Supporting Information for:

The $S_3$ State of the $O_2$-Evolving Complex of Photosystem II: Insights from QM/MM, EXAFS and Femtosecond X-ray Diffraction

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Section I. Description of QM/MM Models.

The choice of the high layer and the MM sphere was done according to the scheme described in our previous models.\textsuperscript{1} We were able to optimize the two spin isomers of the $S_2$ state that give rise to $g = 2.0$ and $g = 4.1$ EPR signals. The main difference is the position of O5: in $g = 2.0$ structure it is coordinated to Mn4 stabilizing oxidation states pattern III, IV, IV, IV; in the $g = 4.1$ structure it is coordinated to Mn1 stabilizing oxidation states pattern IV, IV, IV, III. This is in agreement with previously reported computational models.\textsuperscript{2}

Section II. EXAFS Analysis

Computational Details

FEFF 8.30\textsuperscript{3} combined with IFEFFIT\textsuperscript{4} v.1.2.11d programs were used to compute the EXAFS spectra of the $S_1$ and $S_0$ models. Only the QM layers were taken into account for computing the EXAFS of the QM/MM optimized structures. For the current calculations, we considered all paths with lengths up to eight scattering legs and the extremely small contribution from hydrogen atoms was not considered. A value of 0.003 Å for the Debye–Waller factors was employed in all calculations. The energy ($E$) axis was converted into photoelectron wave vector ($k$) space by using the following transformation; $k = (2m_e/(h/2\pi)^2)(E - E_0)$ where $m_e$ is the mass of the electron and $h$ is Planck’s constant. A value of $E_0 = 6540.0$ eV for the Fermi energy has been employed for the calculations involving the QM/MM model. A fractional cosine-square (Hanning) window with $\Delta k = 1$ was applied to the $k^3$-weighted EXAFS data. The grid of $k$ points, which are equally spaced at 0.05 Å$^{-1}$, was then used for the Fourier transformation (FT).
to $R$ space. A $k$ range of $2.29 - 11.5 \text{ Å}^{-1}$ for the FT for the isotropic EXAFS data was employed. The FT magnitude and EXAFS $\chi k^3$ values were appropriately scaled to match the experimental data.

**The ammonia bound $S_2$ State**

We use the ammonia bound $S_2$ state as the starting point for the S3 state preparation.

![Figure S1. QM/MM optimized structure of the NH$_3$-bound OEC $S_2$ state (III, IV, IV, IV) including coordination of water ligands as well as D1-D61, D1-His337 and CP43-R357. For clarity, D1-D170 is not shown.](image)

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Two interconvertible $S_3$ isomers

**Figure S2.** QM/MM optimized structure of the $S_3$ state (IV, IV, IV, IV) for the open [A] and closed [B] isomers, including coordination of water ligands as well as D1-D61, D1-His337 and CP43-R357. For clarity, D1-D170 and W3 are not shown.

Currently, the models for the $S_3$ state proposed in the literature, rely on either the “open” 5, 6, or the “closed” 5, 7 structure of the CaMn$_4$O$_5$ cubane. One possible way to assess the relevance of those models is to compare the simulated EXAFS to the experimentally available data using RMSD calculated on the same grid. In these calculations, we allow both the simulated intensity of the EXAFS signal and the shift in the $k$-space to relax and find the best fit using a least square fitting procedure. We find that the QM/MM optimized model of $S_3$ state (Figure S2, A) agrees best with experimental EXAFS data. Even better agreement can be reached using Monte Carlo (MC) refinement.
Figure S2. $k$-space EXAFS spectra, simulated in using the optimized model of $S_3$ state, the weighted average of multiple $S$-states as well as the MC refined model simulated.
compared to the recently reported models of $S_3$ state.$^{5-7}$ The number in the legend corresponds to the RMSD to the experimental EXAFS.$^8$

**Section III. Electron Density Maps Analysis**

In simulated difference electron density maps, there was single negative peak near the center of the OEC with no negative peak nearby (Fig. S3). This is consistent with a contraction motion of the OEC, but not rigid-body displacements or other motions. Although some other features away from the OEC were also observed in simulated differences electron density maps and some were even consistent with the features of the observed difference electron density maps, interpretations of these features are beyond the scope of this manuscript and require additional considerations. In the observed difference maps after least-square superposition of two observed electron density maps, a consistent negative peak was also observed for both molecules “A” and “a” at similar location of the OEC as in simulated maps (Fig. 2, S4). In addition to this feature, other positive and negative features nearby were clearly visible at ±3.5 $\sigma$ contour levels, some of which are consistent with those reported by the Arizona group who analyzed differences visually.
Figure S3. Simulated $S_3$-minus-$S_1$ difference Fourier maps calculated using the QM/MM $S_3$ and $S_1$ models.
**Figure S4.** The observed $S_3$-minus-$S_1$ electron density difference maps for monomer “a”.

Panels A and B refer to the same map at a different angle: view B is rotated along the horizontal axis by 45° relative to A.
Section IV: REFERENCES


