Supporting Information

Structural Coupling of Extrinsic Proteins with the Oxygen Evolving Center in Red Algal Photosystem II As Revealed by Light-induced FTIR Difference Spectroscopy

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MATERIALS AND METHODS

Samples. Cells of *Cyanidium caldarium* RK-1 were cultured as described previousely.[1] Preparation of the PSII core complexes from *C. caldarium* was performed following the mothods by Adachi et al.[2] with some modification. Briefly, thylakoid membranes suspended in a buffer (pH 6.0) containing 40 mM Mes, 10 mM $CaCl_2$, and 25% (w/v) glycerol (buffer A) was solubilized with 1.2% *n*-dodecyl-β-D-maltoside (DM). The sample was diluted with an equivolume of buffer A and centrifuged at 40,000 × *g* for 10 min at 273 K. The supernatant was loaded onto a DEAE TOYOPEARL 650 M anion-exchange column. PSII complexes were eluted with a buffer (pH 6.0) containing 40 mM Mes, 190 mM NaCl, 3 mM $CaCl_2$, 25% (w/v) glycerol, and 0.03% DM. The resulting PSII fraction was centrifuged at 40,000 × *g* for 10 min at 277 K after addition of 10% (w/v) polyethylene glycol (PEG) 6000. The precipitate was suspended in buffer A.

Reconstitution experiments were performed according to Enami et al.[3] and Okumura et al.[4] The PSII complexes were treated with 1 M $CaCl_2$ for 30 min on ice in the dark to remove all of the four extrinsic proteins (PsbO, PsbQ′, PsbV, and PsbU). The extrinsic proteins were purified as described previously.[3] The PSII complexes were reconstituted with various combinations of the extrinsic proteins by incubating the mixture of $CaCl_2$-treated PSII complexes and extrinsic proteins with the molar ratio of 1:3 for 30 min on ice in the dark. The reconstituted PSII complexes were centrifuged at 40,000 × *g* for 20 min at 277 K after addition of 10% (w/v) PEG 6000. The precipitation was suspended in buffer A.

FTIR measurements. For FTIR measurements, PSII core complexes in buffer A in the presence of 10% PEG 10,000 were centrifuged at 174,000 × *g* for 5 min and the precipitate was washed once with buffer A without PEG by centrifugation. The suspension of the PSII complexes in buffer A in the presence of 45 mM potassium ferrocyanide and 5 mM potassium ferricyanide was centrifuged at 174,000 × *g* for 35 min. The resultant pellet was loaded on a CaF_2 plate (25 mm in diameter) and sealed with another CaF_2 plate using silicone grease as described previously.[5] A piece of aluminum foil (~1 mm ×~1 mm; ~15 μm in thickness) was placed as a spacer in the outer part of the infrared cell.

FTIR spectra were recorded using a Bruker IFS-66/S spectrophotometer equipped with an MCT detector (InfraRed D316/8). The sample temperature was adjusted to 10 ˚C by circulating cold water in a copper holder and the sample was stabilized for 2 h before starting measurements. Flash illumination was performed using a Q-switched Nd:YAG laser (Quanta-Ray GCR-130; wavelength, 532 nm; pulse width, ~7 ns fwhm; intensity, ~7 mJ $pulse^{-1}$ cm^{-2} at the sample surface). Single-beam spectra were recorded twice before and once after single-flash illumination, followed by dark adaptation, and this cycle was repeated to increase the signal-to-noise ratio of the spectra. The scan time for one single-beam spectrum, the duration of dark adaptation, and the number of repetition (summarized in Table S1) were determined for each sample taking account of the relaxation time of the S_2 state. The difference between the two spectra before and after the flash provides an S_2-minus-S_1 difference spectrum, while the difference between the two spectra before illumination provides a dark-minus-dark spectrum representing a noise level. The spectral resolution was 4 cm^{-1}.

Spectral fitting was performed using Igor Pro (WaveMetrics Inc.). The S_2-minus-S_1 difference spectra PSII complexes depleted of all the extrinsic proteins and then reconstituted with various combinations of the extrinsic proteins (Figure 1a,c, red and blue lines; Figure S2, red lines) were normalized to minimize the difference from the spectrum of untreated PSII in the 1450−1350 cm^{-1} region (symmetric COO^- stretching

region). The double difference spectra (Figures 2 and S3) were calculated by subtracting these normalized spectra from the untreated spectra.

