Supporting Information:

Chromophore Dynamics in the PYP Photocycle from Femtosecond Stimulated Raman Spectroscopy

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PYP Preparation

The PYP gene from *Halorhodospira halophila BN9626* was cloned into the pQE-80L plasmid (QIAGEN) between BamHI and HindIII sites to attach a 6-histidine tag and enterokinase cleavable site to the N-terminal side of the target protein. The plasmid for protein expression was transformed into *E. coli* BL21 (DE3) (Stratagene). The cells were cultured at 37 °C in LB medium containing 50 □g/mL ampicilin overnight. Expression of *apo*-PYP was induced by the addition of isopropyl-thio-*β*-galactoside (IPTG; final concentration 1 mM) after 16–18 hr. After extra incubation for 4 hr, the cells were harvested by centrifugation at 4000 pm for 10 min (Sorvall Evolution® RC, SLC-6000 angle rotor) The harvested *E. coli* pellet was disrupted with 8 M urea (pH 8.0), and the cell debris removed by ultracentrifugation at 25,000 rpm for 20 min (Beckman Coulter, Optima™ L-90K Ultracentrifuge, Type-70Ti angle rotor).[1, 2] The extracted water-soluble fraction was diluted with 20 mM Tris/Cl pH 7.5 until the urea concentration was 4 M, followed by the addition of *p*-coumaric anhydride as described.[3] The solution turned yellow with continuous stirring for 30 min at 4°C. The reconstituted crude PYP was dialyzed with buffer to remove the urea, and applied to Ni-NTA resin (QIAGEN). The column was washed with buffer, 5 mM imidazol and eluted with 200 mM imidazol. The Ni-NTA purified PYP was further purified with a DEAE-Sepharose CL6B column (GE Healthcare). After the column was washed with buffer, PYP was eluted with 100 mM NaCl in the same buffer. This process was repeated three times, and the purity was checked by UV-visible absorption spectrum until the ratio of A278nm/A446nm was < 0.45. PYP was then concentrated with an ultrafiltration membrane (Centriprep 10, Amicon) and diluted with buffer for use. Dilution and concentration steps were repeated several times to remove NaCl.[2]

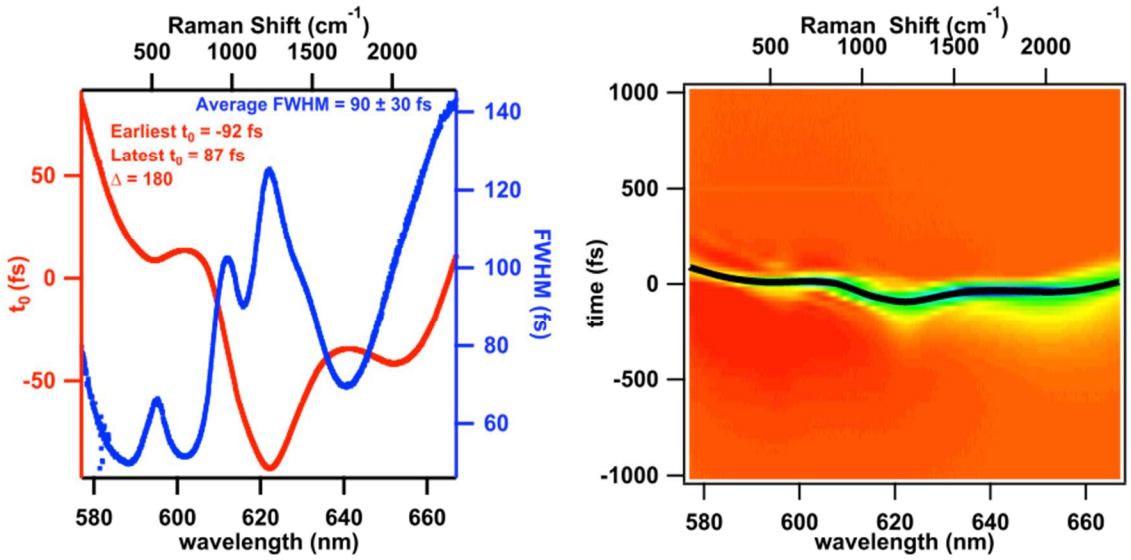

Figure S1 Cross correlation measured using the optical Kerr effect. The graph on the left shows t = 0, the maximum of the Kerr signal (red), and the FWHM of the Kerr signal (blue) as a function of wavelength. The 2D plot at the right shows the measured Kerr signal from -1 ps to 1 ps as a function of wavelength, with t = 0 marked in black.

