## Supporting Information for:

# Computational Insights on Crystal Structures of the OxygenEvolving Complex of Photosystem II with either $\mathrm{Ca}^{2+}$ or $\mathrm{Ca}^{2+}$ Substituted by $\mathrm{Sr}^{2+}$ 

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## S7. QM/MM optimized coordinates

QM/MM coordinates for $\mathrm{Ca}^{2+}$ and $\mathrm{Sr}^{2+}$ models in $\mathrm{S}_{1}, \mathrm{~S}_{0}, \mathrm{~S}_{-1}$, and $\mathrm{S}_{-2}$ states can be found in the accompanying supporting information file.

Figures in this manuscript were prepared using VMD ${ }^{1}$ using colors selected from ColorBrewer 2.0. ${ }^{2}$

## ABBREVIATIONS

DFT, Density Functional Theory; DMRG, Density Matrix Renormalization Group; EXAFS, Extended X-ray Absorption Fine Structure; OEC, Oxygen-Evolving Complex; ONIOM, "our own n-layered integrated molecular orbital and molecular mechanics"; PDB, Protein Data Bank; PSII, Photosystem II; QM/MM, Quantum Mechanics/Molecular Mechanics; RMSD, Root Mean Square Deviation; VMD, Visual Molecular Dynamics; XRD, X-ray Diffraction

## S1. Method details for QM/MM

## QM/MM model selection

The $\mathrm{QM} / \mathrm{MM}$ models reported in this manuscript were constructed starting from the coordinates in the $1.9 \AA$ XRD structure of PSII, ${ }^{3}$ as in our previously reported work. ${ }^{4}$
Each model includes residues with Ca atoms within $15 \AA$ of the atoms in the CaMn 4 O 5 cluster and the two chloride ions near the OEC. Oxygen atoms of water molecules that fall within the $15 \AA$ boundary were also included ( 85 total). The extent of the selection can be seen in Figure S1. Where the selection caused a gap of up to two residues in a peptide chain, the missing residues were added to provide continuity. Neutral capping groups (ACE/NME) were added for each chain break, with positions determined by the backbone atoms of neighboring residues. A few residues on the periphery of the selection were removed because their side chains extended away from the rest of the selection.

The final protein selection includes the following residues (capping residues in parenthesis use only the backbone atoms):

D1 (chain A): (57)-58-67-(68), (81)-82-91-(92), (107)-108-112-(113), (155)-156-192-(193), (289)-290-298-(299), (323)-324-344:C-terminus
CP43 (chain C): (290)-291-(292), (305)-306-314-(315), (334)-335-337-(338), (341)-342-(343), (350)-351-358-(359), (398)-399-402-(403), (408)-409-413-(414)

D2 (chain D): (311)-312-321-(322), (347)-348-352:C-terminus


Figure S1. Atoms included in the QM/MM model belong to residues with Ca atoms within $15 \AA$ of the OEC. A) Selected residues from monomer A of the $1.9 \AA$ structure are shown with colored surfaces (chain A: blue, chain C: purple, chain D: orange, HOH O atoms: red) and the rest of the protein is indicated in grey. B ) An expanded view of the selection with the OEC (shiny spheres with Mn (pink), Ca (grey), O (red)) visible in the center of the model. Two chloride ions (green spheres) are also included in the model. (Figure reproduced from SI of our previous paper. ${ }^{4}$ )

## Initial hydrogen atom placements

As reported previously, ${ }^{4}$ the hydrogen atom coordinates were guessed using the AmberTools 12 software package. ${ }^{5}$ All acidic residues (ASP, GLU) were modeled as anions. Histidine protonation patterns were determined by inspection: D1-His190 is protonated at $\mathrm{N}_{\delta}$ to be a hydrogen bond acceptor from D1-Tyr161, D1-His332 is protonated at $\mathrm{N}_{\delta}$ to leave $\mathrm{N}_{\varepsilon}$ as a ligand to Mn2, and in this work D1-His337 is protonated at both $\mathrm{N}_{\delta}$ and $\mathrm{N}_{\varepsilon}$ (donating a hydrogen bond to O 3 ) and has a positive charge.

We note that although D1-His337 has an additional proton relative to our previous model, ${ }^{4}$ the OEC structure for $\mathrm{Ca}^{2+}$-OEC $\mathrm{S}_{1}$ state is essentially the same, with $\mathrm{Mn}-\mathrm{Mn}$ distances elongated by $0.08 \AA$ on average (see Section S3 for comparison). However, the His $337^{+}$structure in the $\mathrm{S}_{1}$ state fits the $\mathrm{Ca}^{2+}$ XRD atom positions better than with neutral

His337 (see Section S6). For consistency, the positively charged imidazole is used for all structures reported in this work, including re-optimizations of the $\mathrm{Ca}^{2+}-\mathrm{OEC} \mathrm{S}_{0}$ state with either O 4 or O 5 protonated.