Table S1: Conditions for measurements of S_2-minus-S_1 FTIR difference spectra of the PSII complexes from *Cyanidium caldarium*

	scans (s)	dark adaptation (min)	repetition (times)
untreated	50	120	4
all depleted	10	5	108
+PsbQ′	10	10	54
+PsbO	10	15	36
+PsbO/Q′	10	10	36
+PsbO/Q′/U	30	30	10
+PsbO/Q′/V	20	20	15
+PsbO/V/U	50	120	4
+PsbO/Q′/V/U	50	120	4

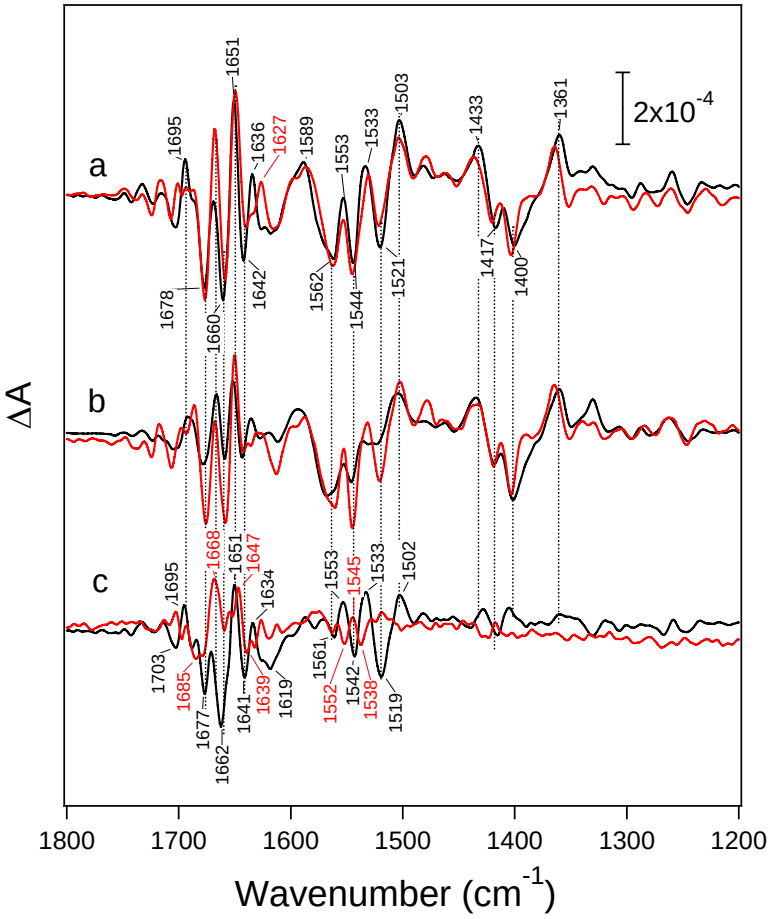

Figure S1. FTIR spectra of of PSII core complexes from *C. caldarium* (black lines) compared with the corresponding spectra of PSII membranes from spinach[6] (red lines). (a) S_2-minus-S_1 FTIR difference spectra of untreated PSII preparations. (b) S_2-minus-S_1 FTIR difference spectra of PSII preparations depleted of all the extrinsic proteins (PsbO, PsbQ′, PsbV, and PsbU for *C. caldarium* and PsbO, PsbP, and PsbQ for spinach). (c) Double difference spectra between the spectra of PSII complexes untreated and depleted of all the extrinsic proteins (untreated-minus-all-depleted).

