

Supporting Information

Role of the O4 Channel in Photosynthetic Water Oxidation as Revealed by Fourier Transform Infrared Difference and Time-Resolved Infrared Analysis of the D1-S169A Mutant

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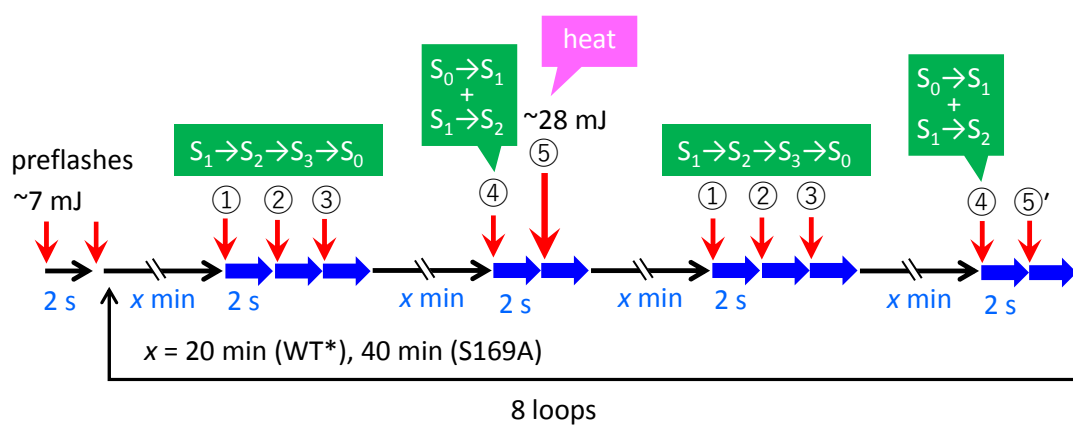


Figure S1. Scheme of the TRIR measurement.

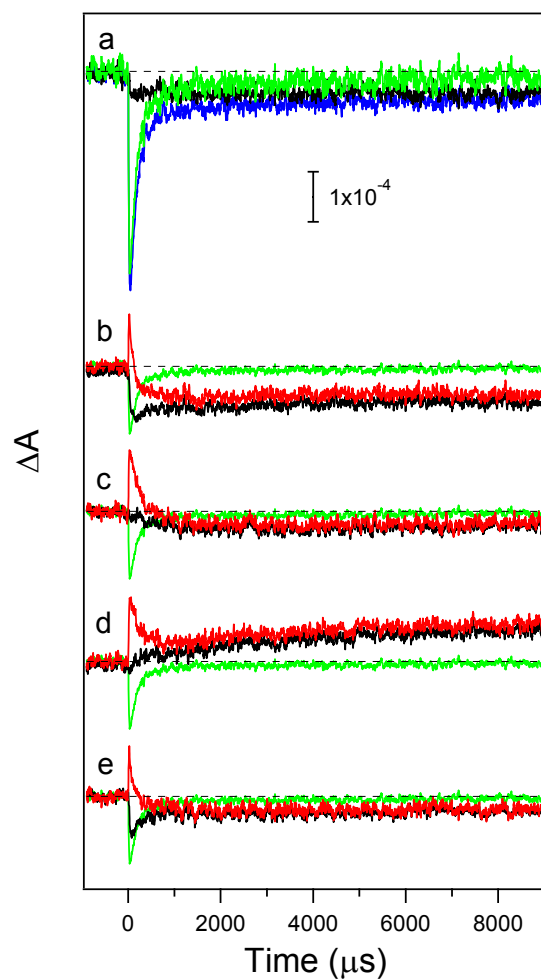


Figure S2. Example of heat-signal correction of the TRIR traces. (a) A heat signal corresponding to a laser power of $21 \text{ mJ cm}^{-2} \text{ pulse}^{-1}$ (green line) was calculated by subtraction of a TRIR trace upon a flash with $7 \text{ mJ cm}^{-2} \text{ pulse}^{-1}$ (black line) from that upon a flash with $28 \text{ mJ cm}^{-2} \text{ pulse}^{-1}$ (blue line). (b–e) Heat-corrected TRIR traces (red lines) obtained by subtraction of a heat signal corresponding to a laser power of $7 \text{ mJ cm}^{-2} \text{ pulse}^{-1}$ (green lines; the 1/3 intensity of the heat signal obtained in a) from raw TRIR traces (black lines) upon the (b) 1st, (c) 2nd, (d) 3rd, and (e) 4th (after dark interval) flashes with $7 \text{ mJ cm}^{-2} \text{ pulse}^{-1}$. The TRIR traces were recorded at 1410 cm^{-1} with the WT* PSII core complexes.

