Supporting Information

Removal of Ca^{2+} from the Oxygen-Evolving Complex in Photosystem II Has Minimal Effect on the Mn_4O_5 Core Structure: A Polarized Mn X-ray Absorption Spectroscopy Study

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S1.   Drawbacks of the NaCl washing method for Ca²⁺-depletion from the OEC

As compared to the low pH/citrate treatment used in this work to remove Ca^{2+} from the OEC, the NaCl treatment[1] has the disadvantage of not only extracting the Ca^{2+} ion, but also washes out PsbP and PsbQ, the 23- and 17-kDa lumenal extrinsic polypeptides that stabilize the OEC. Their removal makes the metal cluster more exposed to the surrounding medium and therefore more prone to modification.[2-3] A modified approach[4] in the presence of chelators (2–20 mM EGTA) makes use of an additional dialysis step to reconstitute the extrinsic polypeptides to ~80–85 %. However, because Ca^{2+}-depletion via the NaCl wash requires concomitant ambient illumination, the samples are initially prepared in the S_2' state (corresponding to S_2 in native PS II), which is metastable. As a consequence, the S_1' state (dark-stable state, S_1 state in native PS II, see Figure 1 in the main text) is only accessible after dark-adaptation for many hours, which might cause damage to the OEC after Ca^{2+} depletion over time.

S2.   Determination of the mosaic spread

Since the PS II complexes are not perfectly aligned in the membrane samples, EPR spectroscopy was used to assess their extent of orientation. Factors contributing to the irregularity could be imperfect stacking of tilted membrane fractions and disorder of the PS II complexes in the membrane. It is necessary to get an estimate of the degree of disorder, because this parameter affects the dichroism of the polarized XAS spectra and must be especially accounted for in the analysis of polarized EXAFS data (eqs. 1 and 2 in the main text). For the purpose of these experiments, a Gaussian distribution of the angles between the OEC and the substrate normal is assumed.[5-7] Thus its half-width Ω, called mosaic spread, is a direct measure for the degree of disorder of a sample. Ω can in turn be determined by making use of the anisotropy of the so-called Signal II from the oxidized tyrosine $Y_D{}^{\bullet-}$ centered at g = 2.00.[8-10] The signal possesses a pronounced angular dependence, especially its feature at ~328 mT in Figure S2, where it exhibits a maximum at a sample orientation of 90° between the substrate plane and the direction of the magnetic field, but not at 0°. The ratio of its signal amplitude at 90° to that at 0°, termed dichroic ratio, was determined for all oriented samples. It is inversely related to Ω and can also be related to the angular dependence of the EPR signal of the cytochrome cyt b_{559}, a cofactor of PS II. Although the correlation of the dichroism of the cyt b_{559} signal to Ω is known,[5] owing to the laborious collection of the cyt b_{559} EPR signal at several orientations,[5-6, 11] the indirect approach to Ω via Signal II is preferred. The correlation between the two EPR signals has been investigated in a previous study from this laboratory[8] in order to construct a calibration curve, which allows for the immediate assessment of the mosaic spread Ω from the Signal II ratio.

S3.   The EXAFS equation

$$\chi(k) = S_0^2 \sum_j \frac{N_{app,j} \left| f_j\left(\pi, k, R_j\right) \right|}{kR_j^2} e^{-2\sigma_j^2 k^2} e^{-2R_j/\lambda_j(k)} \sin\left[2kR_j + a_j(k)\right]$$

(S1) $k = \dfrac{2\pi}{h} \sqrt{2m_e\left(E - E_0\right)}$

(S2)

