Supporting Information for "Heterogeneous Photodynamics of the P_{fr} State in the Cyanobacterial Phytochrome Cph1" by Peter W. Kim,[†] Nathan C. Rockwell,[‡] Shelley S. Martin,[‡] J. Clark Lagarias,[‡] Delmar S. Larsen[*,†]

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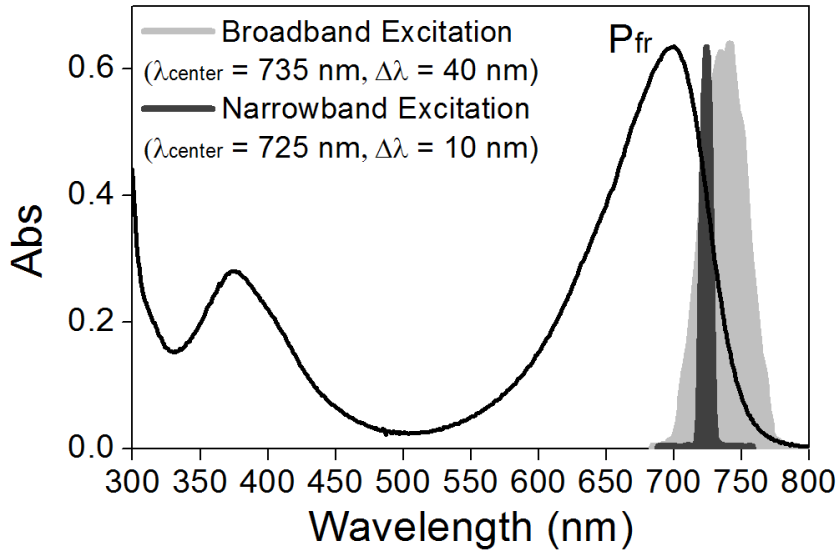

Figure S1. Comparison between excitation pulse spectra of current and previous works[1] (dark grey and grey, respectively) overlaid with Cph1Δ P_{fr} absorbance spectrum (black curve). The current work demonstrates spectrally narrower excitation of Cph1Δ P_{fr}.

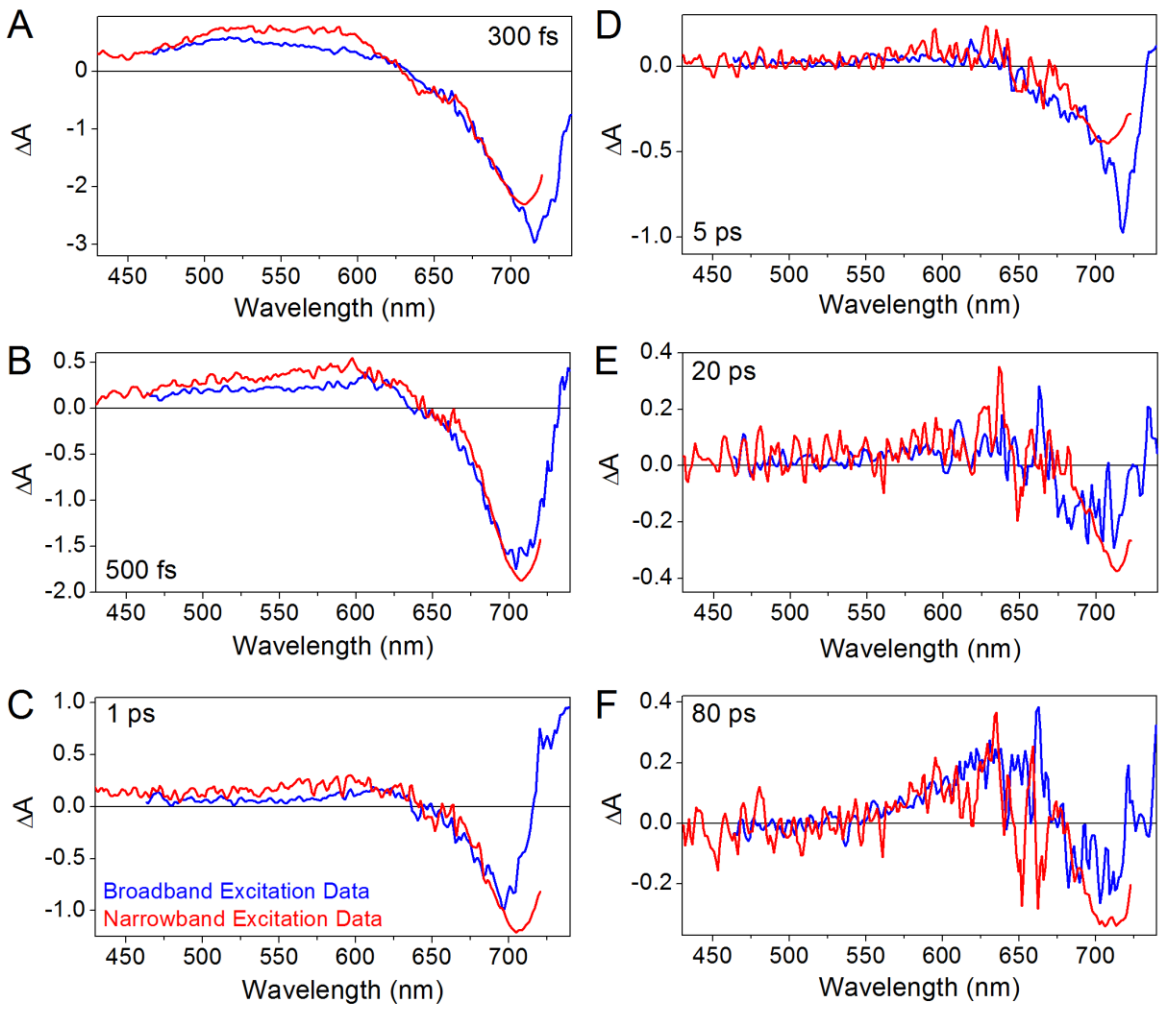

Figure S2. Comparison of transient absorption spectra at various probe times for broadband and narrowband excitation data (blue and red curves, respectively). Transient absorption spectra from the narrowband excitation were scaled by × 0.55.