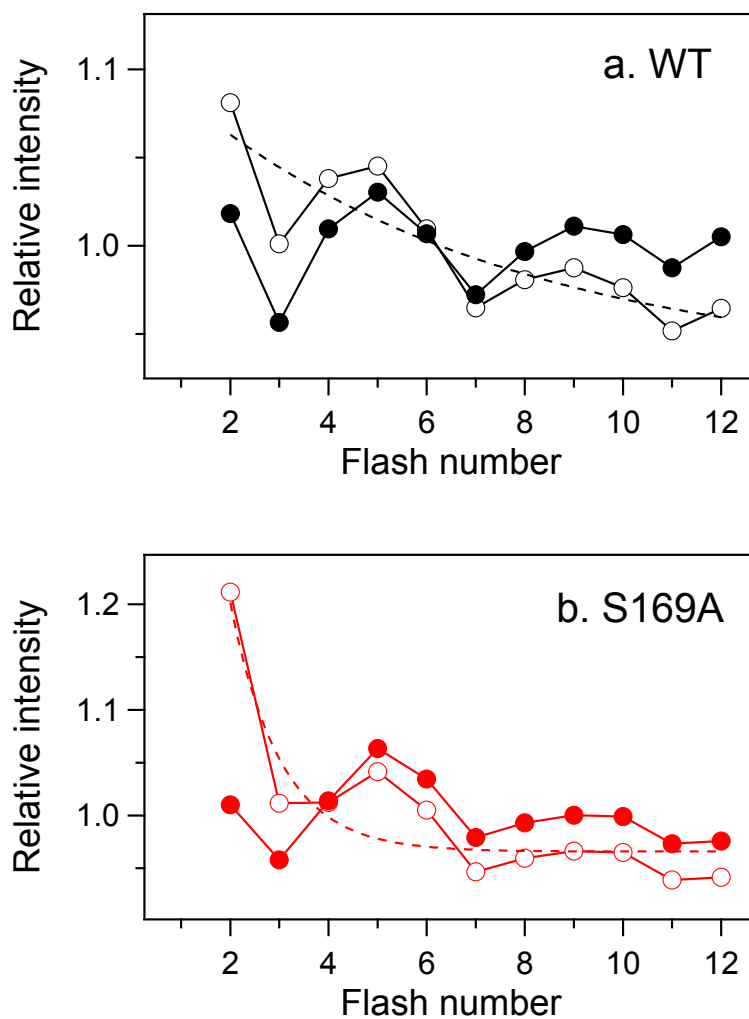


Figure S3. Baseline correction of the oscillation pattern of the ferrocyanide/ferricyanide FTIR signal of (a) WT* and (b) D1-S169A PSII core complexes. The original data (open circles) were corrected (closed circles) using a baseline (dotted line) obtained by a single exponential fit.

