Supporting Information

Ammonia binding in the second coordination sphere of the oxygen-evolving complex of Photosystem II

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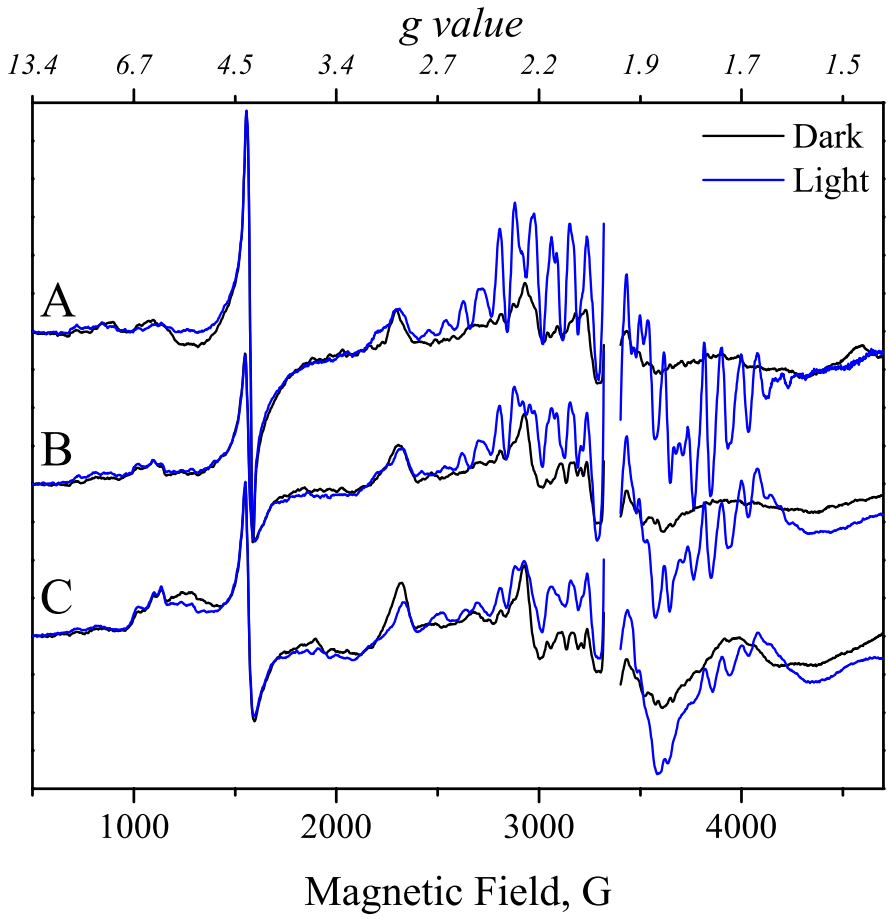

Figure S1. EPR spectra of (A) WT PSII core complexes in elution buffer containing 10% (v/v) glycerol and 1.2 M betaine at pH 6.0, (B) WT PSII core complexes in 1 M sucrose buffer at pH 7.5, and (C) K317A PSII core complexes in 1 M sucrose buffer at pH 7.5. Spectra were recorded at 6.2 - 6.4 K using 2 mW microwave power. Light-*minus*-dark spectra are shown in Figure 2 in the main text.