Sodium counter ions were added based on the electrostatic potential outside the protein in order to neutralize the system in the $\mathrm{S}_{1}$ state. Hydrogen atom placements were then refined by 500 steps of minimization using NAMD v. $2.8^{6}$ using molecular mechanics force field parameters, with all heavy atoms fixed in their X-ray assigned positions. Charges on the OEC atoms were assigned as previously reported. ${ }^{7}$

## QM/MM structure optimization

As in our previous work, ${ }^{4}$ the $\mathrm{QM} / \mathrm{MM}$ optimizations for each state was performed using the two layer ONIOM method with hydrogen link atoms, ${ }^{8}$ as implemented in Gaussian09 v.C01 or v.D01. ${ }^{9}$ The QM layer was modeled at the B3LYP ${ }^{10,11}$ level of theory using the LANL2DZ pseudopotential ${ }^{12,13}$ for $\mathrm{Mn}, \mathrm{Ca}$, and Sr and the 6-31G* basis set ${ }^{14}$ for $\mathrm{H}, \mathrm{C}, \mathrm{N}$, and O. The QM region was chosen to include the OEC, all directly ligated side chains (D1-D170, D1-E189, D1-H332, D1-E333, D1-D342, and CP43-E354), the C-terminus of D1-A344, hydrogen-bonded residues D1-H337 and CP43-R357, residue D1-D61, and ten water molecules, as shown in Figure S2. The anionic ligands were modeled as acetate, D1 C-terminus as propanoate, histidines as methylimidazoles, and arginine as methylguanidinium in the QM layer. The AMBER force field ${ }^{5}$ was used to model the MM region (Figure S2). All the atoms were allowed to relax during the QM/MM optimizations except the chloride ions, heavy atoms of neutral capping groups (ACE/NME), and oxygen atoms of water molecules in the MM region.


Figure S2. Heavy atoms described using DFT in the QM/MM model are shown in CPK representation (G: grey, N: blue, O: red, Mn: pink, Ca: orange, hydrogens omitted for clarity) with the Molecular Mechanics layer shown in grey lines. (Figure reproduced from SI of our previous paper. ${ }^{4}$ )

## S2. Spin Densities for $\mathrm{Ca}^{2+}$ and $\mathrm{Sr}^{2+}$ QM/MM models in the $\mathrm{S}_{1}, \mathrm{~S}_{0}, \mathrm{~S}_{-1}$, and $\mathrm{S}_{-2}$ states

Each state of the OEC is optimized with antiferromagnetic couplings between the Mn atoms in the ABAB form (ordered as in the $1.9 \AA$ XRD structure ${ }^{3}$ ). The resulting Mulliken spin densities for the four Mn are reported in Tables S1 and S2 for each state analyzed in this work. Regardless of whether $\mathrm{Ca}^{2+}$ or $\mathrm{Sr}^{2+}$ is included in the OEC, the resulting oxidation states in the optimized QM/MM structures are as follows:

$$
\begin{array}{lll}
\mathrm{S}_{1} & \mathrm{Mn}_{4}[\text { III,IV,IV,III }] & \\
\mathrm{S}_{0} & \mathrm{Mn}_{4}[\text { III,IV,III,III }] & \text { (with either } \mathrm{O} 4 \text { or O5 protonated) } \\
\mathrm{S}_{-1} & \mathrm{Mn}_{4}[\mathrm{III}, \mathrm{IV}, \mathrm{III}, \mathrm{II}] & \text { (with both } \mathrm{O} 4 \text { and O5 protonated) } \\
\mathrm{S}_{-2} & \mathrm{Mn}_{4}[\mathrm{III}, \mathrm{III}, \mathrm{III}, \mathrm{II}] & \text { (with both } \mathrm{O} 4 \text { and O5 protonated) }
\end{array}
$$

Table S1. Mulliken spin densities (a.u.) for QM/MM optimized structures in different oxidation states with $\mathrm{Ca}^{2+}$ as the heterocation

| $\mathrm{Ca}^{2+}$-OEC | $\mathrm{S}_{1}$ | $\mathrm{S}_{0}{ }^{\text {O4H }}$ | $\mathrm{S}_{0} \mathrm{O} 5 \mathrm{H}$ | $\mathrm{S}_{1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ | S-2 ${ }^{\text {O4H, }}$ O5H |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Mnl | 3.88 | 3.88 | 3.86 | 3.87 | 3.83 |
| Mn2 | -2.92 | -2.85 | -2.89 | -2.89 | -3.82 |
| Mn3 | 2.84 | 3.82 | 3.84 | 3.90 | 3.90 |
| Mn4 | -3.87 | -3.84 | -3.80 | -4.83 | -4.85 |

