

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

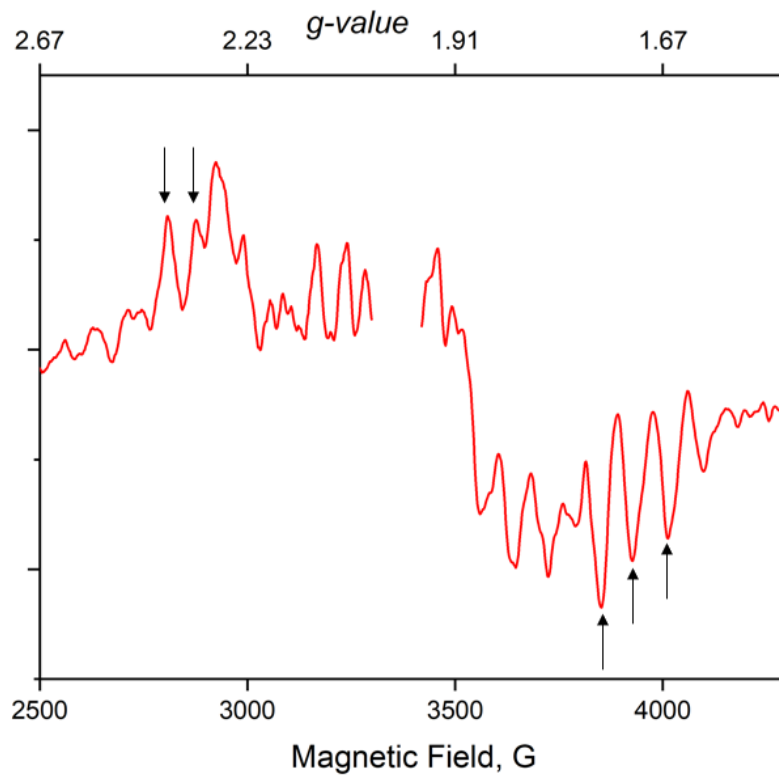


Figure S1. S_2 -minus- S_1 state EPR spectra of D1-S169A PSII core complexes. The black arrows indicate the peaks chosen for quantifying the S_2 state for studying the decay kinetics

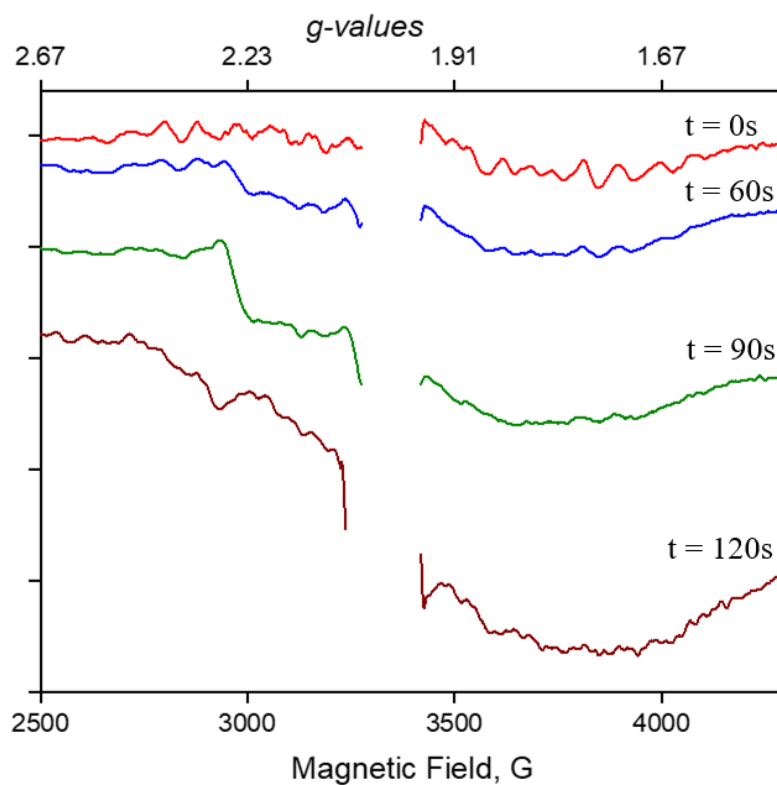


Figure S2. S_2 -minus- S_1 state EPR spectra of wild-type PSII core complexes illuminated at 200 K ($t = 0\text{ s}$) and then incubated at 261 K in the dark. The EPR spectra were collected at different time points, $t = 60\text{ s}$, 90 s and 120 s , as labelled.