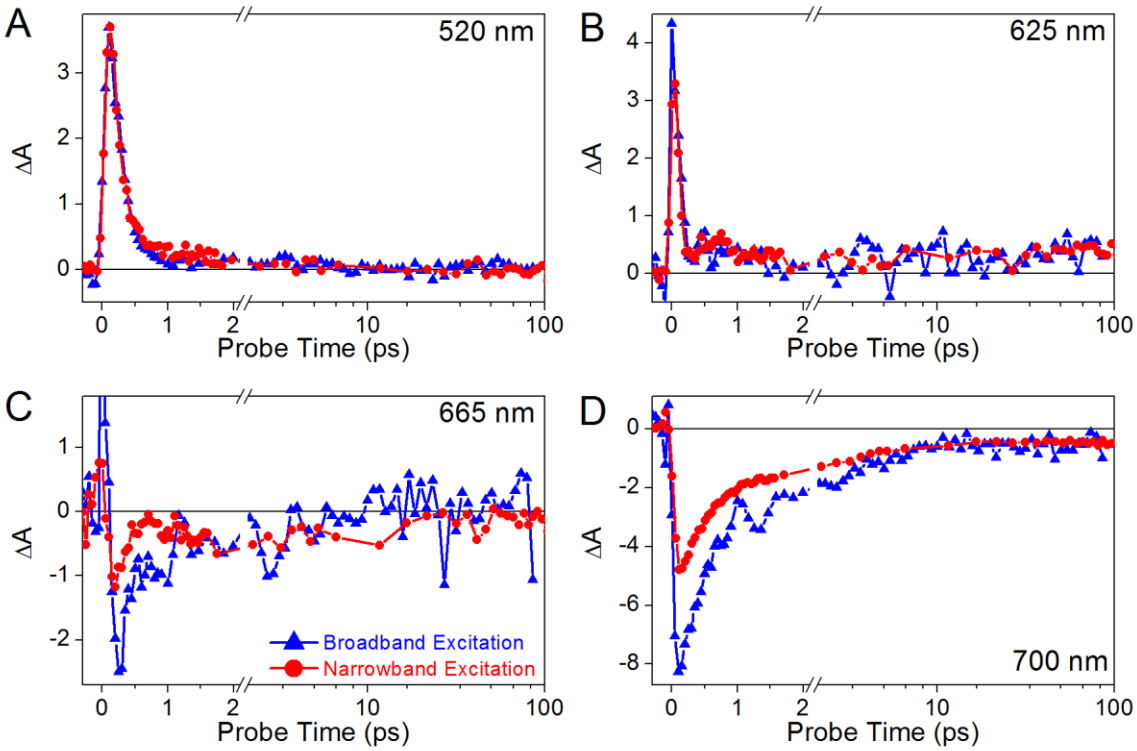

Figure S3. Comparison of kinetic traces at various probe wavelengths for broadband and narrowband excitation data up to 100-ps probe time (blue and red curves, respectively). The traces are scaled by × 3.2 on the broadband excitation signals.

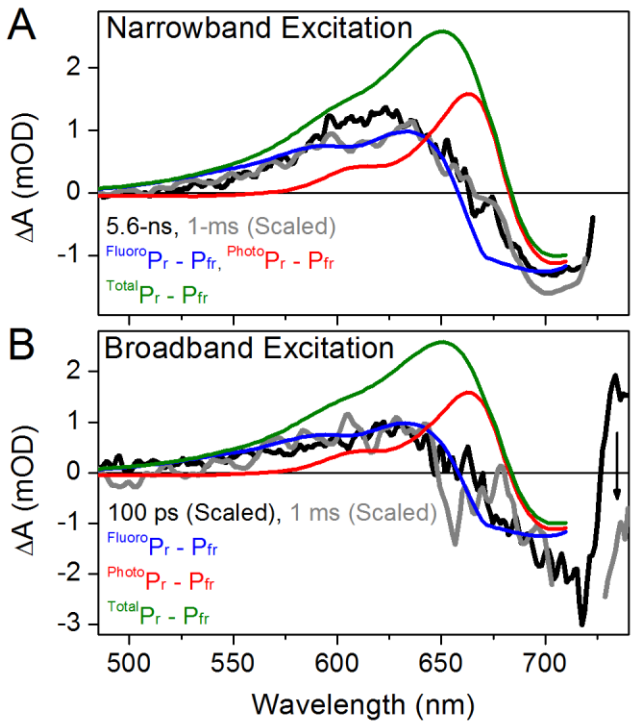

Figure S4. (A) Comparison of P_r minus P_{fr} difference spectra to 5.6-ns and 1-ms difference spectra of P_{fr} to P_r dynamics (black and grey curves, respectively) from the narrowband excitation data. The P_r minus P_{fr} difference spectra has three kinds depending on P_r states: the Fluorescent P_r, Photoactive P_r, and combined Total P_r (blue, red and green curves, respectively) based on previous temperature-dependent absorption spectra and SVD analysis.[2] (B) The same comparison (except the 100-ps spectrum is presented instead of 6-ns one) for the broadband excitation data.