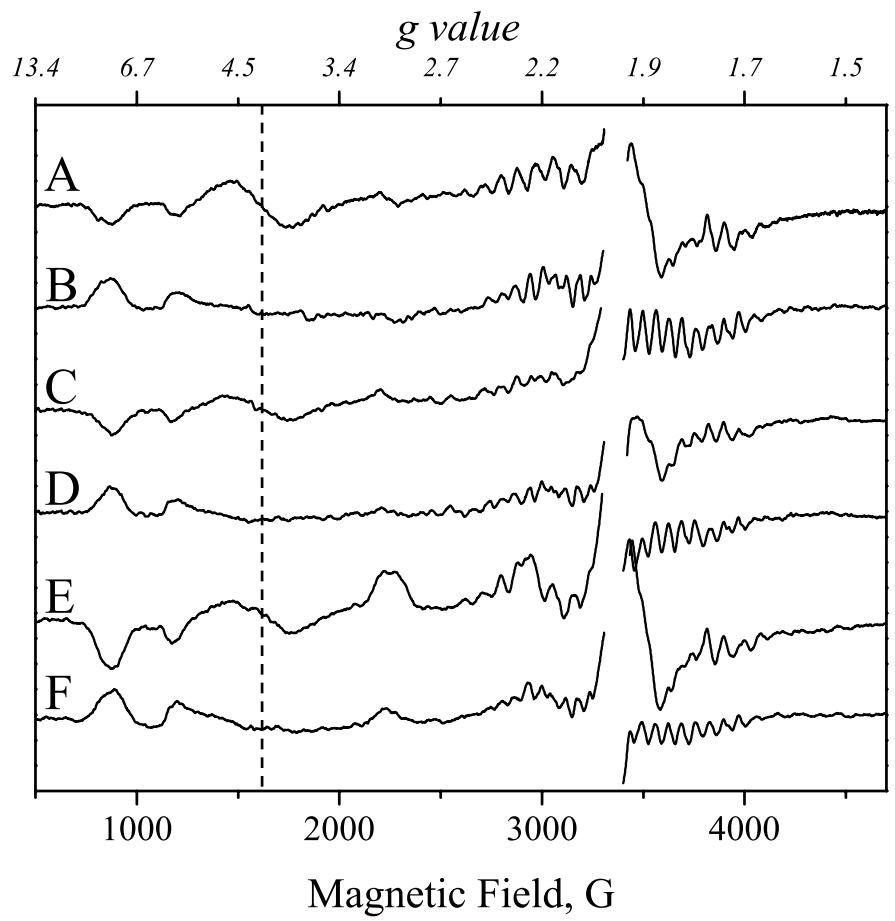

Figure S2. Dependence of cryoprotectant on ammonia binding. WT PSII core complexes were buffer exchanged into 1 M sucrose (A and B), 1.2 M betaine (C and D), or 25% glycerol (E and F) and then all were treated with 100 mM NH_4Cl at pH 7.5. A, C, and E represent light-*minus*-dark spectra following illumination at 200 K. B, D, and F represent light-*minus*-dark spectra after samples were annealed in darkness at 258 K. A dashed line at $g = 4.1$ is shown for clarity. Spectra were recorded at 6.6 - 6.8 K using 5 mW microwave power.