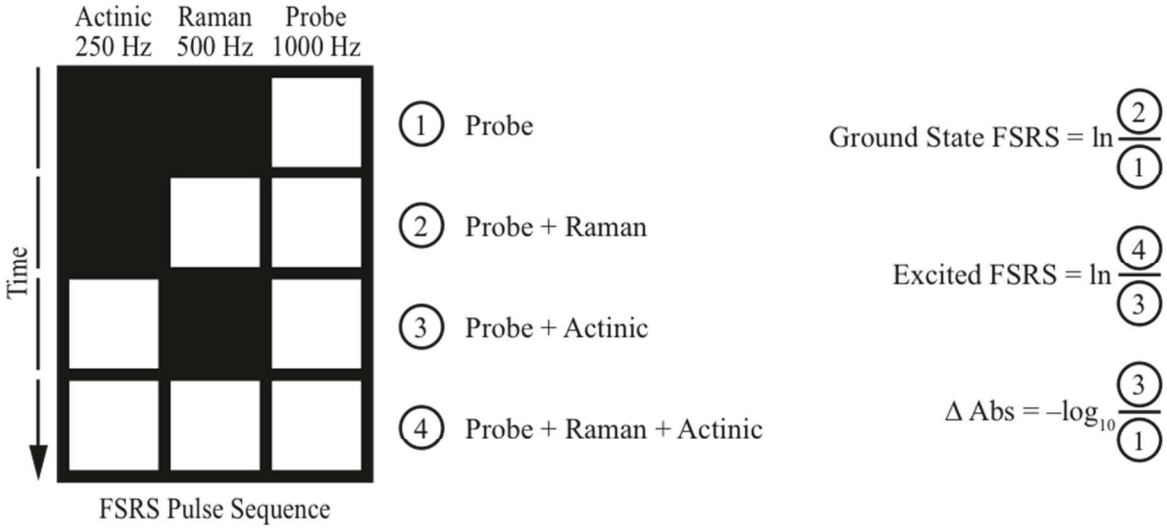

Figure S2 Pulse-timing used for FSRS data acquisition. In the pulse sequence diagram (left) the pulse labels appear across the top with their respective chopping frequencies. White boxes denote the presence of a pulse, while black indicates blocking by an optical chopper. The detector is synced to the 1kHz pulse train of the amplifier, and reads out the dispersed probe signal shot by shot. By modulating the Raman and Actinic pulses as illustrated above, it is possible to collect ground state FSRS, excited state FSRS, and transient absorption spectra for a given time delay in 4 laser shots (4 ms). The general equations for producing FSRS gain and transient absorption spectra from the collected signals are shown at right.