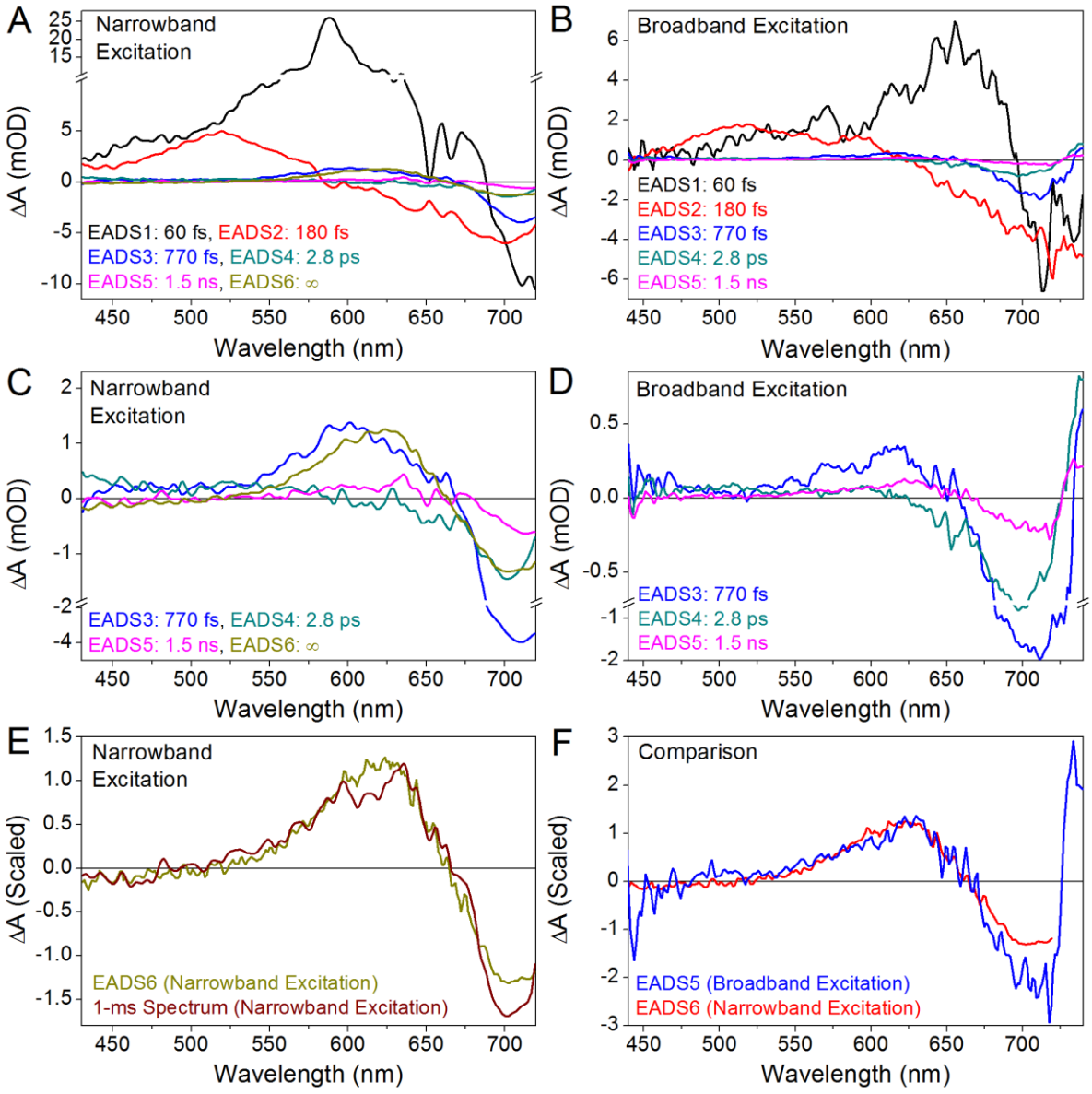

Figure S5. (A) The sequential evolution-associated-difference-spectra (EADS) extracted from the sequential model of the newer narrowband excitation data. The lifetime associated with each EADS is described in the inset. (B) The EADS extracted from the sequential model of the previous broadband excitation data. (C) The EADS3 to EADS6 from the narrowband excitation signals. (D) The EADS3 to EADS5 from the broadband excitation signals. (E) Comparison between the EADS6 and 1-ms transient spectra from the narrowband excitation data. (F) Comparison between the EADS5 of broadband and EADS6 of narrowband excitation data.

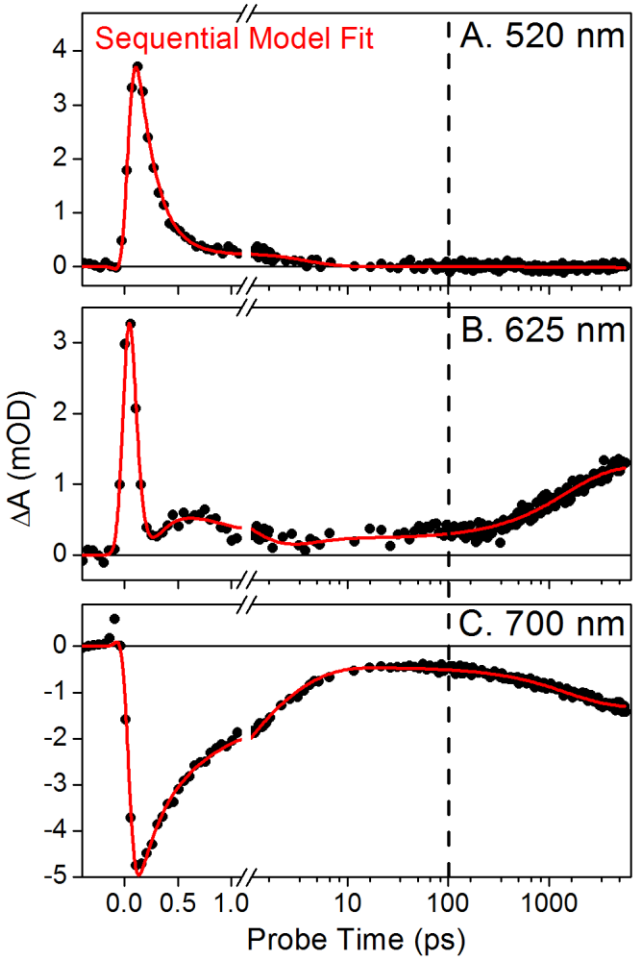

Figure S6. The fit of the global sequential model (Figure S5A) on the narrowband excitation data probed at (A) 520 nm, (B) 625 nm, and (C) 700 nm.

