Supporting Information

Dasgupta *et al.* 10.1073/pnas.0812056106

SI Materials and Methods

Protein Purification. *Escherichia coli* strain LMG194 (Invitrogen) was cotransformed with two plasmids, pBAD-Cph1(N514)-CBD and pPL-PCB, to permit production of holo-Cph1 in vivo. Transformed cells were selected on rich medium (RM) plates containing 100 mg/mL ampicillin and 50 mg/mL kanamycin. An individual colony was cultured overnight in RM ($AMP_{50}KAN_{25}$) and inoculated with 1:500 in 100 mL of RM. Upon reaching an optical density (OD) of 0.5 at 600 nm, the culture was transferred to 900 mL of LB (Luria–Bertani medium) solution with 50 mg/mL ampicillin, 25 mg/mL kanamycin, and 1 mM IPTG for a total expression of 6 L. After 1 h at 37 °C and in a shaker-incubator (Innova 4340 from New Brunswick Scientific) with shaking at 250 RPM, spin, arabinose was added to a final concentration of 0.002% and incubated for 1 additional h before the temperature was reduced to 20 °C for 16 h. Subsequently, the cells were harvested by centrifugation at 5,000 × *g* for 10 min and stored at −80 °C. The pellet was resuspended at 1 g wet weight per 4 mL of lysis buffer A [25 mM Hepes-Na (pH 8.0), 500 mM NaCl, 1 mM EDTA] plus 0.1% Triton X-100. Cells were lysed by two passes through a microfluidizer (M-110Y) at 15,000 psi, and all subsequent steps were carried out at 4 °C. Crude lysate was clarified by centrifugation (35,000 rpm, Ti-60 Beckman, 30 min) and applied to a 30-mL chitin column, which was washed thoroughly with buffer-A at 1 mL/min. Intein-mediated cleavage was induced by flushing the column with 1 bed volume of elution buffer (buffer A + 1 mM DTT). Fractions containing Cph1(N514) were pooled and concentrated in Amicon spin prep columns (10,000-kDa cut-off) to an OD_{656} of 10. The purified protein was then dialyzed against 1 L of buffer B [25 mM Tes-KOH (pH 8.0), 25 mM KCl, 10% glycerol] overnight.

Femtosecond Stimulated Raman Spectroscopy (FSRS) Data Analysis. The ground-state Raman spectrum of Cph1 is shown in Fig. S2 trace c after the subtraction of the Tes buffer spectrum (trace b) from trace a. After photoexcitation by the 635-nm actinic pump, the Raman bands corresponding to the chromophore structure after a 600-fs time delay are shown in trace d. The spectrum is dominated by ground-state peaks, but excited-state features are revealed in the one-to-one subtraction (trace e) of the ground-state Cph1 spectrum to remove the unpumped ground-state contribution. The one-to-one subtraction, which eliminates systematic noise trace, has negative features that are centered on the ground-state peaks. The intensity ratio of the negative features with respect to the absolute ground-state intensity represents the fractional ground-state depletion and repopulation at each time point. The buffer peaks are completely eliminated by subtracting trace a from trace d. Upon adding back a scaled amount of fitted ground-state Raman spectrum of Cph1, a complete Raman spectrum of the excited state at 600 fs after photoexcitation is revealed (in trace g). After baseline correction, the full Raman spectrum is shown in trace h. Subsequently, a broad sloping baseline arising from stimulated emission was subtracted to reveal the individual excited-state FSRS spectrum. This trace at Δt = 600 fs and the other time resolved spectra are compared with the P_r ground state in Figs. 3 and 4.