S_0^2 is an overall amplitude reduction factor accounting for intrinsic inelastic losses. $N_{app,i}$ is the apparent number of equivalent backscattering atoms j at a distance R_j from the absorbing atom, $f_j(\pi,k,R_j)$ is the backscattering amplitude, which is a function of the atomic number of the backscattering element j, and $a_j(k)$ includes the phase shift from the central atom absorber as well as the backscattering element j. The phase shift is caused by the atomic potentials of the absorber and the backscatterer that the electron experiences when passing through. The Debye-Waller term $e^{-2\sigma_j^2 k^2}$ describes static and dynamic disorder due to an inherent distribution of distances R_j and thermal vibrations of the absorbing and scattering atoms, where σ_j is the root-mean-square deviation. The term $e^{-2R_j/\lambda_j(k)}$ describes damping due to extrinsic inelastic losses, with $\lambda_j(k)$ being the mean-free path of the photoelectron. The EXAFS contribution from each backscattering atom j is a damped sine wave in k space with both phase and amplitude depending on k. If the phase shift $a_j(k)$, which depends on the nature of both absorber and backscatterer, is known, their distance R_j can be determined. The phase shift can be obtained either from theoretical calculations or experimentally from compounds with known distances, for instance from crystal diffraction. In the amplitude function, the Debye-Waller factor and the coordination number $N_{app,i}$ at the distance R_j are highly correlated, making the determination of $N_{app,i}$ difficult without previous knowledge.
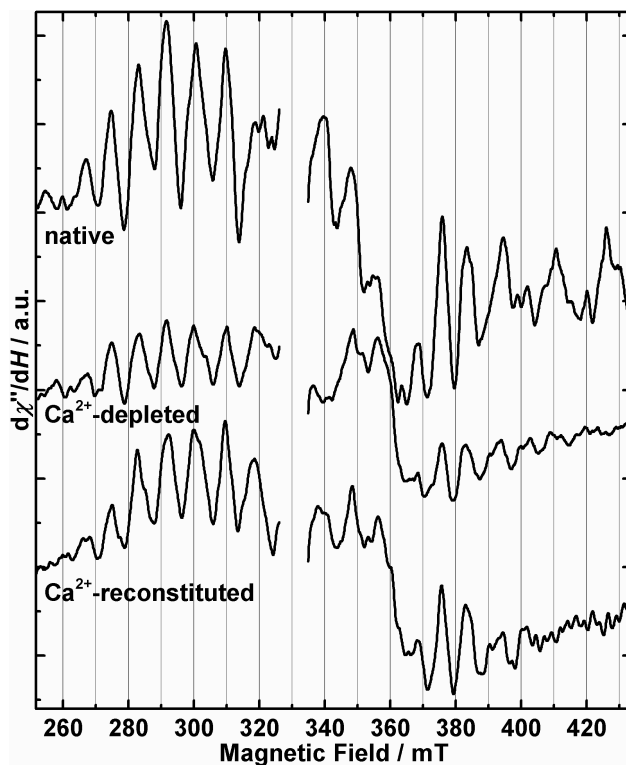