Table S2. Mulliken spin densities (a.u.) for QM/MM optimized structures in different oxidation states with $\mathbf{S r}^{\mathbf{2 +}}$ as the heterocation

| $\mathbf{S r}^{\mathbf{2 +}}$-OEC | $\mathrm{S}_{1}$ | $\mathrm{S}_{0} \mathrm{O} 4 \mathrm{H}$ | $\mathrm{S}_{0} \mathrm{O}^{\text {5H }}$ | $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ | $\mathrm{S}_{-2} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Mnl | 3.87 | 3.87 | 3.85 | 3.86 | 3.82 |
| Mn2 | -2.90 | -2.85 | -2.88 | -2.88 | -3.83 |
| Mn3 | 2.83 | 3.82 | 3.84 | 3.89 | 3.89 |
| Mn4 | -3.87 | -3.83 | -3.80 | -4.84 | -4.83 |

## S3. Interatomic distances for OEC atoms in QM/MM models

Table S3. Interatomic distances $(\AA)$ between metals in the $\mathbf{C a}{ }^{2+}$-OEC

|  | XRD ${ }^{\text {a }}$ |  | QM/MM |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | A | B | $\mathrm{S}_{1}{ }^{\text {b }}$ | $\mathrm{S}_{1}$ | $\mathrm{S}_{0}{ }^{\text {O4H }}$ | $\mathrm{S}_{0} \mathrm{O} 5 \mathrm{H}$ | $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ | S-204H, O5H |
| Mn1-Mn2 | 2.84 | 2.76 | 2.73 | 2.79 | 2.75 | 2.74 | 2.73 | 2.82 |
| Mn2-Mn3 | 2.89 | 2.91 | 2.76 | 2.83 | 2.76 | 2.78 | 2.78 | 2.76 |
| Mn1-Mn3 | 3.29 | 3.30 | 3.26 | 3.34 | 3.30 | 3.23 | 3.22 | 3.28 |
| Mn3-Mn4 | 2.97 | 2.91 | 2.68 | 2.72 | 2.86 | 2.94 | 3.08 | 3.07 |
| Mnl-Mn4 | 5.00 | 4.95 | 4.76 | 4.90 | 4.58 | 4.66 | 4.75 | 4.61 |
| $\mathrm{Ca}-\mathrm{Mn} 1$ | 3.51 | 3.46 | 3.55 | 3.67 | 3.54 | 3.39 | 3.45 | 3.42 |
| Ca-Mn2 | 3.36 | 3.29 | 3.44 | 3.31 | 3.50 | 3.45 | 3.48 | 3.44 |
| Ca-Mn3 | 3.41 | 3.44 | 3.58 | 3.44 | 3.68 | 3.77 | 3.74 | 3.69 |
| Ca-Mn4 | 3.79 | 3.80 | 3.68 | 3.71 | 3.63 | 3.94 | 3.89 | 3.75 |

${ }^{a}$ Distances from XRD monomers A and B of $\mathrm{Ca}^{2+}$ structure (PDB: 3ARC). ${ }^{3}{ }^{\text {b }}$ Data from Ref. 4 with neutral D1-His337.

Table S4. Interatomic distances (Å) between metals in the $\mathbf{S r}^{2+}$-OEC

|  | XRD $^{\mathbf{a}}$ |  | QM/MM |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | A | B | $\mathrm{S}_{1}$ | $\mathrm{~S}_{0}$ O4H | $\mathrm{S}_{0} \mathrm{O} 5 \mathrm{H}$ | $\mathrm{S}_{-1}$ O4H, O5H | S -2 O4H, O5H |
| Mn1-Mn2 | 2.81 | 2.75 | 2.74 | 2.75 | 2.74 | 2.73 | 2.82 |
| Mn2-Mn3 | 2.94 | 2.91 | 2.78 | 2.77 | 2.78 | 2.78 | 2.79 |
| Mn1-Mn3 | 3.34 | 3.33 | 3.24 | 3.31 | 3.21 | 3.22 | 3.25 |
| Mn3-Mn4 | 2.86 | 2.90 | 2.70 | 2.86 | 2.95 | 3.10 | 3.08 |
| Mn1-Mn4 | 5.03 | 5.08 | 4.73 | 4.67 | 4.75 | 4.82 | 4.79 |
|  |  |  |  |  |  |  |  |
| Sr-Mn1 | 3.55 | 3.56 | 3.63 | 3.63 | 3.49 | 3.54 | 3.52 |
| Sr-Mn2 | 3.55 | 3.45 | 3.56 | 3.63 | 3.58 | 3.62 | 3.59 |
| Sr-Mn3 | 3.63 | 3.65 | 3.69 | 3.78 | 3.84 | 3.83 | 3.73 |
| Sr-Mn4 | 4.01 | 3.99 | 3.77 | 3.70 | 4.05 | 3.91 | 3.80 |

