Supporting Information

D1-S169A Substitution of Photosystem II Perturbs Water Oxidation

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Supplementary Note S1

Measurement of Chlorophyll Fluorescence. Yields of chlorophyll fluorescence were measured with a pulse-amplitude-modulation (PAM) fluorometer (Heinz Walz GmbH, Effeltrich, Germany) as described previously.[1-4] For measurements, concentrated cells (20 µg of Chl) were diluted into 50 mM MES-NaOH, 25 mM $CaCl_2$, 10 mM NaCl, pH 6.5) at 22°C. For measurements of charge recombination between $Q_A^{\bullet-}$ and oxidized donors in PSII, cells were incubated in darkness with 0.3 mM *p*-benzoquinone and 1 mM potassium ferricyanide for 1 min to oxidize $Q_B^{\bullet-}$. DCMU was then added to a concentration of 40 µM (the final concentration of ethanol was 2%) and the fluorometer's weak measuring flashes were switched on. In response to these flashes, the fluorescence yield rose from an initial level of F_0 to a steady-state level denoted "F_{eq}".[1] Actinic illumination (a single flash or 5 s of illumination) was applied approximately 2 min later, after a stable F_{eq} level had been achieved. To measure the total yield of variable chlorophyll fluorescence (F_{max} - F_0), samples were incubated in darkness with 0.3 mM *p*-benzoquinone and 1 mM potassium ferricyanide for 5 min to ensure full oxidation of $Q_B^{\bullet-}$. DCMU was then added to a concentration of 40 µM, followed 1 min later by hydroxylamine to a concentration of 20 mM (fresh a fresh solution of 0.5 M hydroxylamine hydrochloride adjusted to pH 6.5 with NaOH). The fluorometer's weak measuring flashes were switched on 20 s after the addition of hydroxylamine. This was followed 0.5 s later by ten saturating xenon flashes, followed by 5 s of continuous illumination. The difference between the maximum chlorophyll fluorescence yield produced by the continuous illumination (F_{max}) and the initial chlorophyll fluorescence produced by the measuring flashes (F_0) provides a relative measure of PSII content.[1]

Oxygen Evolution and PSII Contents of Cells. The light-saturated O_2-evolution rates of wild-type and D1-S169A cells were 790 ± 60 and 610 ± 30 µmol O_2 (mg of Chl)$^{-1}$ h^{-1}, respectively. Consequently, D1-S169A cells evolved O_2 at 77 ± 7 % the rate of wild-type cells (Table S1). The maximum fluorescence yields (F_{max} -F_0) of these wild-type, D1-N87A, and D1-N87D cells were 1.32 ± 0.04 and 1.46 ± 0.05, respectively, corresponding to a PSII content of 111 ± 5 % for D1-S169A cells compared to wild-type cells (Figure S1, Table S1).

Charge recombination between $Q_A^{\bullet-}$ and PSII electron donors. The charge recombination kinetics of wild-type and D1-S169A cells in response to a single flash are compared in the left panels of Figure S2. The kinetics were fit with three exponentially-decaying components (fewer components yielded non-random residuals). The amplitudes and rates of these components are presented in Table S1. In wild-type cells, the kinetics correspond to charge recombination between $Q_A^{\bullet-}$ and the S_2 state of the Mn_4CaO_5 cluster. In D1-S169A cells, the kinetics were slowed slightly compared to wild-type cells, with the amplitudes of the two slower components increasing at the expense of the fastest component (from 36% and 27 % in wild-type to 48% and 31% in D1-S169A, respectively) and the time constant of the slowest component increasing 2.6-fold. The slowing of the kinetics implies a decrease in the S_2/S_1 midpoint potential.