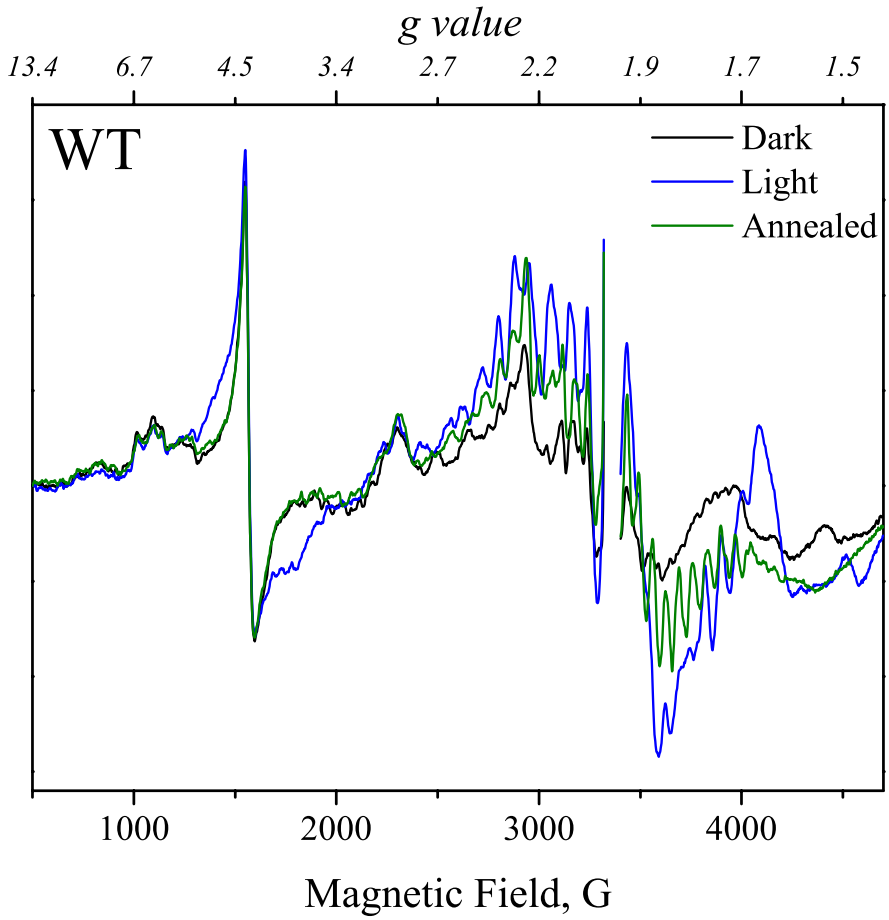

Figure S3. EPR spectra of WT PSII core complexes in the presence of 100 mM NH_4Cl and pH 7.5. Spectra were recorded at 6.2 - 6.4 K using 2 mW microwave power. Light-*minus*-dark spectra are shown in Figure 3 (A and B) in the main text.

