Supporting Information

D1-S169A substitution of photosystem II reveals a novel S2-state structure

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Supplemental Material

Supplementary Figures S1-S4

Supplementary Note 1
Figure S1. $S_2$-minus-$S_1$ state EPR spectra of D1-S169A PSII core complexes. The black arrows indicate the peaks chosen for quantifying the $S_2$ state for studying the decay kinetics.
**Figure S2.** S<sub>2</sub>-minus-S<sub>1</sub> state EPR spectra of wild-type PSII core complexes illuminated at 200 K (t = 0 s) and then incubated at 261 K in the dark. The EPR spectra were collected at different time points, t = 60 s, 90 s and 120 s, as labelled.
Figure S3. Effect of ammonia treatment on the steady-state O₂-evolution activity of D1-S169A PSII (100% activity = 400 µmol O₂ (mg Chl)⁻¹ h⁻¹). The effect of ammonia was studied in the presence of 100 mM NH₄Cl. The average and the standard error of 3 readings are reported.
Figure S4. Effect of bicarbonate treatment on the steady-state \( O_2 \)-evolution activity of D1-S169A PSII. The effect of bicarbonate was studied in the presence of 8.4 mM NaHCO\(_3\). The average and the standard error of 3 readings are reported.

Supplementary Note 1

Assignment of the spin state for the \( S_2^X \) structure of S169A PSII

Although the Wx-bound \( S_2 \) state seems to resemble the closed cubane structure of high-spin \( S_2 \) isomer, its electronic structure actually mirrors that of the open cubane, low-spin isomer. When attempting to assign the sextet (Mn\( ^{IV} \) Mn\( ^{IV} \) Mn\( ^{IV} \) Mn\( ^{III} \) Mn\( ^{IV} \) Mn\( ^{III} \)) electronic state to the Wx-bound structure, the spin states are extremely unstable. Despite atom-specific charge/multiplicity assignments, the quartet on Mn\( ^{IV} \) crashes down to a doublet and the energy jumps up over 23 kcal/mol compared to the low-spin state. Conversely, the assignment of the doublet (Mn\( ^{III} \)
Mn$^{2\text{IV}}$ Mn$^{3\text{IV}}$ Mn$^{4\text{IV}}$) electronic structure is readily stabilized and maintained throughout optimization.

Theoretical energetics estimates for $S_1$ to $S_2$ transition

We performed two pairs of single point calculations using the $S_1$ and $S_2$ ($g = 2$) electronic states with both the WT $S_2$, $g = 2$ structure and the novel S169A $S_2^x$ structure. (Here, superscripts indicate structure and subscripts indicate the electronic state.) The $S_1$ to $S_2$ transition in the WT $S_2$, $g = 2$ structure was 0.5 kcal/mol uphill, making the $S_1$ state slightly more energetically favorable. With the S169A $S_2^x$ structure, the difference was 5.4 kcal/mol in favor of the $S_2$ state. Likely a significant rearrangement of atoms would be required for the $S_1$ electronic structure to be the more favorable state in the S169A mutant, whereas in WT, the optimized $S_1$ and $S_2$, $g = 2$ structures are nearly identical, with only a slight change in Mn-Mn distance. This indicates that this novel structure could hinder the decay of the $S_2$ state in the S169A mutant.

References