An assay for PSII reaction centers lacking Mn_4CaO_5 clusters is provided by the kinetics of charge recombination measured after a brief period of actinic illumination in the presence of DCMU.[1-4] The basis for this assay is that both cytochrome *b*-559 and Y_D reduce $P_{680}^{\bullet+}$ with low quantum yields.[5] During continuous illumination in the presence of DCMU, the states

$S_2Q_A^{\bullet-}$and $Y_Z^{\bullet}Q_A^{\bullet-}$ form and recombine repeatedly until the stable states $cyt^{ox}Q_A^{\bullet-}$ and $Y_D^{\bullet}Q_A^{\bullet-}$photoaccumulate. The subsequent oxidation of $Q_A^{\bullet-}$ is a slow process (t > 20 s). The rates of electron transfer from cytochrome *b*-559 and Y_D to $P_{680}^{\bullet+}$ are determined by the equilibrium concentration of $P_{680}^{\bullet+}$. Consequently, $cyt^{ox}Q_A^{\bullet-}$ and $Y_D^{\bullet}Q_A^{\bullet-}$photoaccumulate much more rapidly in PSII centers lacking Mn_4CaO_5 clusters than in PSII centers containing Mn_4CaO_5 clusters.[1-3] The charge recombination kinetics of wild-type and D1-S169A cells in response to 5 s of continuous illumination are compared in the right panels of Figure S2. The kinetics were fit with three exponentially-decaying components (fewer components yielded non-random residuals). The amplitudes and rates of these components are presented in Table S1. The two faster components correlate approximately with the two slower components of charge recombination measured after a single flash. In wild-type cells, the slowest component, 6.6 ± 0.2 % of the total, exhibited a decay rate of 24 ± 6 s, a percentage comparable that those found in previous measurements of wild-type cells.[1-4] In earlier measurements, the slowest component in a mutant that assembles no Mn_4CaO_5 clusters (D1-D170A) was 72 ± 6%.[1] In D1-S169A cells, the slowest component was 14.5 ± 1 % of the total decay (Figure S2, Table S1). We conclude that 6.6 ± 0.2 % of wild-type and 14.5 ± 1 % of D1-S169A PSII reaction centers photoaccumulate $Q_A^{\bullet-}$ during 5 s of illumination. On the basis of comparing these percentages with the extents of $Q_A^{\bullet-}$photoaccumulated during 5 s of illumination in numerous mutant strains examined previously,[1-3] we estimate that 10-14% of PSII reaction centers in D1-S169A cells lack Mn_4CaO_5 clusters *in vivo*.

Table S1. Characteristics of wild-type and mutant cells

Strain	O_2 evolution[a] (% of wt)	PSII content[b] (% of wt)	Kinetics of $Q_A^{\bullet-}$ oxidation[c]			
			after a single flash		after 5 s of illumination	
			(%)	k^{-1} (s)	(%)	k^{-1} (s)
Wild-type	100	100	37±2 36±2 27±4	0.11±0.02 0.69±0.14 2.2±0.3	51±4 42±4 6.6±0.2	0.22±0.01 1.55±0.05 24±6
D1-S169A	77±7	111±5	21±2 48±2 31±4	0.07±0.01 0.71±0.12 5.8±0.4	52±1 34±1 14.5±1	0.60±0.03 4.1±0.2 40±9

[a]Wild-type cells exhibited 790 ± 60 µmol O_2 (mg of Chl)$^{-1}$ h^{-1}.
[b]Estimated from the total yield of variable chlorophyll fluorescence (F_{max}-F_0).
[c]Measured in the presence of DCMU and analyzed assuming three exponentially decaying components. For each component, the relative amplitude (%) and the inverse of the rate constant are reported.