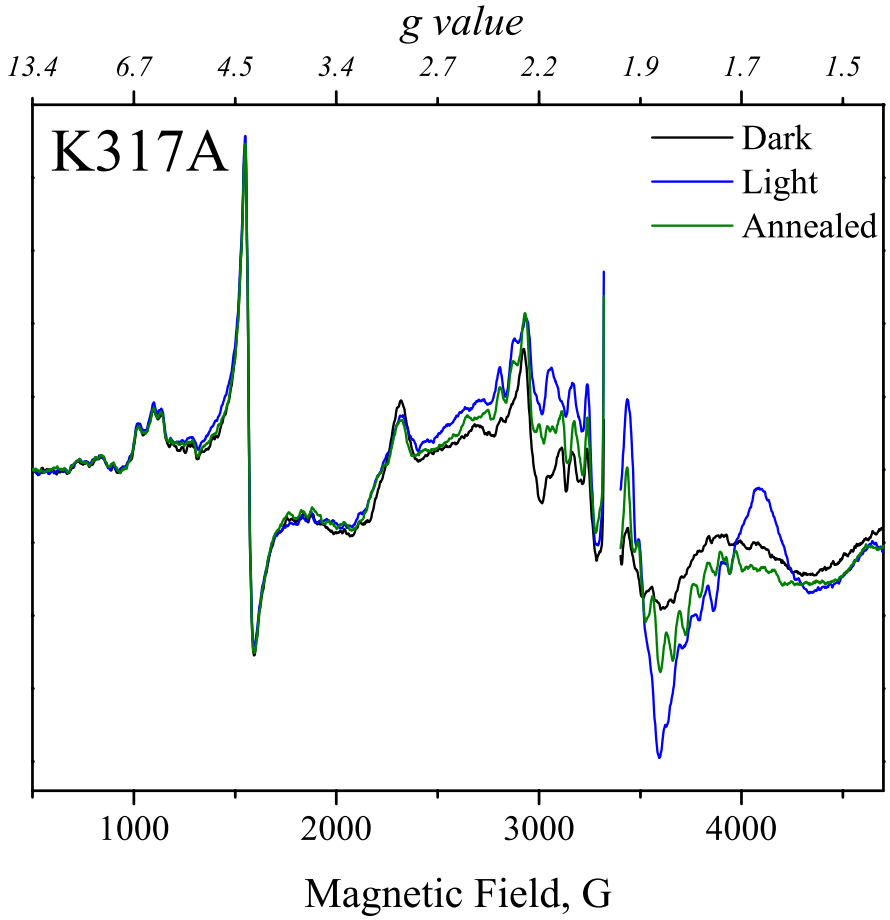

Figure S4. EPR spectra of K317A PSII core complexes in the presence of 100 mM NH_4Cl and pH 7.5. Spectra were recorded at 6.2 - 6.4 K using 2 mW microwave power. Light-*minus*-dark spectra are shown in Figure 3 (C and D) in the main text.

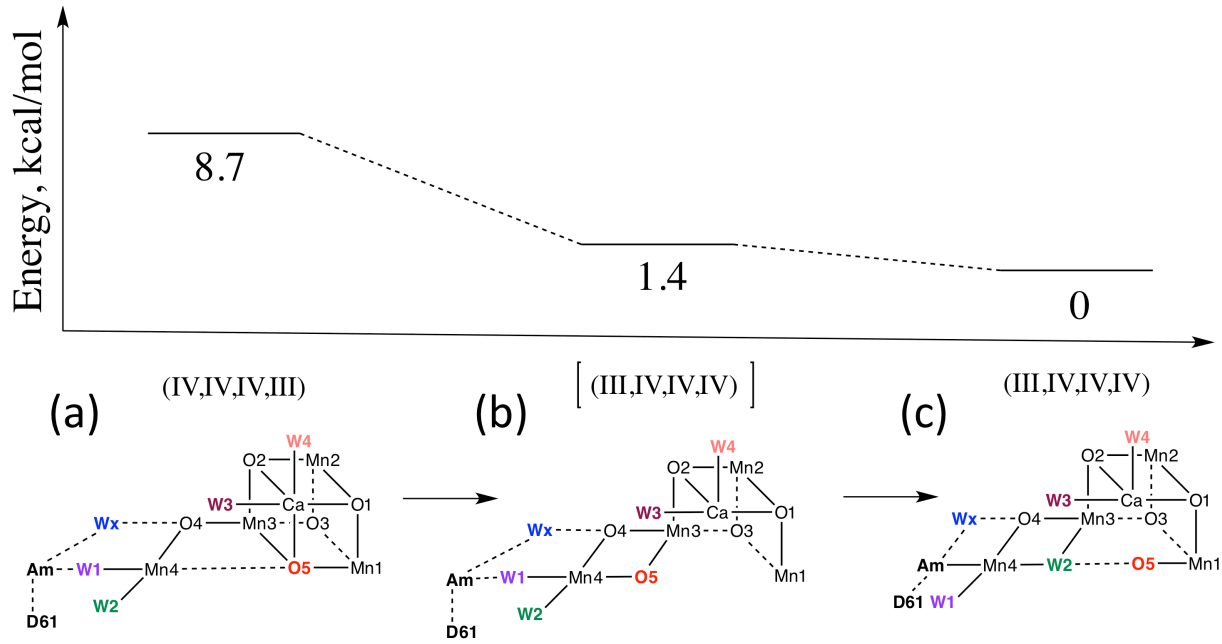

Figure S5. QM/MM calculated energy diagram relating the energetics of the secondary and primary ammonia binding sites in the S_2 state. When the S_2 state is generated by illumination at low temperature, ammonia remains bound to the secondary binding site (a) stabilizing the S = 5/2 spin isomer with (IV,IV,IV,III) oxidation states. Upon annealing, ammonia moves to bind as an additional 6th ligand to Mn4 (c), leading to an 8.7 kcal/mol additional stabilization and the (III,IV,IV,IV, S = 1/2) oxidation/spin state pattern. Structure (b) could be an unstable intermediate in this transformation.