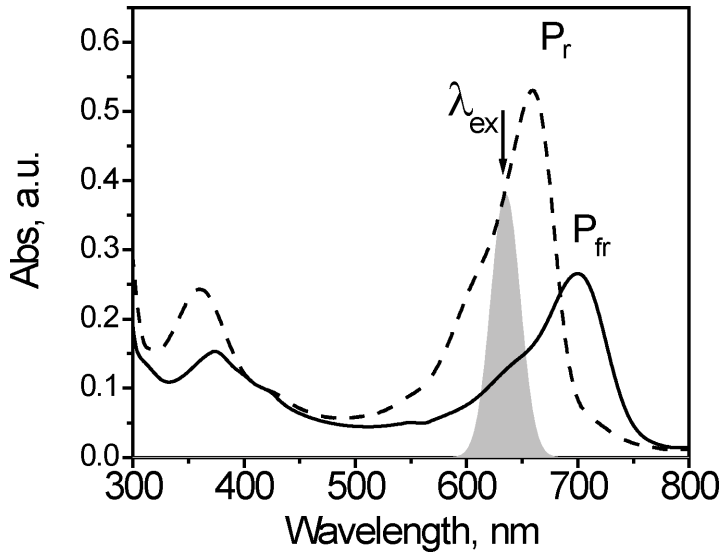

Fig. S1. Electronic absorption spectra of Cph1 in P_r (dashed line) and P_{fr} (solid line) forms. The Gaussian profile (shaded in gray) of the 635-nm photoexciting pump is also shown.

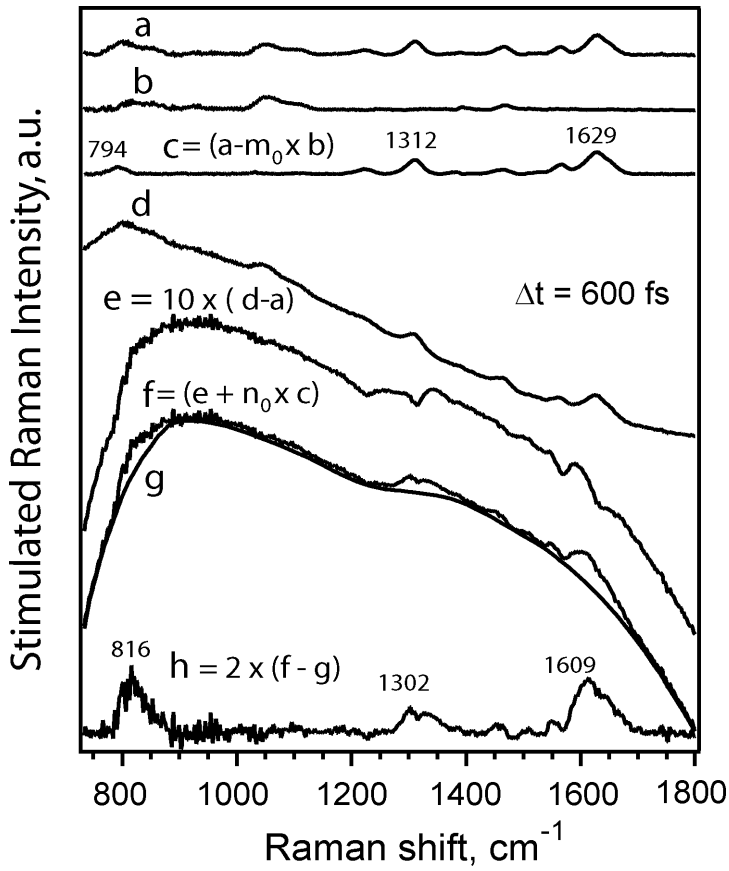

Fig. S2. Subtraction procedure used to produce the time-resolved femtosecond stimulated Raman spectra of Cph1. The raw FSRS spectrum of Cph1 in TES buffer is presented in trace a. The Raman peaks of the TES (trace b) are subtracted with a scale factor (m_0) to yield a buffer-free ground-state spectrum of Cph1 (trace c). A time-resolved FSRS spectrum (trace d) is produced by employing an actinic pulse with a delay time (here Δt = 600 fs) relative to the Raman probe pulse. The ground-state and buffer peaks are removed from trace d by subtracting trace a one-to-one, resulting in the transient difference spectrum, trace e. Negative Raman peaks caused by residual ground-state population are removed by adding back a scaled ground-state spectrum ($n_0\times$ trace c) to produce the pure transient Raman spectrum, trace f. A broad baseline (trace g) was subtracted to give the background-free transient FSRS spectrum (trace h). (Spectra magnification in traces e, f, and h, 10×.)

