on the basis of their compatibility with the overall electron density and the positioning of the protein ligands in the 3.0 Å resolution X-ray crystal structure.⁶ The Mn atoms are shown in red. The distance between Mn_C and Mn_D atoms is ~ 2.8 Å (indicated by blue oxo bonds), and the distance between the Mn_A and Mn_B atoms, as well as the Mn_B and Mn_C atoms, is ~ 2.7 Å. The distance between Mn_B and Mn_D is ~ 3.3 Å. The Ca atom (green sphere) is ~ 3.4 Å from two Mn atoms. The bridging motif to Ca is not well defined by our experiments; therefore, dashed lines connect the Ca atom to the two Mn atoms at ~ 3.4 Å.

states) can be studied, which may be difficult to study with XRD at high resolution. The structural model from polarized EXAFS from the S_1 state presented here, and from the other S states, will provide a reliable foundation for the investigation of the mechanism of photosynthetic water oxidation and for the design of biomimetic catalysts for water splitting.

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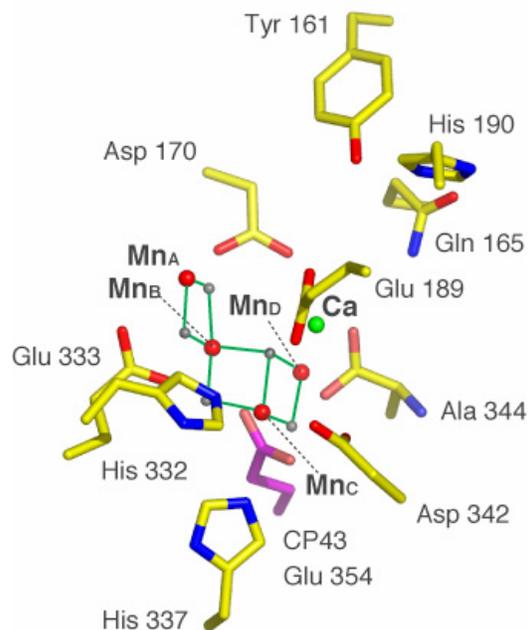


Figure 3. Placement of model II for the Mn_4Ca cluster derived from polarized Mn EXAFS in relation to the putative ligands obtained from the 3.0 Å resolution X-ray crystal structure.⁶ The spheres represent Mn (red), Ca (green), and the bridging oxygen ligand atoms (gray). The assignment of ligands is tentative because it is based on the electron density of the Mn_4Ca cluster, and its immediate environment may be altered by X-ray damage.

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