Figure S1. S_2 state MLS of native, Ca^{2+}-depleted and Ca^{2+}-reconstituted PS II, normalized with respect to the amount of the $Y_D^{\bullet}$ signal (not shown). The spectra are light-minus-dark difference spectra measured before and after sample illumination at 195 K for 20 min. The intensity of the normalized MLS serves as an indication of Ca^{2+} depletion and Ca^{2+} reconstitution. EPR conditions: microwave frequency, 9.28 GHz; microwave power, 30 mW; modulation amplitude, 32 G; temperature, 8 K.
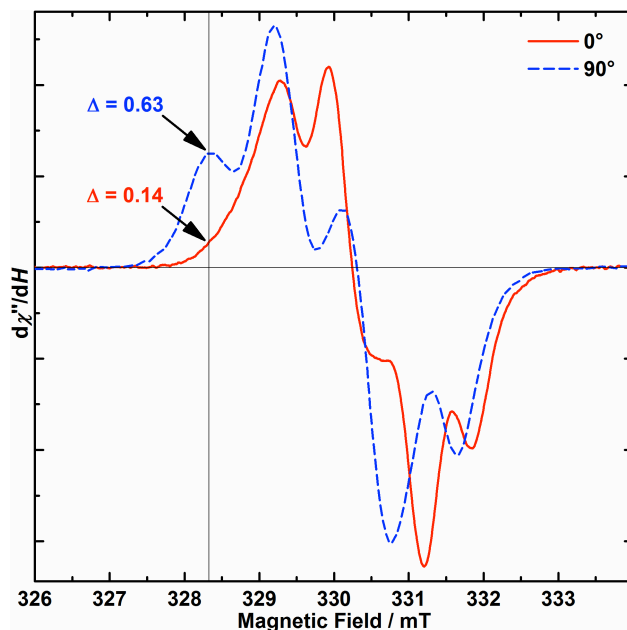
Figure S2. EPR Signal II spectra of $Y_D^{\bullet -}$ from one-dimensionally ordered PS II membrane layers at angles of 0° (solid red lines) and 90° (dashed blue lines) between the sample plane and the direction of the external magnetic field. A vertical line is drawn at the abscissa of the feature at ~328 mT, which exhibits a peak at a sample orientation of 90°. Its angular dependence, used to determine the mosaic spread Ω is clearly visible. In this example, the dichroic ratio, the ratio of the two delta values marked, was 4.5. EPR conditions: microwave frequency, 9.28 GHz; microwave power, 25 µW; modulation amplitude, 2.5 G; temperature, 20 K.
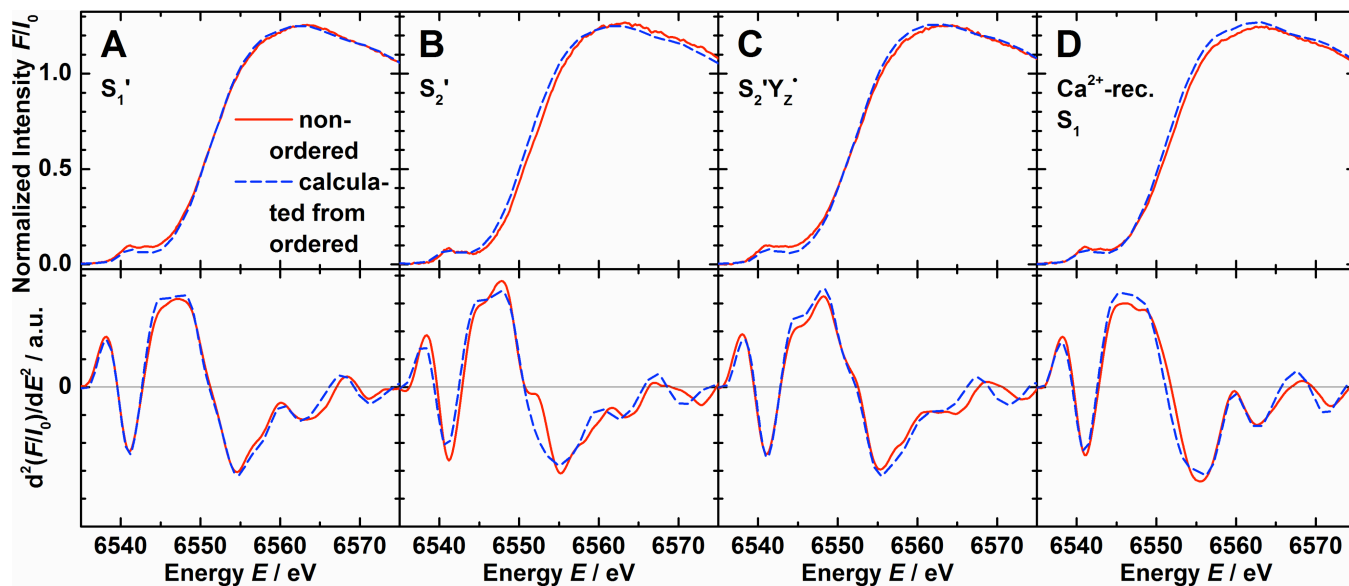
Figure S3. Isotropic Mn K edge XANES spectra (top) and corresponding second derivatives (bottom) of Ca^{2+}-depleted S_1' (A), S_2' (B) and $S_2'Y_Z^{\bullet-}$ (C) state samples and Ca^{2+}-reconstituted S_1 state (D) samples. Shown is a comparison of spectra recorded from non-ordered samples (solid red lines) and curves calculated on the basis of the oriented data (Figure 3 in the main text) at 10° and 80° (dashed blue lines).
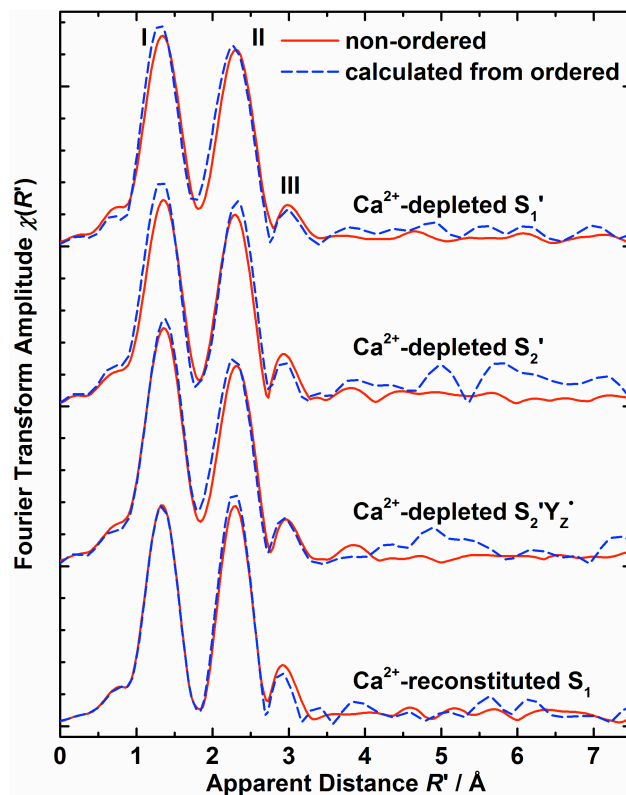
Figure S4. Isotropic Fourier transforms of Ca^{2+}-depleted S_1', S_2' and $S_2'Y_Z^{\bullet-}$ state samples and Ca^{2+}-reconstituted S_1 state samples (from top). Shown is a comparison of curves recorded from non-ordered samples (solid red lines) and curves calculated on the basis of the oriented data (Figure 5, panel B in the main text) at 10° and 80° (dashed blue lines).

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