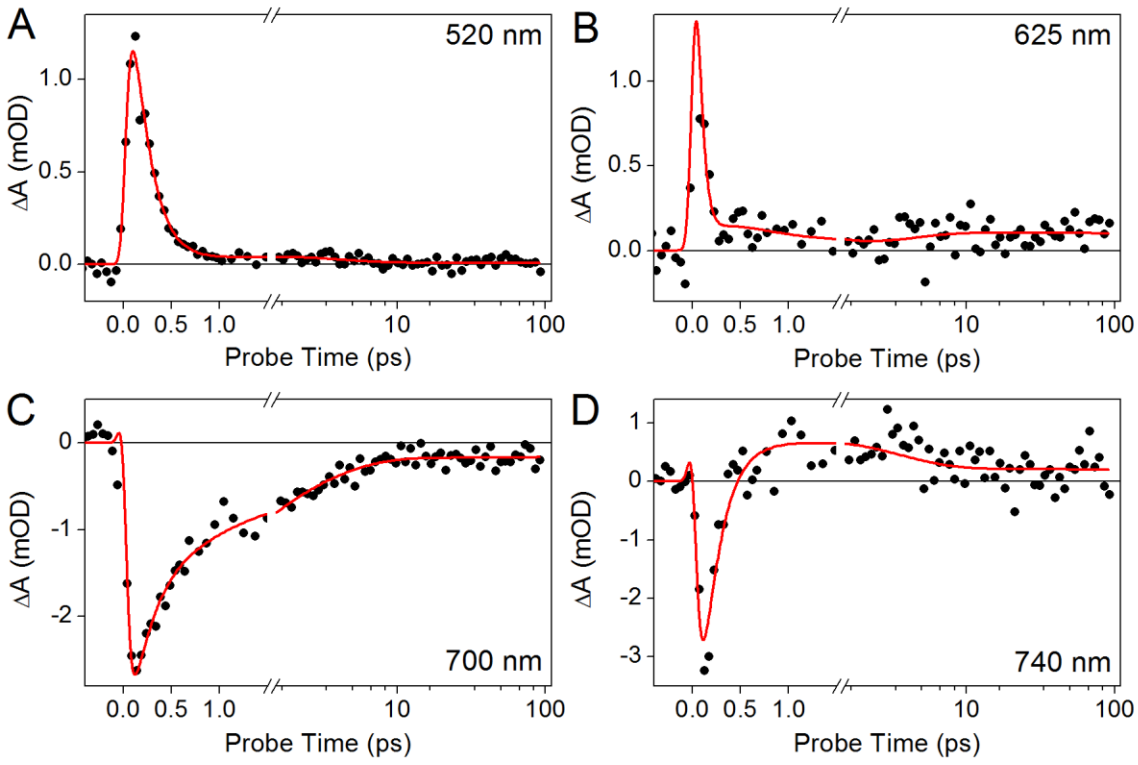

Figure S7. The fit of the global sequential model (Figure S5B) on the broadband excitation data probe at (A) 520 nm, (B) 625 nm, (C) 700 nm, and (D) 740 nm.