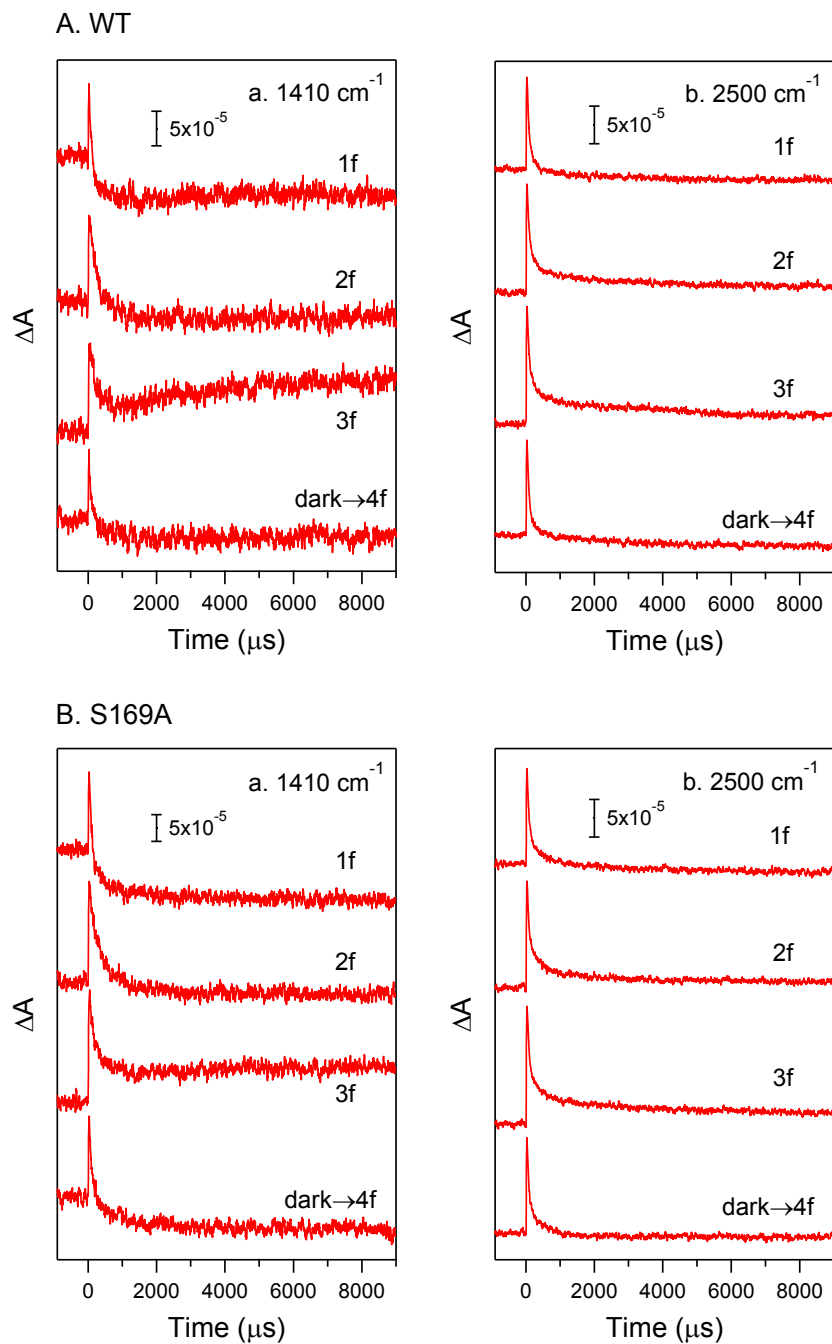


Figure S4. TRIR traces at 1st, 2nd, 3rd, and 4th (after dark interval) flashes detected at (a) 1410 and (b) 2500 cm^{-1} in (A) WT* and (B) D1-S169A PSII core complexes. All the traces were corrected with a heat signal (see Figure S2).

Estimation of the pure TRIR trace of the $S_0 \rightarrow S_1$ transition

The procedure follows that in Shimizu et al.¹ except for using individual efficiencies of the S-state transitions. The intensities at 6 ms (average value between 5.7 and 6.3 ms) in the traces at 1410 cm^{-1} (Figure S4) at the 1st, 2nd, and 3rd flashes, A_1 , A_2 , and A_3 , respectively, which were corrected with a baseline shift in the simulation of the oscillation pattern, are expressed using the true ΔA amplitudes, I_{12} , I_{23} , and I_{30} , and the efficiencies, α_1 , α_2 , and α_3 , of the $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_3 \rightarrow S_0$ transitions, respectively:

$$A_1 = \alpha_1 I_{12}$$

$$A_2 = (1 - \alpha_1)\alpha_1 I_{12} + \alpha_1 \alpha_2 I_{23}$$

$$A_3 = (1 - \alpha_1)^2 \alpha_1 I_{12} + (2 - \alpha_1 - \alpha_2)\alpha_1 \alpha_2 I_{23} + \alpha_1 \alpha_2 \alpha_3 I_{30}$$

Thus, I_{12} , I_{23} , and I_{30} , and the true amplitude of the $S_0 \rightarrow S_1$ transition, I_{01} , are expressed as:

$$I_{12} = A_1 / \alpha_1$$

$$I_{23} = \{A_2 - (1 - \alpha_1)A_1\} / (\alpha_1 \alpha_2)$$

$$I_{30} = \{A_3 + (1 - \alpha_1)(1 - \alpha_2)A_1 - (2 - \alpha_1 - \alpha_2)A_2\} / (\alpha_1 \alpha_2 \alpha_3)$$

$$I_{01} = -(I_{12} + I_{23} + I_{30})$$

The base-line corrected intensity at the 4th flash, A_4 , is expressed as:

$$A_4 = \{r + (1 - r)(I_{01}/I_{12})\}A_1$$

where r is the contribution of the $S_1 \rightarrow S_2$ transition in the intensity at the 4th flash. Thus, r is obtained as:

$$r = (A_4/A_1 - I_{01}/I_{12}) / (1 - I_{01}/I_{12})$$

The values of r were estimated to be 0.68 and 0.79 in WT* and D1-S169A, respectively.

The pure trace of the $S_0 \rightarrow S_1$ transition, f_{01} , is obtained from the traces at the 1st and 4th flashes, f_1 and f_4 , as:

$$f_{01} = (f_4 - r f_1) / \{(1 - r)\alpha_3\}$$

Reference

(1) Shimizu, T.; Sugiura, M.; Noguchi, T. Mechanism of proton-coupled electron transfer in the S_0 -to- S_1 transition of photosynthetic water oxidation as revealed by

time-resolved infrared spectroscopy. *J. Phys. Chem. B* **2018**, *122*, 9460-9470.