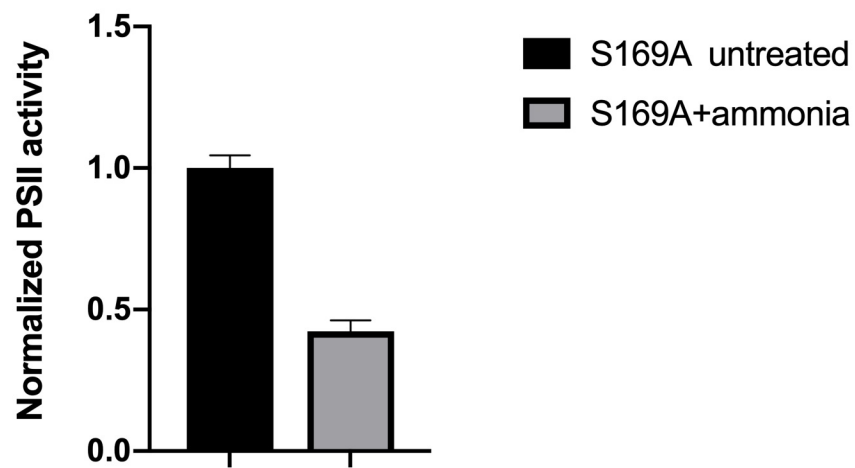


Figure S3. Effect of ammonia treatment on the steady-state O₂-evolution activity of D1-S169A PSII (100% activity = 400 $\mu\text{mol O}_2$ (mg Chl)⁻¹ h⁻¹). The effect of ammonia was studied in the presence of 100 mM NH₄Cl. The average and the standard error of 3 readings are reported.

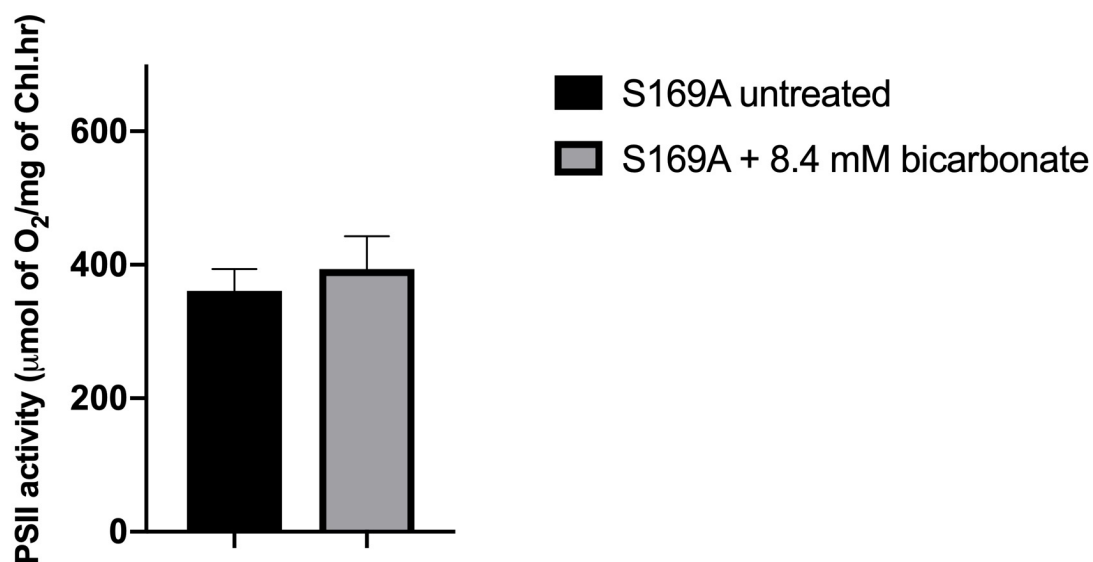


Figure S4. Effect of bicarbonate treatment on the steady-state O₂-evolution activity of D1-S169A PSII. The effect of bicarbonate was studied in the presence of 8.4 mM NaHCO₃. The average and the standard error of 3 readings are reported.

Supplementary Note 1

Assignment of the spin state for the S₂^X structure of S169A PSII

Although the W_x-bound S₂ state seems to resemble the closed cubane structure of high-spin S₂ isomer, its electronic structure actually mirrors that of the open cubane, low-spin isomer. When attempting to assign the sextet (Mn1^{IV} Mn2^{IV} Mn3^{IV} Mn4^{III}) electronic state to the W_x-bound structure, the spin states are extremely unstable. Despite atom-specific charge/multiplicity assignments, the quartet on Mn2^{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 (Mn1^{III}

Mn^{2IV} Mn^{3IV} Mn^{4IV}) electronic structure is readily stabilized and maintained throughout optimization.

Theoretical energetics estimates for S₁ to S₂ transition

We performed two pairs of single point calculations using the S₁ and S₂ (g = 2) electronic states with both the WT S₂, g = 2 structure and the novel S169A S₂^x structure. (Here, superscripts indicate structure and subscripts indicate the electronic state.) The S₁ to S₂ transition in the WT S₂, g = 2 structure was 0.5 kcal/mol uphill, making the S₁ state slightly more energetically favorable. With the S169A S₂^x structure, the difference was 5.4 kcal/mol in favor of the S₂ state. Likely a significant rearrangement of atoms would be required for the S₁ electronic structure to be the more favorable state in the S169A mutant, whereas in WT, the optimized S₁ and S₂, 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 S₂ state in the S169A mutant.

References

[1] Z. Li, R.L. Burnap, Mutations of basic arginine residue 334 in the D1 protein of photosystem II lead to unusual S₂ state properties in *Synechocystis* sp. PCC 6803, *Photosynth. Res.*, 72 (2002) 191-201.