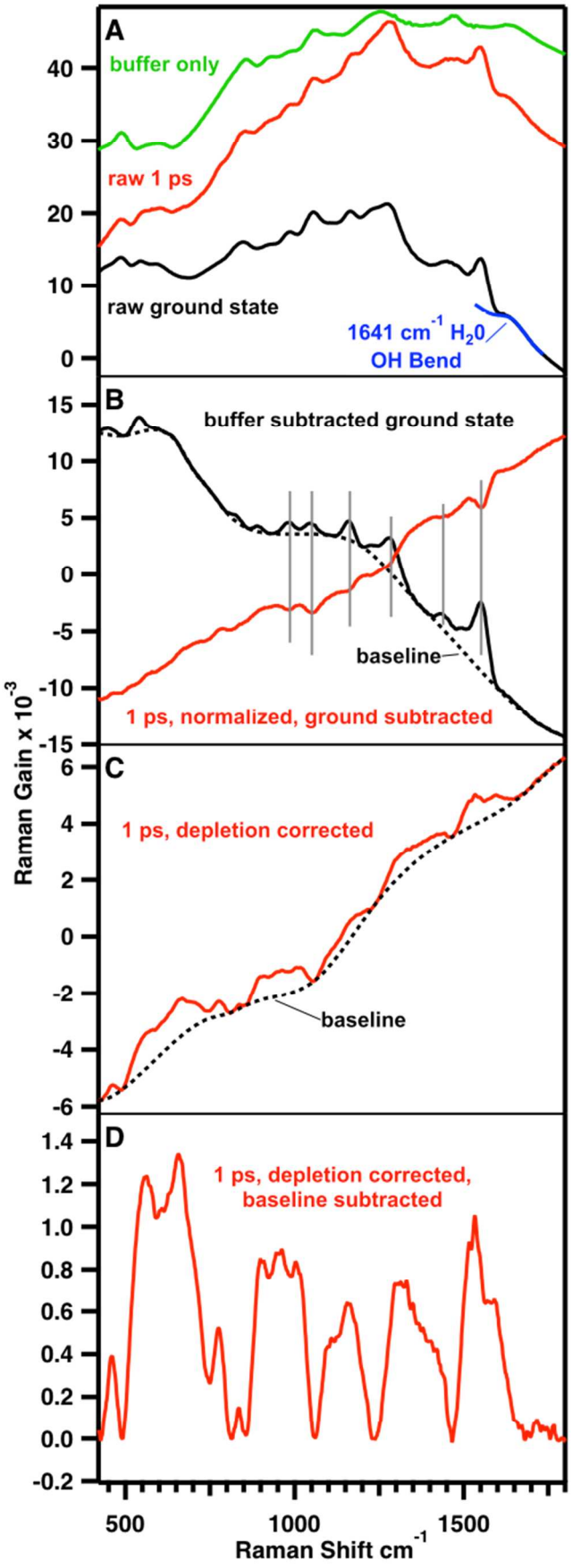

Figure S3 General steps for FSRS data analysis. The process begins in panel A. In each of the steps (A-D) processing of the ground state FSRS spectrum is shown in black while processing of the excited FSRS spectrum (using the 1 ps timepoint as an example) is shown in red. Panel A shows the raw FSRS spectra for the ground state (A, black), the 1 ps time-point (A, red) and buffer only (A, green). The ground state signals of PYP are revealed by subtracting a scaled buffer-only spectrum (A, green) from the raw ground state spectrum (A, black). Contributions from the ground state and buffer are removed from the excited spectra by first normalizing each timepoint to the raw ground state spectrum using the intensity of the 1641 cm^{-1} OH bend of water (A, blue) and then subtracting the raw ground state (A, black) from each normalized time-point. Panel B shows the buffer subtracted ground state data (B, black) along with the normalized 1 ps data after subtraction of the raw ground state signals (B, red). The 1 ps spectrum now contains features of the photo-excited species along with negative features arising from ground state depletion (marked with vertical grey lines). The depletion is removed by adding a scaled, buffer-subtracted ground state spectrum (B, black) to each timepoint. Panel C shows the result of the ground state depletion correction for the 1 ps timepoint (C, red). The remaining baseline (C, dotted black) is subtracted to produce the excited FSRS spectrum seen in Panel D (D, red).

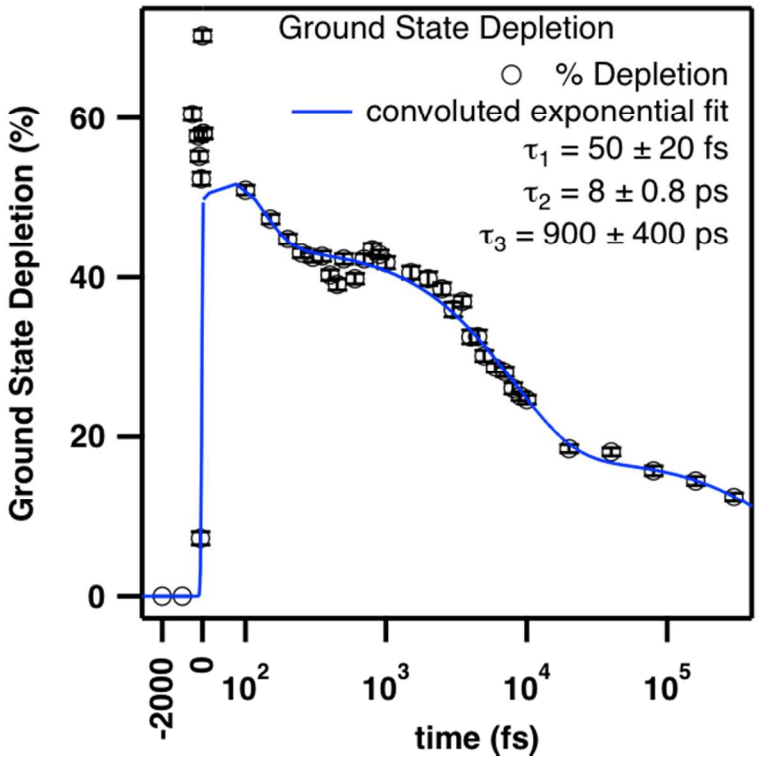

Figure S4 Kinetic plot of GS depletion as a function of log time. Following photoexcitation the population of PYP in the ground state decreases, and therefore there is less ground state PYP signal in these spectra. Thus subtracting the raw ground state FSRS spectrum (which is necessary to remove peaks from the non-photoactive buffer) from excited timepoints results in over-subtraction features at the ground state frequencies. These are removed by adding a scaled ground-state-PYP-only spectrum (Figure S3, panel B, black) back to each normalized, subtracted timepoint (Figure S3, panel B, red). The amount of depletion reports on the recovery of the ground state species. The ground state depletion was fit to a convoluted exponential decay with three time constants; 50 fs, 8 ps, and 900 ps.