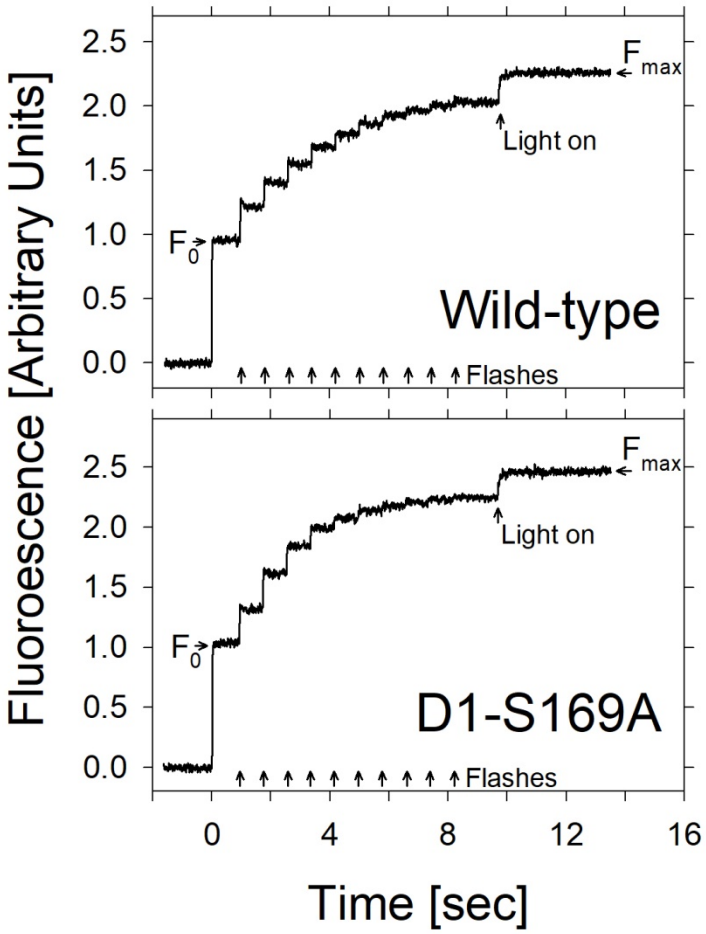

Figure S1. Formation of $Q_A^{\bullet -}$ in response to 10 saturating flashes (arrows) given at 800 ms intervals in the presence of DCMU and hydroxylamine to wild-type or D1-S169A cells, followed by continuous illumination. Continuous illumination (applied to obtain F_{max}) was applied 1.1 s after the 10th flash (arrow). The initial fluorescence yields produced by the weak measuring flashes (F_0) are indicated.