[^0]Table S5. Interatomic distances $(\AA)$ between Mn and O atoms in the $\mathrm{Ca}^{2+}$-OEC

|  | XRD ${ }^{\text {a }}$ |  | QM/MM |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | A | B | $\mathrm{S}_{1}{ }^{\text {b }}$ | $\mathrm{S}_{1}$ | $\mathrm{S}_{0} \mathrm{O}^{4 \mathrm{H}}$ | $\mathrm{S}_{0} \mathrm{O}^{\text {5 }}$ | $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ | $\mathrm{S}_{-2} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ |
| Mnl-O1 | 1.87 | 1.79 | 1.85 | 1.84 | 1.83 | 1.86 | 1.85 | 1.77 |
| Mnl-O3 | 1.81 | 1.83 | 1.87 | 1.90 | 1.89 | 1.90 | 1.90 | 1.95 |
| Mn1-O5 | 2.60 | 2.60 | 2.90 | 3.01 | 2.90 | 2.26 | 2.34 | 2.49 |
| Mn2-O1 | 2.06 | 2.03 | 1.85 | 1.86 | 1.87 | 1.85 | 1.85 | 2.15 |
| $\mathrm{Mn} 2-\mathrm{O} 2$ | 2.13 | 2.06 | 1.78 | 1.78 | 1.74 | 1.78 | 1.77 | 1.82 |
| Mn2-O3 | 2.10 | 2.13 | 1.85 | 1.89 | 1.89 | 1.86 | 1.87 | 1.93 |
| Mn3-O2 | 1.87 | 1.94 | 1.84 | 1.85 | 1.85 | 1.85 | 1.84 | 1.81 |
| Mn3-O3 | 2.13 | 2.02 | 2.00 | 2.07 | 2.00 | 2.01 | 2.01 | 1.94 |
| Mn3-O4 | 2.09 | 2.07 | 1.78 | 1.78 | 1.89 | 1.83 | 1.88 | 1.92 |
| Mn3-O5 | 2.38 | 2.40 | 1.81 | 1.80 | 2.17 | 2.50 | 2.38 | 2.58 |
| Mn4-O4 | 2.11 | 2.08 | 1.85 | 1.84 | 2.11 | 1.76 | 2.11 | 2.12 |
| Mn4-O5 | 2.50 | 2.47 | 1.88 | 1.91 | 1.75 | 2.52 | 2.48 | 2.23 |
| Mn4-W1 | 2.22 | 2.13 | 2.07 | 2.02 | 2.09 | 2.23 | 2.24 | 2.35 |
| Mn4-W2 | 2.08 | 2.16 | 2.08 | 2.09 | 2.20 | 2.06 | 2.18 | 2.22 |

${ }^{a}$ Distances from XRD monomers A and B of $\mathrm{Ca}^{2+}$ structure (PDB: 3ARC). ${ }^{3}{ }^{\mathrm{b}}$ Data from Ref. 4 with neutral D1-His337.

Table S6. Interatomic distances $(\AA)$ between Mn and O atoms in the $\mathbf{S r}^{\mathbf{2 +}}{ }^{-} \mathbf{O E C}$

|  | XRD ${ }^{\text {a }}$ |  | QM/MM |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | A | B | $\mathrm{S}_{1}$ | $\mathrm{S}_{0}{ }^{\text {O4H }}$ | $\mathrm{S}_{0} \mathrm{O}^{\text {5 }}$ H | $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ | $\mathrm{S}_{-2} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ |
| Mnl-O1 | 2.06 | 1.90 | 1.84 | 1.82 | 1.85 | 1.84 | 1.76 |
| Mn1-O3 | 1.94 | 2.02 | 1.87 | 1.89 | 1.90 | 1.90 | 1.94 |
| Mn1-O5 | 2.64 | 2.68 | 2.83 | 2.98 | 2.26 | 2.36 | 2.56 |
| Mn2-O1 | 1.94 | 1.90 | 1.85 | 1.87 | 1.84 | 1.84 | 2.15 |
| Mn2-O2 | 1.75 | 1.83 | 1.76 | 1.74 | 1.78 | 1.77 | 1.84 |
| Mn2-O3 | 2.01 | 1.95 | 1.87 | 1.89 | 1.86 | 1.87 | 1.92 |
| Mn3-O2 | 1.72 | 1.71 | 1.84 | 1.85 | 1.85 | 1.84 | 1.85 |
| Mn3-O3 | 2.01 | 1.88 | 2.02 | 2.01 | 2.00 | 2.01 | 1.95 |
| Mn3-O4 | 1.73 | 1.86 | 1.78 | 1.90 | 1.84 | 1.89 | 1.89 |
| Mn3-O5 | 2.48 | 2.36 | 1.79 | 2.15 | 2.40 | 2.32 | 2.35 |
| Mn4-O4 | 1.87 | 1.92 | 1.82 | 2.09 | 1.76 | 2.10 | 2.07 |
| Mn4-O5 | 2.58 | 2.53 | 1.93 | 1.75 | 2.59 | 2.50 | 2.28 |
| Mn4-W1 | 2.09 | 2.12 | 2.08 | 2.09 | 2.21 | 2.23 | 2.32 |
| Mn4-W2 | 2.04 | 2.08 | 2.07 | 2.19 | 2.06 | 2.17 | 2.20 |