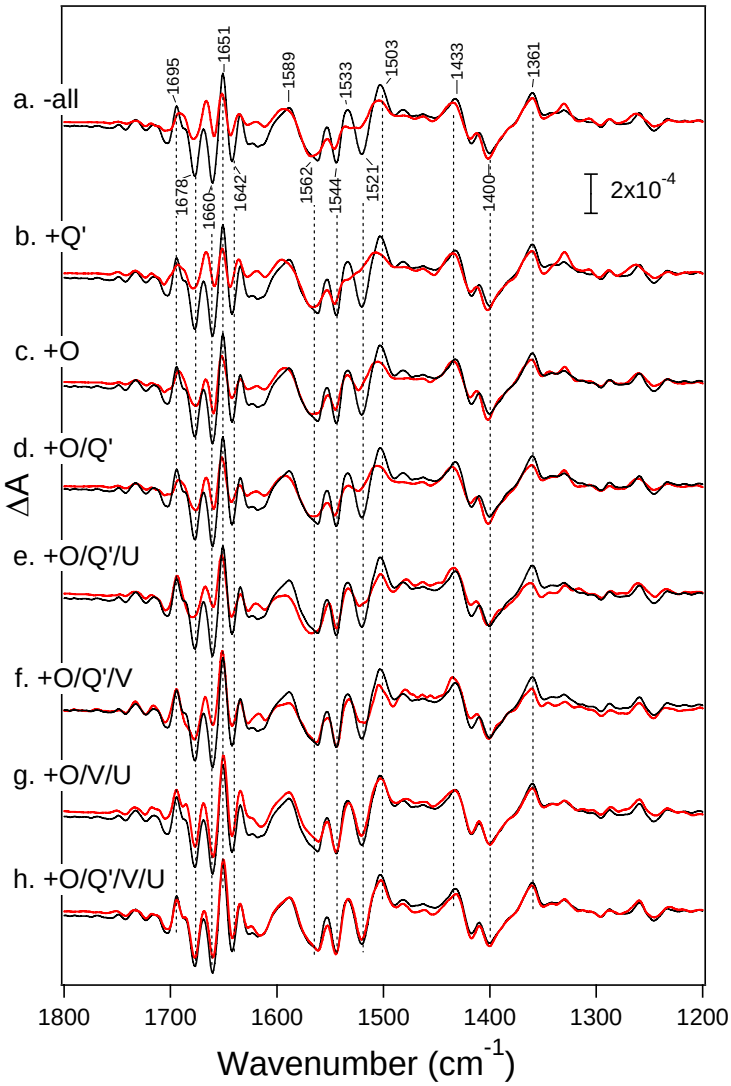

Figure S2. (a) S_2-minus-S_1 FTIR difference spectra of PSII core complexes from *C. caldarium* (red lines) depleted of all the four extrinsic proteins (a), and then reconstituted with PsbQ′ (b), PsbO (c), PsbO/Q′ (d), PsbO/Q′/U (e), PsbO/Q′/V (f), PsbO/V/U (g), and PsbO/Q′/V/U (h), in comparison with the spectrum of untreated PSII (black lines).

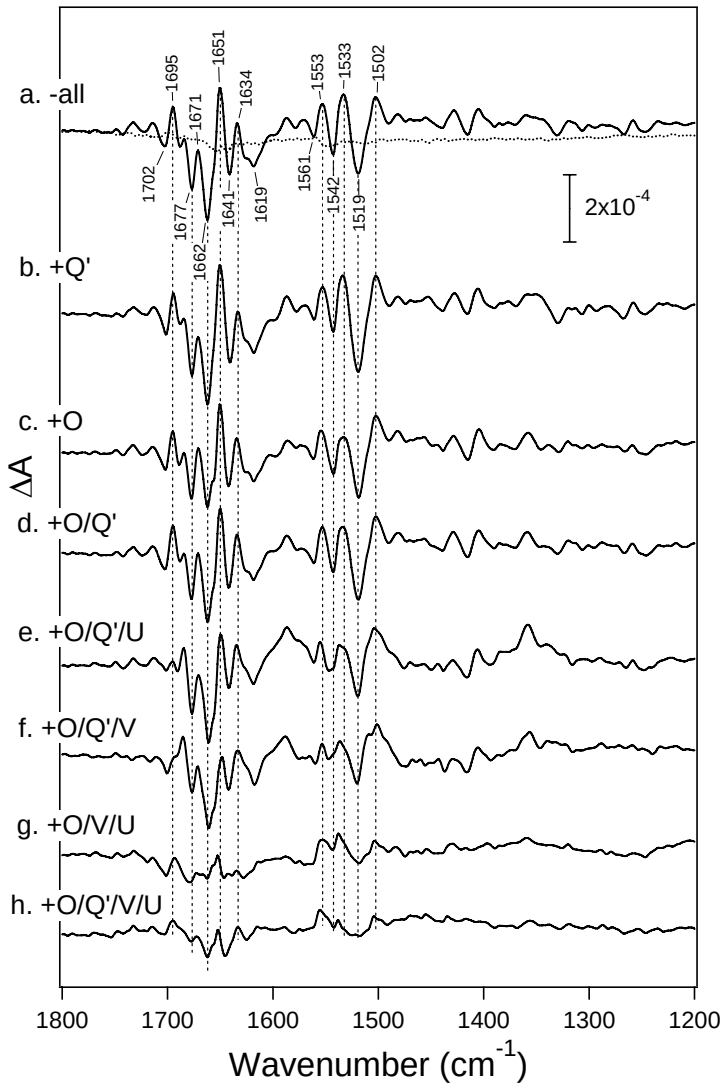

Figure S3. The 1800–1200 cm^{-1} region of untreated-minus-treated double difference spectra between the S_2-minus-S_1 spectrum of untreated PSII and that of PSII depleted of all the four extrinsic proteins (a), then reconstituted with PsbQ′ (b), PsbO (c), PsbO/Q′ (d), PsbO/Q′/U (e), PsbO/Q′/V (f), PsbO/V/U (g), and PsbO/Q′/V/U (h). The dotted line in panel a is a double difference spectrum of dark-minus-dark spectra representing a noise level.

References

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