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Supplementary Information

Characterization of Ammonia Binding to the Second Coordination Shell of the Oxygen-Evolving Complex of Photosystem II

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Charges for ammonia:

Charges of ammonium (NH_3):

N -1.125, 1H 0.375, 2H 0.375, 3H 0.375

Charges of ammonium cation (NH_4^+):

N -0.744, 1H 0.436, 2H 0.436, 3H 0.436, 4H 0.436

Charges of ammonium anion (NH_2^-):

N -1.50, 1H 0.250, 2H 0.250

Table S1. Equilibrium Charge States of Important Protein Residues.

	Asp61	Glu65	Glu329
Without Cl^-	-1.0	-0.85	-0.8
With Cl^-	-0.9	-0.2	-0.1
Lys317Ala, no Cl^-	-0.85	-0.1	-0.6
S2_g2.0(3444)	-1.0	-0.7	-0.6

Calculations run at pH 7.5 with OEC in the S_2_g4.1 (4443) redox state

Table S2. Free energy (kcal/mol) of ammonia binding (sphere vs full protein) to six high affinity binding sites at pH 7.5.

	Am1	Am2	Am3	Am4	Am5	Am6
S_2 g = 4.1 (full protein)	-2.1	-1.7	-1.4	-0.9	-0.8	-0.1
S_2 g = 4.1 (sphere)	-2.2	-1.7	-1.2	-0.8	-1.0	-0.1
S_2 g = 2.0 (full protein)	3.4	-1.0	-0.9	-0.8	-0.9	-0.1
S_2 g = 2.0 (sphere)	3.2	-1.1	-1.0	-0.9	-1.1	-0.1

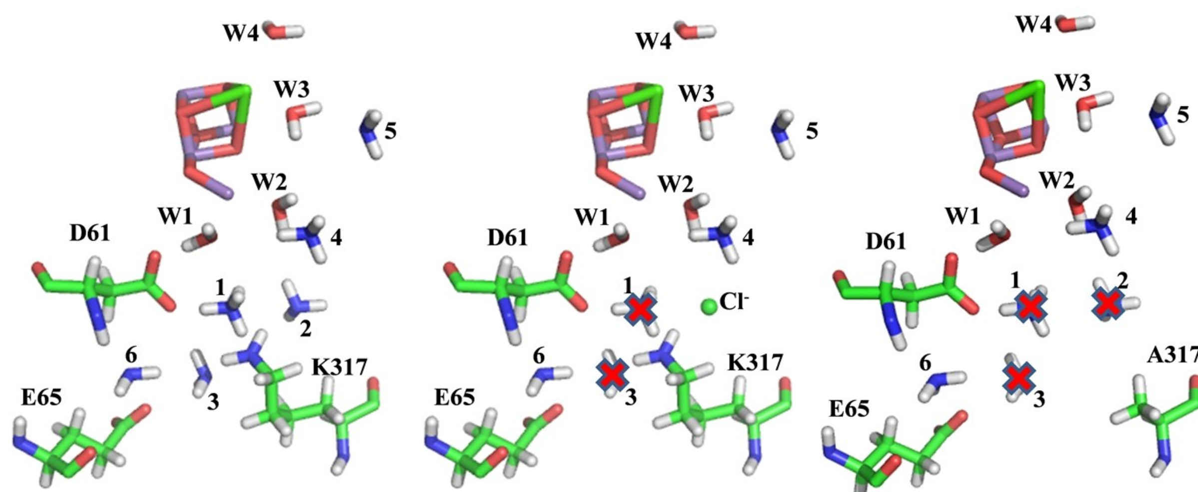

Fig. S1. (a) Positions of all high affinity ammonias near the OEC; (b) Am1 and Am2 are lost in the presence of Cl^-; (c) Am1, Am2 and Am3 are lost in the K317A mutant.

QM/MM Method

The QM/MM models were optimized using the B3LYP functional with the LANL2DZ [1] basis set for calcium and manganese, and the 6-31G*[2] basis set for all other atom[3–6] as in previous studies[7,8] The AMBER force field[9] was used for the MM layer. The model of the ammonia bound S_2 state was confirmed through a comparison of the simulated and experimental[10] EXAFS spectra[3]. The simulated EXAFS spectra were calculated using the *ab-initio* real space Green's function approach as implemented in FEFF (version 8.30)[11].

The initial guess for atom coordinates was taken from the 1.9-Å crystal structure (PDB: 3ARC)[12]. The model includes residues with C_α atoms within 15 Å of the atoms in the $CaMn_4O_5$ cluster and the two chloride ions near the OEC. 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.

Hydrogen atom placements were guessed using the AmberTools12 software package[9]. All acidic residues (ASP, GLU) were modeled as anions. Histidine protonation patterns were determined by inspection: His190 is protonated at N_δ to be a hydrogen-bond acceptor from D1-Tyr161, His332 is protonated at N_δ to leave N_ε as a ligand to Mn2, and His337 is protonated so that N_ε donates a hydrogen bond to O3. Sodium counter ions were added based on the electrostatic potential outside the protein to neutralize the system. In order to get estimates for relative energies of ammonia in various binding sites, the Cl^- ion was replaced with a water molecule.