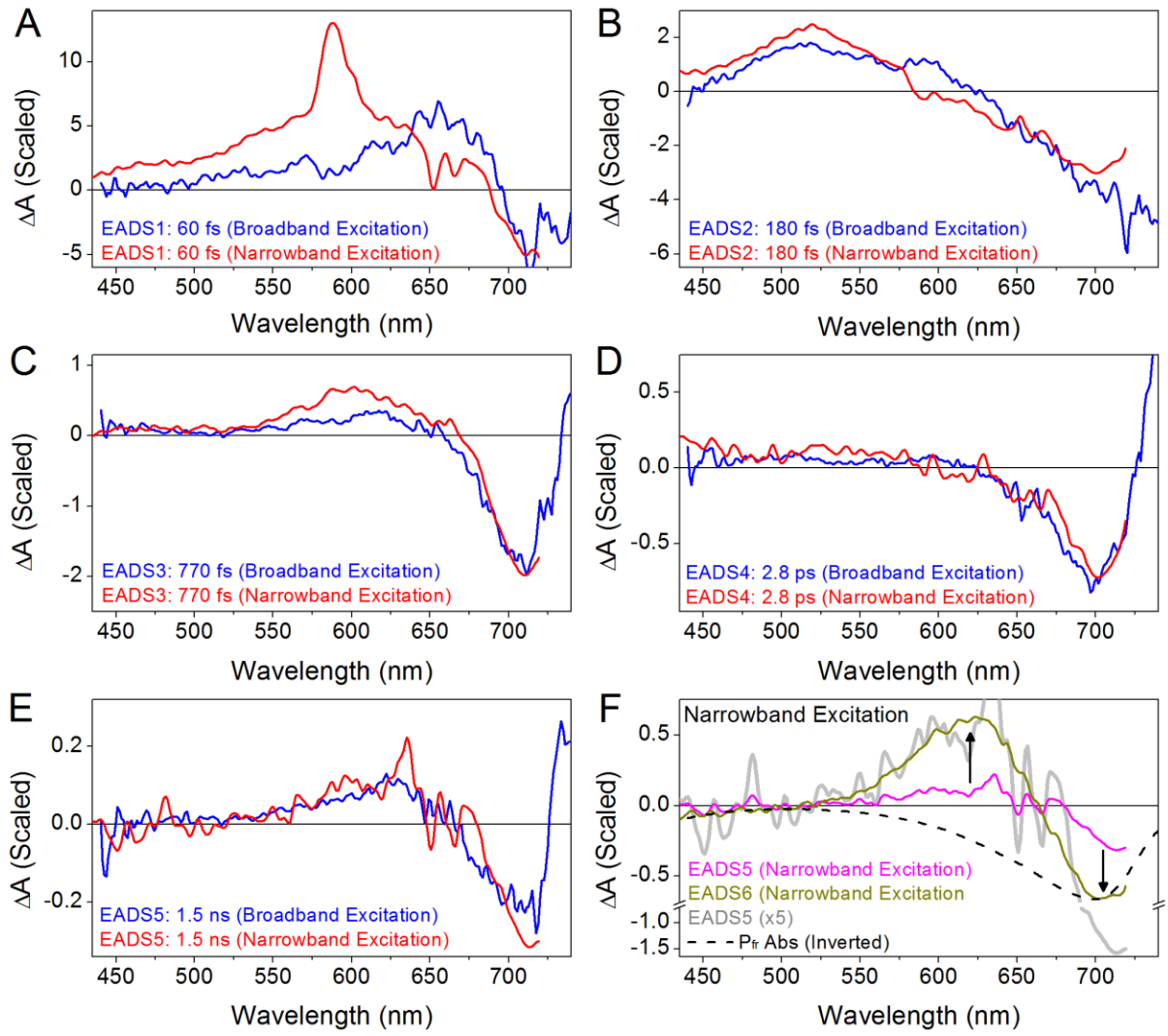

Figure S8. Comparison of EADS from the broadband (blue curve) and narrowband (red curve) excitations from EADS1 to EADS 5 (A-E). All of the narrowband excitation EADS's are scaled by ×0.3, which allows scaling of EADS5 in Panel E. (F) Comparison between the narrowband excitation EADS5 and EADS6, overlaid with the inverted P_{fr} absorbance spectrum as an estimate of bleach band (black dashed line). The arrows indicate the spectral amplitude change from EADS5 to EADS6.

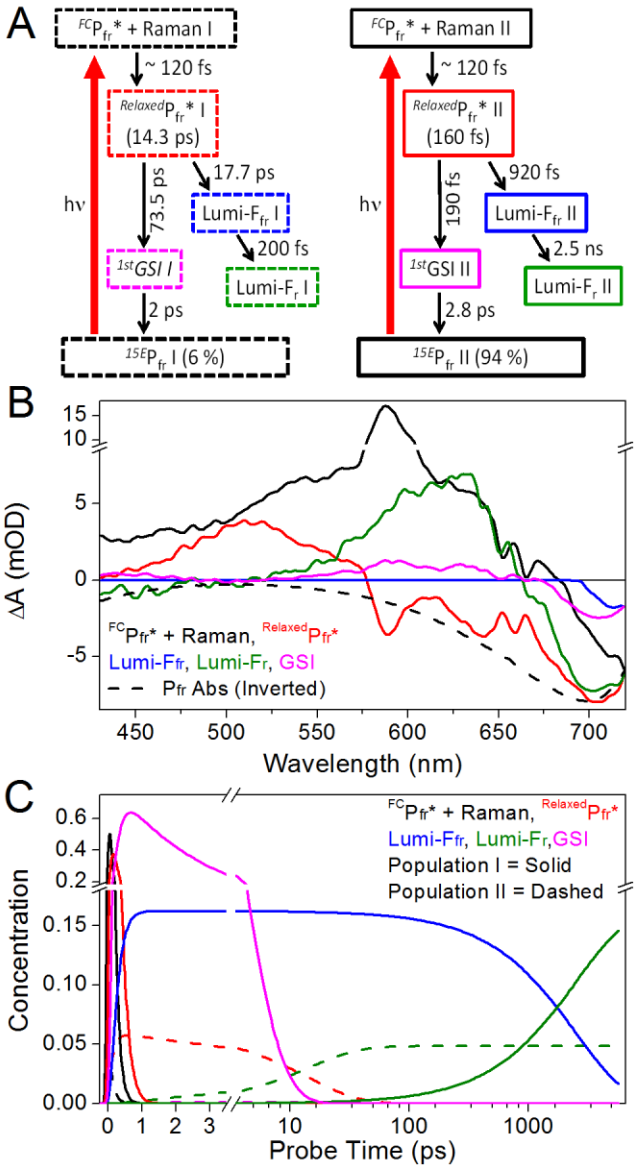

Figure S9. (A) Schematics of a kinetic model with two P_{fr} subpopulations, P_{fr} I and P_{fr} II, and a single GSI population. Each spectral species are represented in a box and its apparent time constant is indicated in parenthesis. (B) The SADS of the kinetic model in panel A. The $^{Relaxed}P_{fr}^{*}$, GSI1, GSI2, Lumi-F_f, and Lumi-F_r from population I and II have identical respective SADS. (C) Concentration profile of each constituent population. The color scheme is the same as Panel A.