[^1]S4. Structural changes due to $\mathrm{Ca}^{2+} / \mathrm{Sr}^{2+}$ substitution in $\mathrm{QM} / \mathrm{MM}$ models of the $\mathrm{S}_{1}, \mathrm{~S}_{0}, \mathrm{~S}_{-1}$, and $\mathrm{S}_{-2}$ states


Figure S3. QM/MM optimized $\mathrm{Ca}^{2+}$-OEC structures with oxidation states $\mathrm{S}_{1}, \mathrm{~S}_{0}$, and $\mathrm{S}_{-1}$. Light colored atoms show positions when $\mathrm{Sr}^{2+}$ is the heterocation ( $\mathrm{S}_{1}$ : orange, $\mathrm{S}_{0} \mathrm{O}^{24 H}$ : blue, $\mathrm{S}_{0} \mathrm{O} 5 \mathrm{H}$ : purple, $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ : pink, $\mathrm{S}_{-2} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ : salmon). Bonds to OEC ligands and terminal waters (W1-W4) are shown as grey and black dashed lines, respectively. For clarity, His332, Arg357, and other waters are not shown. Atom and side chain labels can be found in Figure 1 of the main text.

## S5. EXAFS simulations

EXAFS simulations were performed in the same way as in our previously published work. ${ }^{4}$ FEFF $8.30^{16}$ combined with IFEFFIT ${ }^{17}$ v.1.2.11d programs were used to compute the EXAFS spectra of the $\mathrm{S}_{1}, \mathrm{~S}_{-1}$ and $\mathrm{S}_{-2}$ 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 $0.003 \AA$ 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: $k=\left(2 \mathrm{~m}_{\mathrm{e}} /(\mathrm{h} / 2 \pi)^{2}\right)\left(\mathrm{E}-\mathrm{E}_{0}\right)$ where $\mathrm{m}_{\mathrm{e}}$ is the mass of the electron and h is Planck's constant. A value of $\mathrm{E}_{0}=6540.0 \mathrm{eV}$ for Mn and 16117.0 eV for Sr is as used as the Fermi energy for the calculations involving the $\mathrm{QM} / \mathrm{MM}$ models. A fractional cosine-square (Hanning) window with $\Delta k=1$ was applied to the $k^{3}$-weighted EXAFS data. The EXAFS $\chi k^{3}$ values were appropriately scaled to match the experimental data.

For the $S_{1}$ states, we compare calculated EXAFS spectra for the QM/MM models directly to the experimental data and also refine the coordinates (R-QM/MM) using the Monte Carlo method reported in our previous work. ${ }^{4}$ In the case of Mn K-edge EXAFS, the R-QM/MM refinement improves the residual cost function from $\sim 500$ to 115 by moving the OEC atoms $0.07 \AA$ on average. For the Sr K-edge EXAFS simulations, the R-QM/MM structure improves the residual from 650 to 400 with average OEC atom movements of $0.07 \AA$. (We note that due to differences in signal-to-noise, the residuals cannot be used to compare the quality of the fit between the Mn and Sr results).


Figure S4. Experimental and calculated EXAFS spectra of $\mathrm{S}_{1}$ states. a) Mn K-edge EXAFS spectra of $\mathrm{Ca}^{2+}$-OEC, with QM/MM and refined QMMM structures along with b) the Fourier Transform, and c) corresponding results for the Sr Kedge EXAFS spectra of $\mathrm{Sr}^{2+}$-OEC along with d) the Fourier Transform. Experimental spectra are from Ref. 18 for Ca and Ref. 19 for Sr .