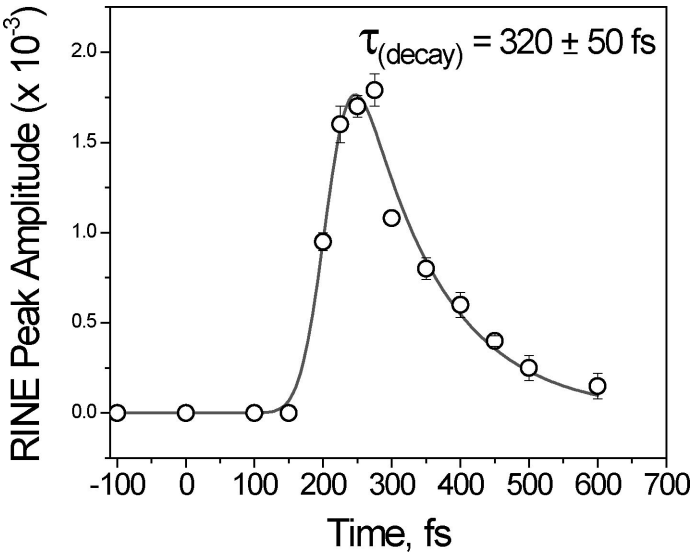

Fig. S3. Kinetic analysis of the RINE feature at 1,668 cm^{-1} representing the C═C stretch. Open circles represent the peak area as a function of time delay. The decay time is 320 ± 50 fs.

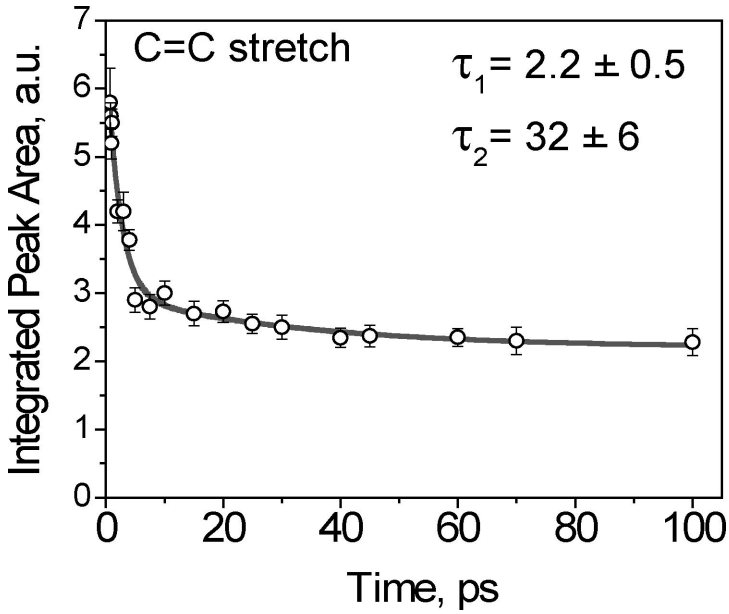

Fig. S4. Temporal profile of peak area of the C═C stretch at 1,612 cm^{-1}. The 32-ps time constant related to the decay is the photoproduct Lumi-R formation.