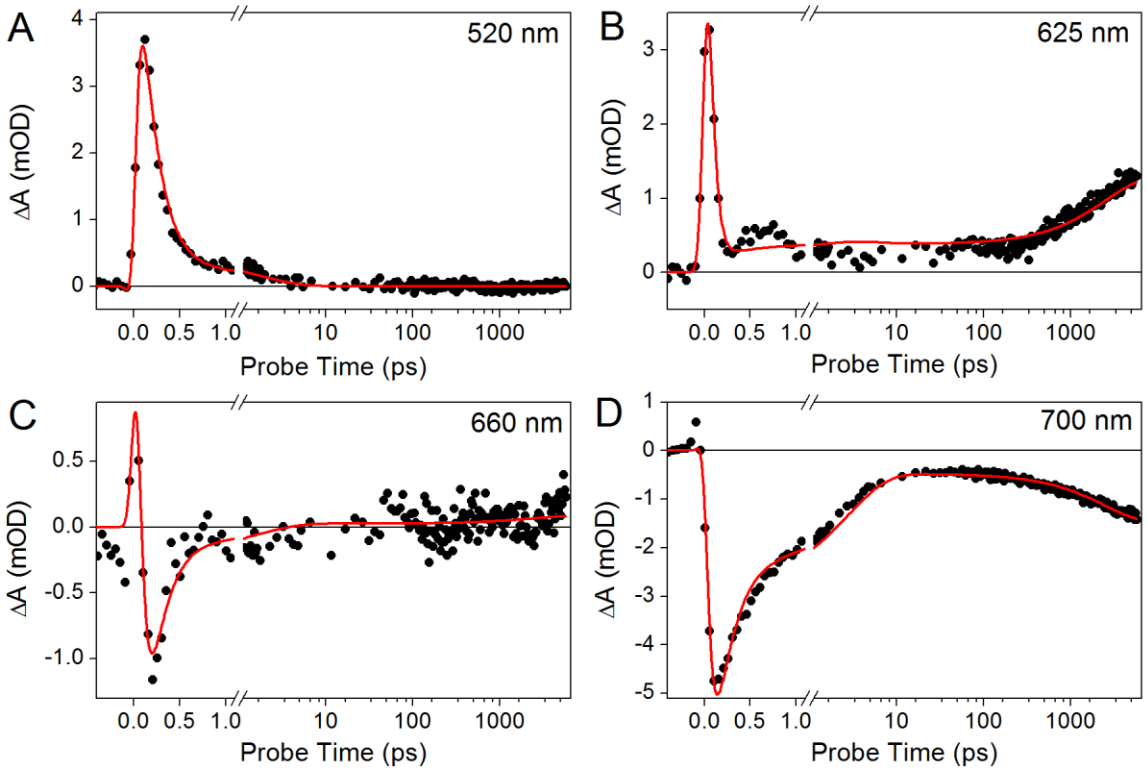

Figure S10. Fit of the kinetic model in Figure S9A to the narrowband excitation data probed at (A) 520 nm, (B) 625 nm, (C) 660 nm, and (D) 700 nm.

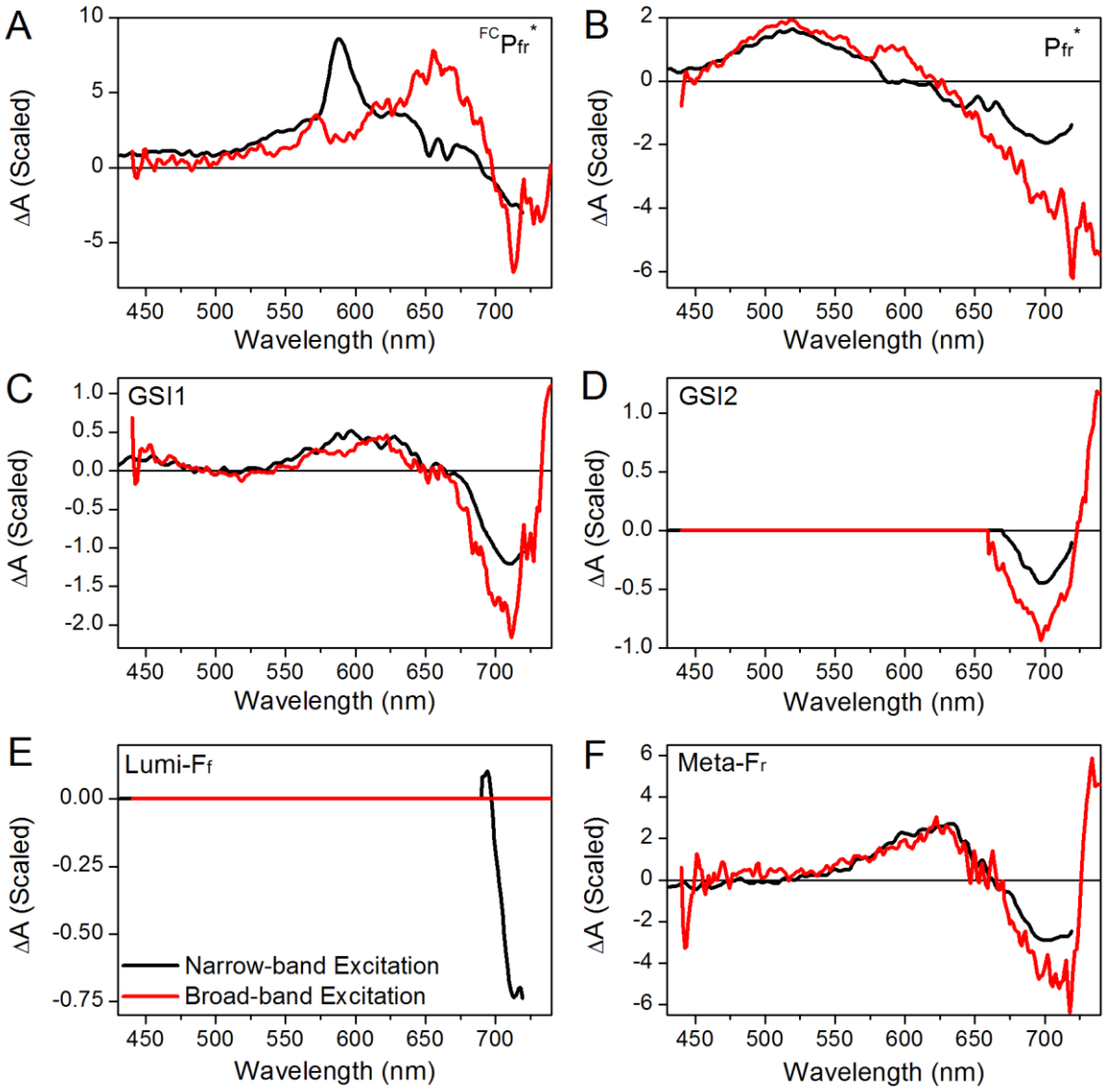

Figure S11. Comparison between SADS extracted from the target model from Figure 8 on the narrowband excitation data (black) and previous broadband excitation data (red). The narrowband excitation SADS are scaled by ×0.3. The Lumi-F_f extracted from the broadband excitation (Panel E, red curve) is zeroed in the spectral region of the experiment.