The oxidation state of the OEC QM/MM model is $\mathrm{Mn}_{4}[\mathrm{III}, \mathrm{IV}, \mathrm{III}, \mathrm{II}]$ in the $\mathrm{S}_{-1}$ state and $\mathrm{Mn}_{4}[\mathrm{IIII}, \mathrm{III}, \mathrm{III}, \mathrm{II}]$ in the $\mathrm{S}_{-2}$ state. In order to determine whether these states are consistent with X-ray radiation damage, we compare the calculated Mn K-edge EXAFS spectra with experimental data from a sample that has been exposed to X-ray radiation. ${ }^{20}$ This spectrum is reported to contain $\sim 25 \% \mathrm{Mn}(\mathrm{II})$, which corresponds to one $\mathrm{Mn}(\mathrm{II})$ per OEC on average. Notably, the dosage reported to obtain this state is somewhat larger than the reported dosage for either $\mathrm{Ca}^{2+}$ or $\mathrm{Sr}^{2+}$ crystal structures of PSII and, therefore, represents a state that is likely to be more reduced on average than the crystal structures.

Figure S 5 shows the comparison between the QM/MM and experimental spectra, suggesting that the experimental data might include contributions from the $\mathrm{S}_{-1}$ and $\mathrm{S}_{-2}$ states, each with one $\mathrm{Mn}(\mathrm{II})$. However, neither $\mathrm{S}_{-1}$ nor $\mathrm{S}_{-2}$ give a complete match since the experimental data come from a sample that is likely to contain a mixture of states and be even more reduced than the XRD structures. However, we can use the EXAFS results to check whether it is appropriate to consider the protonation of both O 4 and O 5 in the $\mathrm{QM} / \mathrm{MM}$ model for more reduced states. We therefore calculated EXAFS spectra of $\mathrm{S}_{-1}$ with only O 5 protonated. The $\mathrm{S}_{-1}$ state has a residual of 412 with only O 5 protonated vs. 511 with both O 4 and O 5 protonated. MC refinement improves the fit of the singly protonated $\mathrm{S}_{-1}$ significantly (to a residual of 61 ), but requires atomic movements of over $0.15 \AA$ that result in a poor fit the XRD coordinates. The maximum movement of OEC atoms in the $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ state during MC refinement is $<0.05 \AA$, so we propose that the second reduction of the OEC is accompanied by protonation to result in the $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ state analyzed in the main text. Of the states reported here, the $\mathrm{S}_{-2} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ state is the best match for the photoreduced spectrum (initial residual of 379 with MC refinement improving to 136 with atom movements of $\sim 0.05 \AA$ ). Further protonation of $\mu$-oxo bridges in the $\mathrm{S}-2$ state results in a poor fit to the XRD data.


Figure S5. Experimental Mn K-edge EXAFS spectra for PSII samples with $\sim 25 \% \mathrm{Mn}(\mathrm{II})$ content due to X-ray exposure ${ }^{20}$ plotted against the calculated EXAFS spectra of the $\mathrm{Ca}^{2+}-\mathrm{OEC}$ in the $\mathrm{S}_{-1}$ state a) with only O 5 protonated, b) with both O 4 and O 5 protonated, or c) the $\mathrm{S}_{-2}$ state with both O 4 and O 5 protonated.

## S6. RMSDs of QM/MM models against XRD coordinates

## QM/MM structures with His337 positively charged fit XRD coordinates better than with His 337 neutral

Previously, we reported optimized $\mathrm{QM} / \mathrm{MM}$ structures for the $\mathrm{S}_{1}, \mathrm{~S}_{0} \mathrm{O} 4 \mathrm{H}$, and $\mathrm{S}_{0}{ }^{\mathrm{O} 5 \mathrm{H}}$ states with D1-His337 in the neutral singly-protonated state, with $\mathrm{N}_{\varepsilon}$ forming a hydrogen bond with O3. ${ }^{4}$ These structures provided a good match to the published EXAFS spectra for the $S_{1}$ and $S_{0}$ states. However, we find that structures optimized with positively charged D1-His 337 result in a slightly better fit to the reported XRD coordinates, with RMSD values (in $\AA$ ) relative to monomer A of the $\mathrm{Ca}^{2+}-\mathrm{OEC}$ structure ${ }^{3}$ of 0.300 for $\mathrm{S}_{1}, 0.301$ for $\mathrm{S}_{0} \mathrm{O} 4 \mathrm{H}$, and 0.285 for $\mathrm{S}_{0} \mathrm{O} 5 \mathrm{H}$, as compared to 0.301 for $\mathrm{S}_{1}, 0.350$ for $\mathrm{S}_{0}{ }^{\mathrm{O} 4 \mathrm{H}}$, and 0.289 for $\mathrm{S}_{0} \mathrm{O} 5 \mathrm{H}$ when D 1 -His 337 is neutral. Since the improved XRD fit with the additional His337 proton is consistent with the protonation of $\mu$-oxo bridges that occurs during X-ray data collection, the positively charged His337 is used for all structures reported here.