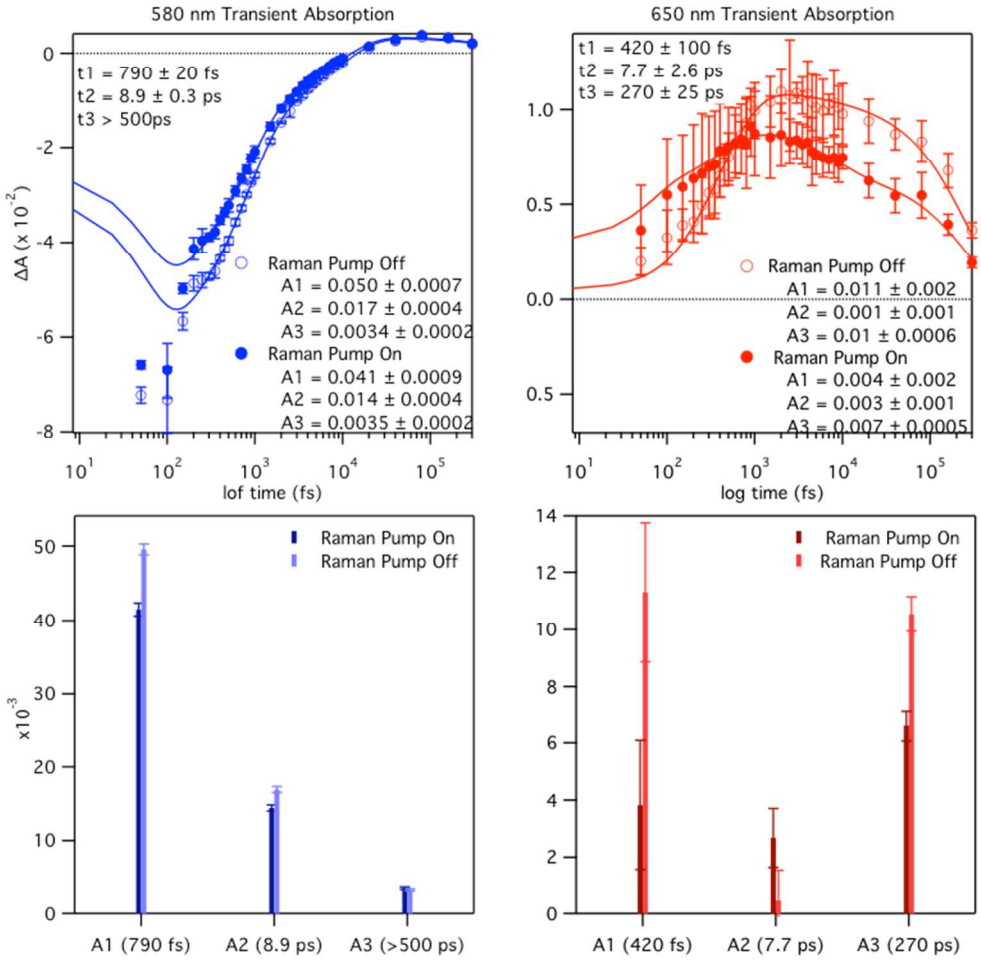

Figure S5 Effects of Raman pump on transient absorption. The top panels show the transient absorption kinetic traces for 580 nm (blue) and 650 nm (red) recorded in the absence (open circles) and in the presence (closed circles) of the Raman pump pulse. The bottom panels compare the amplitudes of the exponential components when the Raman pump is on and off. The most pronounced effects are seen on the amplitude of the 420 fs rise of the absorption signal at 650 nm, which is nearly halved in the presence of the Raman pump.

References

(1) Mihara, K.; Hisatomi, O.; Imamoto, Y.; Kataoka, M.; Tokunaga, F. Functional Expression and Site-Directed Mutagenesis of Photoactive Yellow Protein. *J. Biochem. (Tokyo)* **1997**, *121*, 876–880.

(2) Kumauchi, M.; Hamada, N.; Sasaki, J.; Tokunaga, F. A Role of Methionine100 in Facilitating PYPM-Decay Process in the Photocycle of Photoactive Yellow Protein. *J. Biochem. (Tokyo)* **2002**, *132*, 205–210.

(3) Imamoto, Y.; Ito, T.; Kataoka, M.; Tokunaga, F. Reconstitution Photoactive Yellow Protein from Apoprotein and p-coumaric Acid Derivatives. *FEBS Lett.* **1995**, *374*, 157–160.