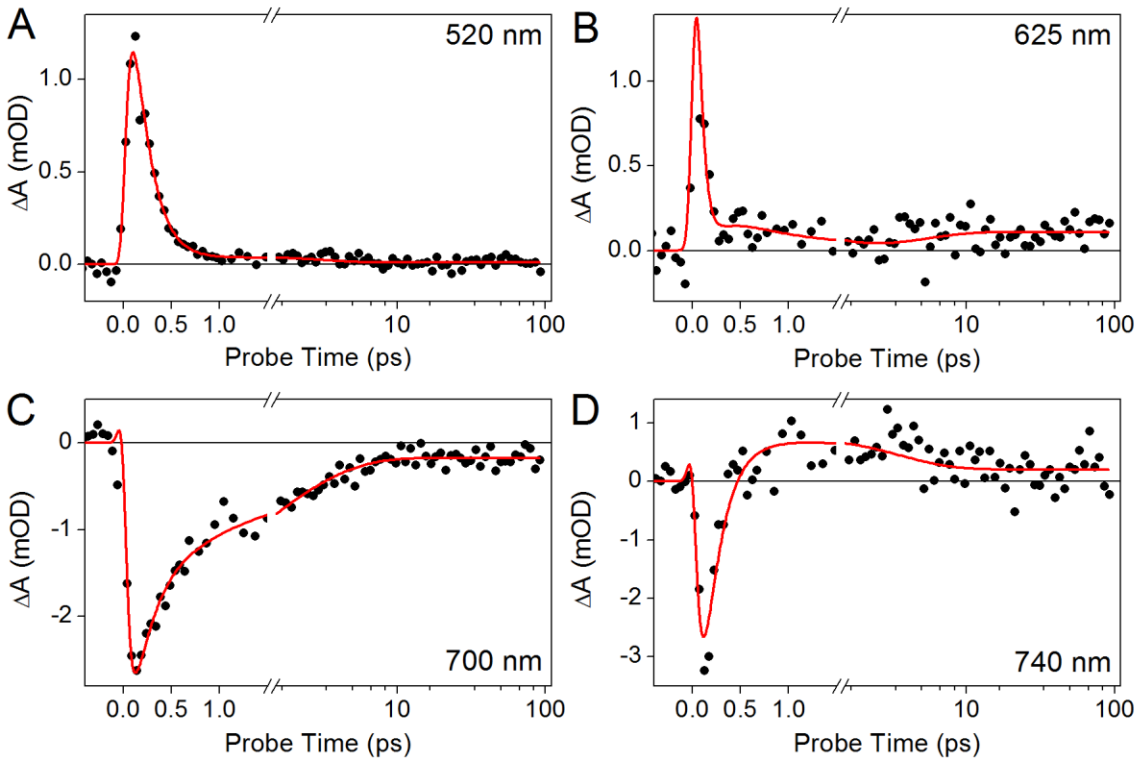

Figure S12. Fit of the kinetic model in Figure 8A to the broadband excitation data probed at (A) 520 nm, (B) 625 nm, (C) 700 nm, and (D) 740 nm.