## Structures aligned by protein backbone atoms in QM/MM model

For general analysis, the QM/MM models are aligned to the corresponding XRD coordinates using the backbone atoms of all residues included in model, including capping groups. The RMSD values of $\mathrm{QM} / \mathrm{MM}$ model backbone atoms vs. corresponding XRD coordinates after alignments are $0.31-0.35 \AA$ for all cases. The RMSDs for the ten atoms in the OEC are determined using the aligned structures and are reported in Figure 2 and Table S7.
Table S7. OEC atom RMSDs ( $\AA$ ) for $\mathbf{Q M} / \mathbf{M M}$ vs. XRD structures

|  | $\mathrm{S}_{1}$ | $\mathrm{~S}_{\mathrm{o}}{ }^{\mathrm{O} 4 \mathrm{H}}$ | $\mathrm{S}_{0} \mathrm{O}^{05} \mathrm{H}$ | $\mathrm{S}_{-1} \mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}$ | $\mathrm{S}_{-2}{ }^{\mathrm{O} 4 \mathrm{H}, \mathrm{O} 5 \mathrm{H}}$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{C a}^{2+}$ a |  |  |  |  |  |
| Monomer A | 0.30 | 0.32 | 0.32 | 0.28 | 0.29 |
| Monomer B | 0.32 | 0.33 | 0.32 | 0.28 | 0.29 |
|  |  |  |  |  |  |
| Sr $^{2+\mathrm{b}}$ |  |  |  |  |  |
| Monomer A | 0.35 | 0.35 | 0.34 | 0.30 | 0.29 |
| Monomer B | 0.30 | 0.29 | 0.26 | 0.24 | 0.23 |

${ }^{a} \mathrm{Ca}^{2+}$ structures from PDB: 3ARC. ${ }^{3}{ }^{\mathrm{b}} \mathrm{Sr}^{2+}$ structures from PDB: 4IL6. ${ }^{15}$

Based on these backbone-aligned structures, it is clear that the more reduced $\mathrm{S}_{-1}$ and $\mathrm{S}_{-2}$ states are a better fit to the XRD coordinates than the $S_{1}$ state. However, since the samples are presumably in the $S_{1}$ state at the start of the data collection, we also looked to characterize the RMSD values for weighted combinations of QM/MM coordinates as compared to the reported structures. For both the $\mathrm{Ca}^{2+}$ and $\mathrm{Sr}^{2+}-\mathrm{OEC}$ structures, the weighted averages produced a better fit than any individual $\mathrm{QM} / \mathrm{MM}$ coordinate set (the results for monomer B follow similar trends in each case). A series of sample cases are shown in Figures S6 and S7, with the composition producing the best RMSD fit indicated by arrows. For the $\mathrm{Ca}^{2+}$-OEC structure, the best fit for monomer A is $40-50 \% \mathrm{~S}_{1}$ and $40-50 \% \mathrm{~S}_{-2}$, with small contributions from $\mathrm{S}_{0} \mathrm{O} 5 \mathrm{H}$ and/or $\mathrm{S}_{-1}$ states. Assuming $100 \%$ occupancy of the Sr site, the best fit for monomer A of the $\mathrm{Sr}^{2+}$ OEC is $40-70 \% \mathrm{~S}_{-2}, 15-40 \% \mathrm{~S}_{0} \mathrm{O} 5 \mathrm{H}$, and the remainder from $\mathrm{S}_{0} \mathrm{O} 4 \mathrm{H}$ and/or $\mathrm{S}_{-1}$ states. The OEC atoms that are matched least well when using the averaged coordinates include $\mathrm{O} 5, \mathrm{O} 4$, and O 2 for $\mathrm{Ca}^{2+}$ - OEC and Mn4 and O 1 for the $\mathrm{Sr}^{2+}$-OEC structure.


Figure S6. RMSD values for all OEC atoms in the $\mathrm{Ca}^{2+}$-OEC structure when using weighted average coordinates. The best fit for the $\mathrm{Ca}^{2+}$-OEC structure relative to the coordinates reported for monomer A of the XRD structure (PDB: 3ARC ${ }^{3}$ ) is $0.21 \AA$. The $\mathrm{QM} / \mathrm{MM}$ structures are aligned to the XRD system using backbone atoms prior to OEC analysis.


Figure S7. RMSD values for all OEC atoms in the $\mathrm{Sr}^{2+}$-OEC structure when using weighted average coordinates. The best fit for the $\mathrm{Sr}^{2+}-\mathrm{OEC}$ structure relative to the coordinates reported for monomer A of the XRD structure (PDB: 4IL6 ${ }^{15}$ ) is $0.27 \AA$. The QM/MM structures are aligned to the XRD system using backbone atoms prior to OEC analysis.