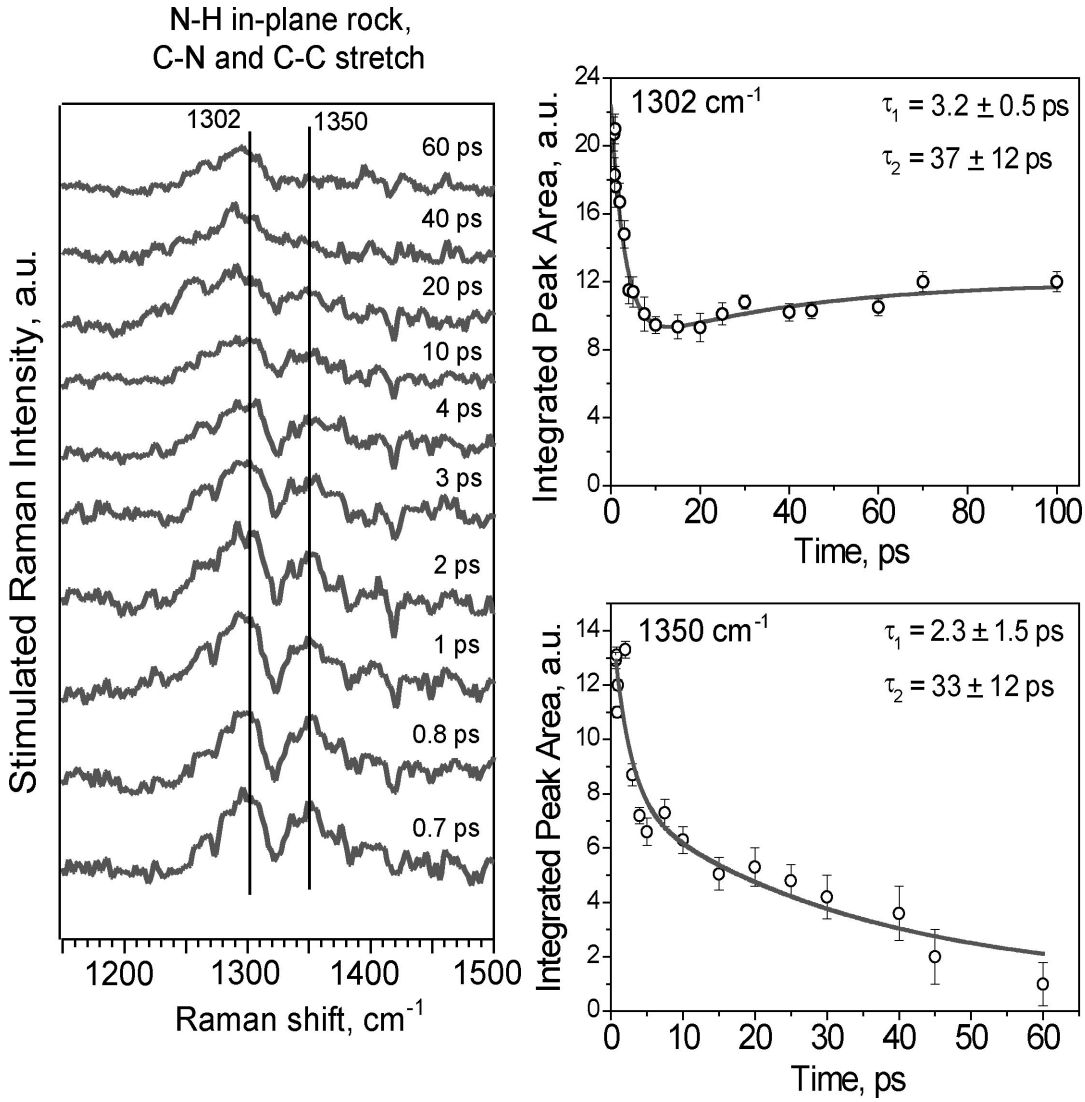

Fig. S5. Kinetic analysis of the Raman bands representing N—H in-plane rock, C—C stretch, and C—N stretch. Open circles represent the peak area as a function of time delay.