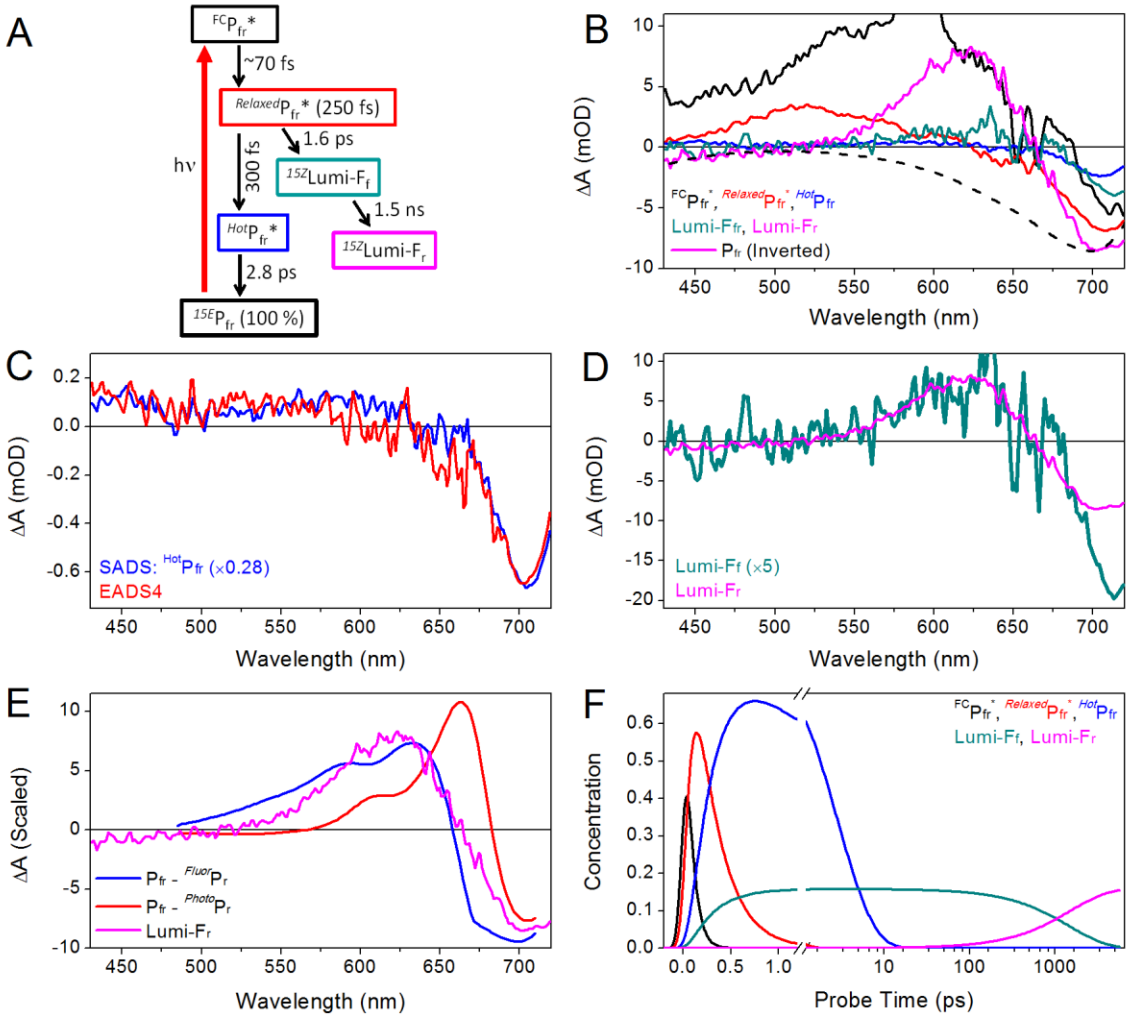

Figure S13. (A) A kinetic model scheme adapted from previous publication.[1] ^{15Z}Lumi-F_r population is added with 1.5 ns formation time. (B) Species-Associated-Difference-Spectra (SADS) of the target model in Panel A. The respective SADS are color-coded with the same color scheme in Panel A. (C) Comparison between $^{Hot}P_{fr}$ SADS with EADS4 from Figure S4A. (D) Comparison between Lumi-F_f and Lumi-F_r spectra. Lumi-F_r is scaled ×5. (E) Comparison between Lumi-F_r spectrum and P_{fr} minus P_r spectra. The P_{fr} minus P_r spectra have two kinds depending on $^{Fluor}P_r$ and $^{Photo}P_r$ spectra extracted from previous temperature-dependence study. (F) Concentration profile of the kinetic model in Panel A. The final Lumi-F_r concentration is ~15%.

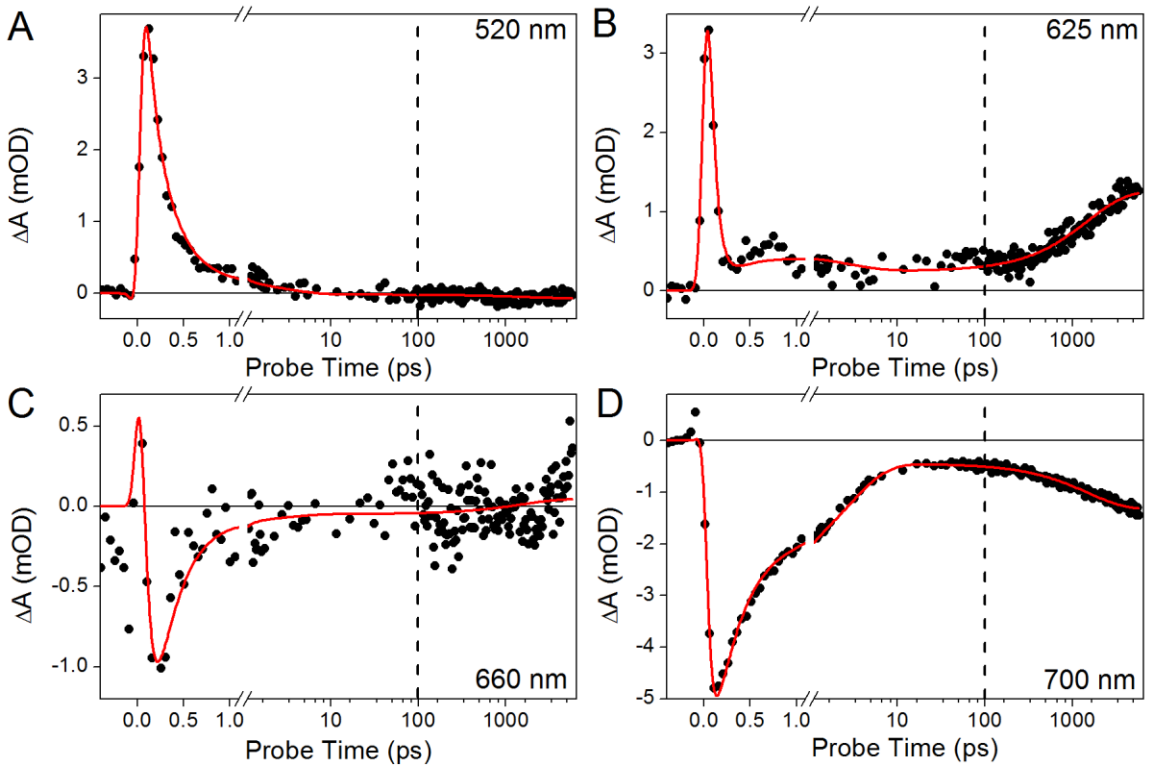

Figure S14. Fit of the kinetic model in Figure S13A to the narrowband excitation data probed at (A) 520 nm, (B) 625 nm, (C) 660 nm, and (D) 700 nm.

REFERENCES

[1] Kim, P. W., Pan, J., Rockwell, N. C., Chang, C.-W., Taylor, K. C., Lagarias, J. C., and Larsen, D. S. (2012) Ultrafast E to Z Photoisomerization Dynamics of the Cph1 Phytochrome, *Chemical Physics Letters 549*, 86-92.

[2] Kim, P. W., Rockwell, N. C., Martin, S. S., Lagarias, J., Clark, and Larsen, D. S. (2014) Dynamic Inhomogeneity in the Photodynamics of Cyanobacterial Phytochrome Cph1, *Biochemistry In press*.