## B-factors from XRD structures

Due to slight movements during optimization in the protein residues ligating the OEC, the QM/MM models do not optimally overlay the OEC atoms when using the protein backbone for alignment. Therefore, for a more detailed comparison of the OEC structures, the QM/MM models are also aligned to the XRD coordinates using the five OEC atoms that have the lowest B-factors in monomer A of the Ca ${ }^{2+}$ structure: O1, Mn1, Mn2, O3, Mn3. ${ }^{3}$ The B-factors for each monomer are shown in Figure S8. These five atoms are among the best-resolved OEC atoms in the other XRD models as well, so for consistency, these atoms are also used for $\mathrm{Ca}^{2+}$ monomer B and $\mathrm{Sr}^{2+}$ structure alignments.


Figure S8. B-factors reported for OEC atoms in the XRD structures containing $\mathrm{Ca}^{2+}\left(\mathrm{PDB}: 3 \mathrm{ARC}^{3}\right)$ or $\mathrm{Sr}^{2+}\left(\mathrm{PDB}^{2}\right.$ : 4L66 $\left.{ }^{15}\right)$. Lines serve only to show patterns in each data series.

## Structures aligned by selected OEC atoms

Once the QM/MM models are aligned to the XRD coordinates using the five relatively well-defined OEC atoms (O1, Mn1, Mn2, O3, Mn3), we then determine the displacement for each OEC atom in each S state individually. For the set of atoms with lower B-factors, most atoms are well within the experimental error for the XRD coordinates (Figure S9). There is no significant change in the position of these atoms upon heterocation substitution or Mn reduction, and in most cases the $\mathrm{S}_{0}$ protonation does not affect the alignment. Therefore, we conclude that these atoms are not affected by changes that result from X-ray radiation damage.
As mentioned in the main text, the position of Ol in the $\mathrm{Sr}^{2+}$ - OEC structure is less well resolved in monomer A relative to other OEC atoms than in the other models. The consistent displacement of this atom in all the S -state $\mathrm{QM} / \mathrm{MM}$ models with $\mathrm{Sr}^{2+}$ is only slightly larger than the reported experimental error. ${ }^{15}$ Since the $\mu$-oxo bridges are difficult to resolve, we propose that the $\mathrm{QM} / \mathrm{MM}$ model coordinates provide a more likely location for the Ol atom in monomer A, leading to a position that is similar to that seen in monomer B.


Figure S9. Displacement of individual QM/MM model OEC atoms relative to XRD coordinates (PDB: 3ARC ${ }^{3}$ or 4IL6 ${ }^{15}$ ) after alignment using the set of these five atoms.

## Comparison with XRD electron density

In addition to comparing the $\mathrm{QM} / \mathrm{MM}$ coordinates with the atomic positions assigned in the crystal structures, we also compare the calculated coordinates to the reported electron density for monomer A of $\mathrm{Ca}^{2+}$-PSII. ${ }^{3}$ After alignment of the $\mathrm{QM} / \mathrm{MM}$ models to the XRD coordinates using the five lowest B -factor atoms ( $\mathrm{O} 1, \mathrm{Mn} 1, \mathrm{Mn} 2, \mathrm{O} 3$, and Mn 3 ), the location of the QM/MM atoms are shown in Figure S10 relative to the reported electron density.


Figure S10. Overlay of $\mathrm{QM} / \mathrm{MM}$ model coordinates relative to XRD coordinates for monomer A of Ca²+_PSII (PDB: $3 \mathrm{ARC}^{3}$ ) after alignment using the set of five OEC atoms (O1, Mn1, Mn2, O3, Mn3). In all panels, the XRD coordinates are shown in green. The mesh isosurface indicates an electron density of $0.2 \mathrm{e} / \AA^{3}$ in panels a) and b) and $0.15 \mathrm{e} / \AA^{3}$ in panels c) and d). The $\mathrm{S}_{1}$ optimized $\mathrm{QM} / \mathrm{MM}$ model is shown in panels a) and c) with Mn in purple and Ca in orange. The $\mathrm{S}_{-2}$ optimized $\mathrm{QM} / \mathrm{MM}$ model is shown in panels b ) and d) with Mn in light purple and Ca in yellow (protons on O4 and O5 are not shown to allow for easier comparison of heavy atom placements).

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[^0]:    ${ }^{a}$ Distances from XRD monomers A and B of $\mathrm{Sr}^{2+}$ structure (PDB: 4IL6). ${ }^{15}$

[^1]:    ${ }^{\text {a }}$ Distances from XRD monomers A and B of $\mathrm{Sr}^{2+}$ structure (PDB: 4IL6). ${ }^{15}$