DFT geometry optimization

The model were optimized using the B97D functional to count for the dispersion forces with the LANL2DZ[1] basis set for calcium and manganese, and the 6-31G*[4]. The initial coordinates of the ammonia bridging D61 and D2-K317 were obtained from the most occupied conformer in the MCCE calculations. The model includes the $Mn_4O_5Ca^{2+}$ cluster, the amino acid ligands, H337, CP43-R357, D61, D2-K317 and 13 crystallographic waters. The model was optimized with and without chloride to study the effect of chloride depletion on the protonation of the ammonia in the Am1 position.

Measurements of the pH dependence of the S_2-state g = 4.1 EPR signal

Spinach PSII membranes were prepared as described previously[13,14]. The isolated membranes were concentrated to 4-6 mg of Chl mL^{-1} in a buffer solution containing 400 mM sucrose, 50 mM MES, 20 mM $Ca(OH)_2$, 10 mM NaCl, and 0.01% Triton X-100. The final pH was adjusted to 6.0 with NaOH.

Oxygen-evolving PSII core complexes were isolated from His-tagged *Synechocystis* PCC 6803 cells. Single colonies were selected for their ability to grow on solid media containing 5 μg/mL kanamycin monosulfate and 20 μg/mL gentamycin sulfate[15]. Cells were grown mixotrophically in liquid media supplemented with 5 mM glucose in the presence of warm white fluorescent lighting at 28 °C lighting (80 μE m^{-2} s^{-1}) and bubbled with 5% CO_2 in air. The PSII core complexes were extracted under dim light conditions at 4 °C using a Ni-NTA superflow affinity resin (Qiagen, Valencia, CA) as described previously[16]. The purified PSII core complexes were stored in a buffer solution containing 1.2 M betaine, 10% (v/v) glycerol, 50 mM MES-NaOH (pH 6.0), 20 mM $CaCl_2$, 5 mM $MgCl_2$, 50 mM histidine, 1 mM EDTA, and 0.03% (w/v) *n*-dodecyl β-D-maltoside at -80 °C.

EPR Measurements

EPR samples were prepared using Amicon Centrifugal Units having 100 kDa cutoff. The samples were washed 6-8 times with buffer containing 400 mM sucrose, 0.1 mM NaCl, 11 mM $Ca(OH)_2$, 60 mM MES (pH 6.0) and 45 mM HEPES (pH 7.5). Additionally, for ammonia-treated samples 350 mM $(NH_4)_2SO_4$ was added to the buffer at pH 6.0 and 11 mM $(NH_4)_2SO_4$ at pH 7.5. The samples were concentrated to 1.5 mg/mL of Chl. The S_2 state was generated by illuminating the sample with red LED (623 nm) in a 200 K acetone/dry ice bath. The measurements were performed using a SHQ resonator and an Oxford ESR-900 continuous flow cryostat at 7.5 K. The EPR parameters used for recording the spectrum are as follows: microwave frequency, 9.38 GHz; modulation frequency, 100 kHz; modulation amplitude, 19.95 G; microwave power, 5 mW; sweep time, 84 s; conversion time, 41 ms; time constant, 82 ms. Each spectrum is the average over two scans.

Spinach PSII membranes exhibit two spin isomers of the S_2 state, g = 4.1 ($S = {}^5/_2$ spin isomer) and g = 2 multiline ($S = {}^1/_2$ spin isomer), generated by illumination at 200 K as shown in Figure S2 (spectrum A). However, PSII core complexes extracted from wild-type (WT) *Synechocystis* PCC 6803 only exhibit the g = 2 multiline signal upon generation of the S_2 state by illumination at 200 K (Figure S2, spectrum B). The WT *Synechocystis* PSII S_2 state spectrum remains unchanged on changing the pH from 6 to 7.5 (Figure S2, spectra B and C (adapted from Vinyard *et al.*, 2016)[17]).

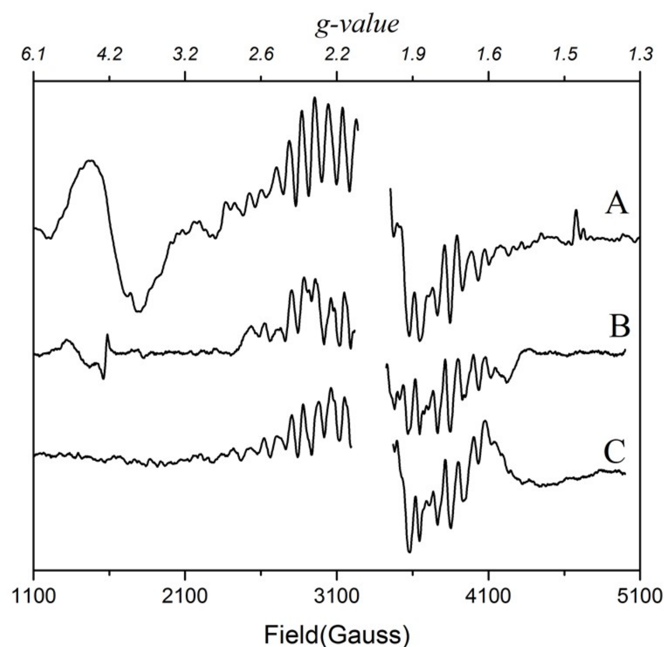

Figure S2: S_2 state light-minus-dark spectrum of: A) spinach PSII membranes at pH 6.0, B) WT *Synechocystis* PSII core complexes at pH 6.0, and C) WT *Synechocystis* PSII core complexes at pH 7.5

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