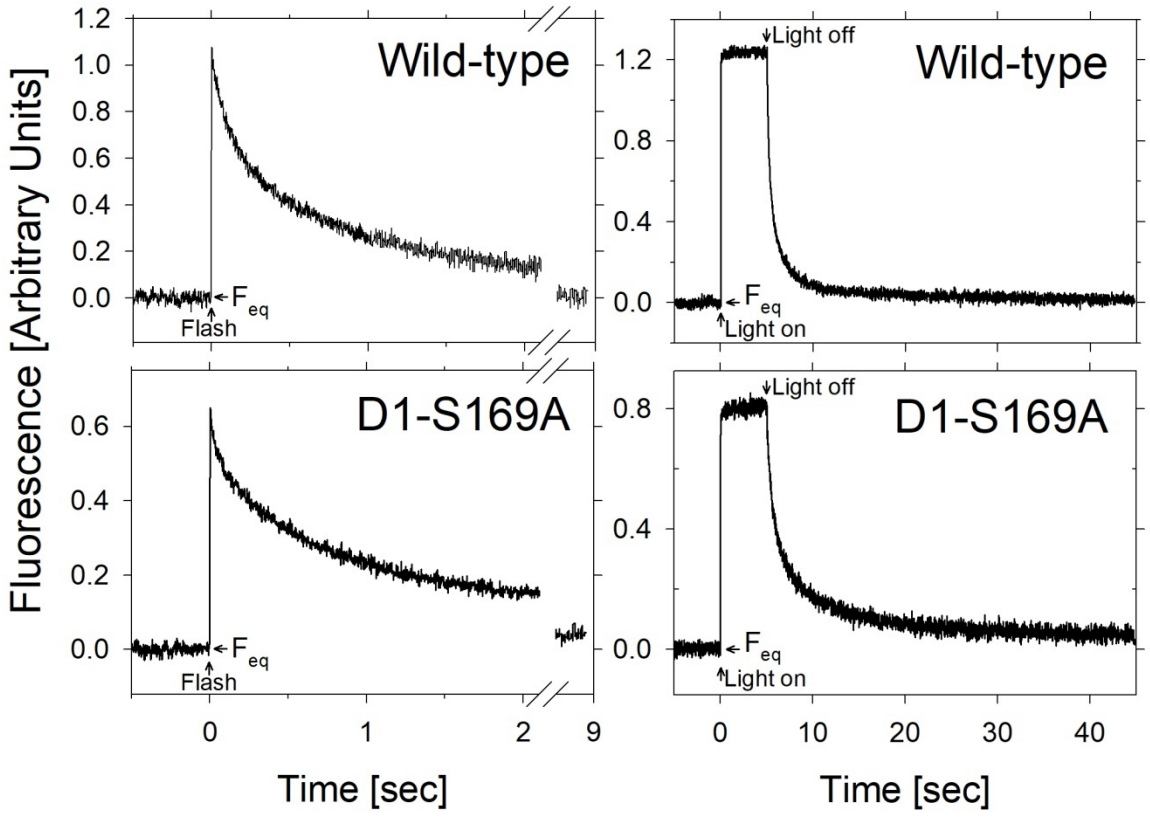

Figure S2. Formation and decay of $Q_A^{\bullet-}$ in response to a saturating flash (left panels) or 5 s of continuous illumination (right panels) given to wild-type or D1-S169A cells in the presence of DCMU, as measured by changes in the yield of chlorophyll fluorescence. In the left panels, the wild-type and D1-S169A traces represent the averages of 4 and 12 traces, respectively. In the right panels, arrows denote the onset and termination of illumination. For a definition of F_{eq}, see Supplementary Note S1. Note the difference in time scales between the left and right panels.

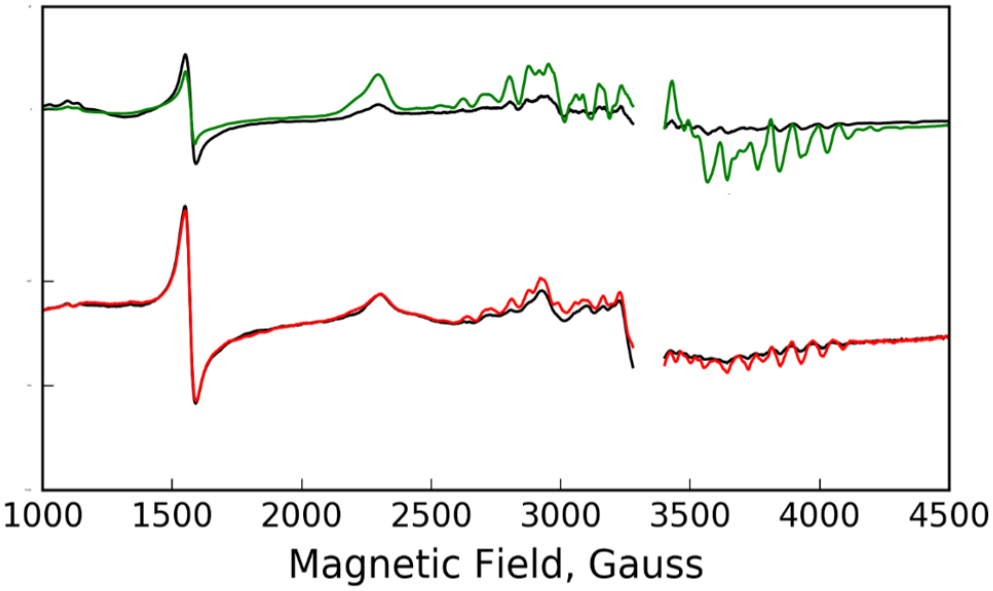

Figure S3. Comparison of the EPR spectra of the S_2 state in wild-type (green), and D1-S169A PSII core complexes (red). The dark spectra are marked in black.

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