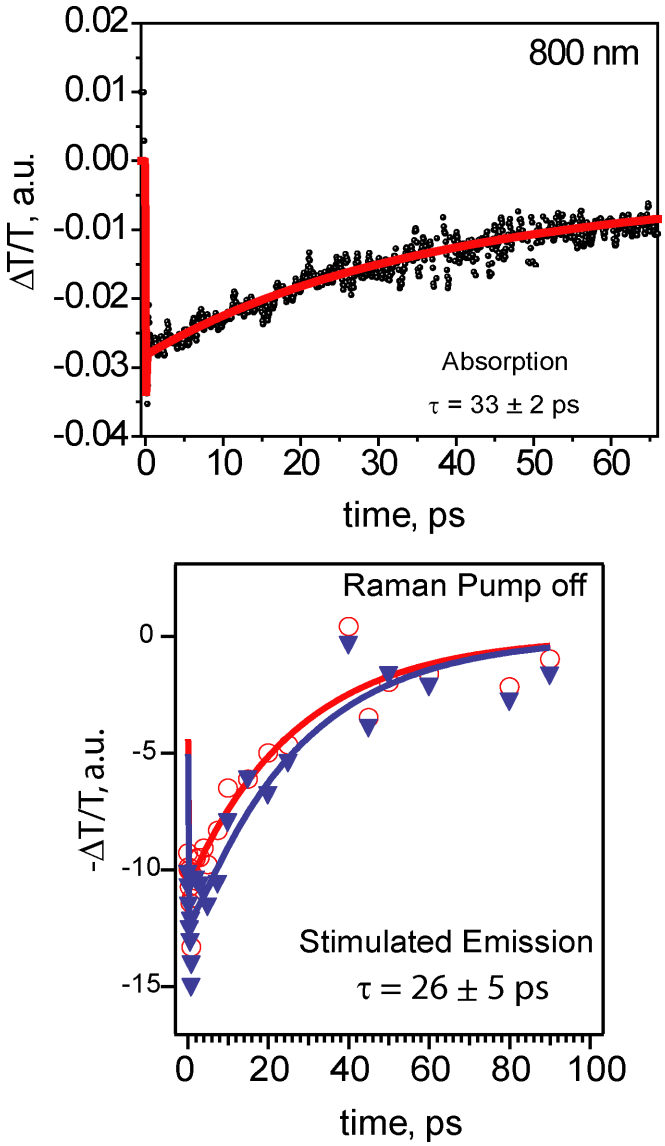

Fig. S6. Kenetic analysis of Lumi-R formation. (*Upper*) Transient absorption of Cph1 probed at 800 nm. Open circles correspond to absorption differences measured at 800 nm as a function of time delay; the solid line represents the best fit to the formation of the Lumi-R state. The excited-state absorption (ESA) decays with a 33-ps time constant and matches the intensity decrease in the Raman bands. (*Lower*) Stimulated emission band probed from 840 to 960 nm. Open circles designate probe wavelength at 840 nm and solid triangles at 880 nm, respectively. The formation of Lumi-R state is seen from the decay of stimulated emission with 26 ± 5-ps time constant.

Table S1. Kinetic time constants corresponding to changes in the different Raman bands from FSRS and transient electronic absorption

Representative features	Short (~0.2–0.5 ps)	Medium (~3 ps)	Long (~30 ps)
HOOP 803 cm^{-1}	RINE decay 0.3 ps; Amplitude rise 0.45 ps	Amplitude decay 4.5 ps	NA
N—H rock 1,302 cm^{-1}	RINE decay 0.3 ps; Amplitude rise 0.5 ps	Amplitude decay 3.2 ps	Amplitude rise 37 ps
C—C, C—N stretch 1,350 cm^{-1}	RINE decay 0.3 ps	Amplitude decay 2.3 ps	Amplitude decay 33 ps
C═C stretch 1,612, 1,645 cm^{-1}	RINE decay 0.32 ps; Amplitude rise 0.5 ps	Amplitude decay 2.2 ps	Amplitude decay 32 ps
GS bleaching	Decay 0.15 ps	Decay 3 ps	NA
Transient Abs	NA	NA	SE decay 26 ps; ESA decay 33 ps

NA, not applicable.