Sub-Picosecond Excited State Proton Transfer Preceeding Isomerization During the Photorecovery of Photoactive Yellow Protein

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Supporting Results

The model used in global analysis is shown in **Fig. S1**. The first panel depicts the transitions modeled by first order rate equations. Traces and spectra obtained from the global fits to this model are shown in the main text in Figure 2. The second panel is a conceptual embellishment including our interpretation of the time constants and inclusion of processes faster than our instrument response time (< 300 fs) which were inferred from spectral analysis. P_{430}, the photoproduct identified from global analysis of power dependent data (Fig. S2 & S3) on the terminal transient spectrum was assigned to a ground state photocycle intermediate, but its connectivity with the excited states is conjecture. A second long-lived photoproduct P_{350} was identified, but an assignment was not conclusively made. We speculate that P_{350} is a protonated *p*-coumaric acid that either forms directly from relaxation of S_1, or may form from partial protonation of P_{430}. Alternatively, P_{350} may be a radical species formed from resonant two-photon ionization of *p*CA.

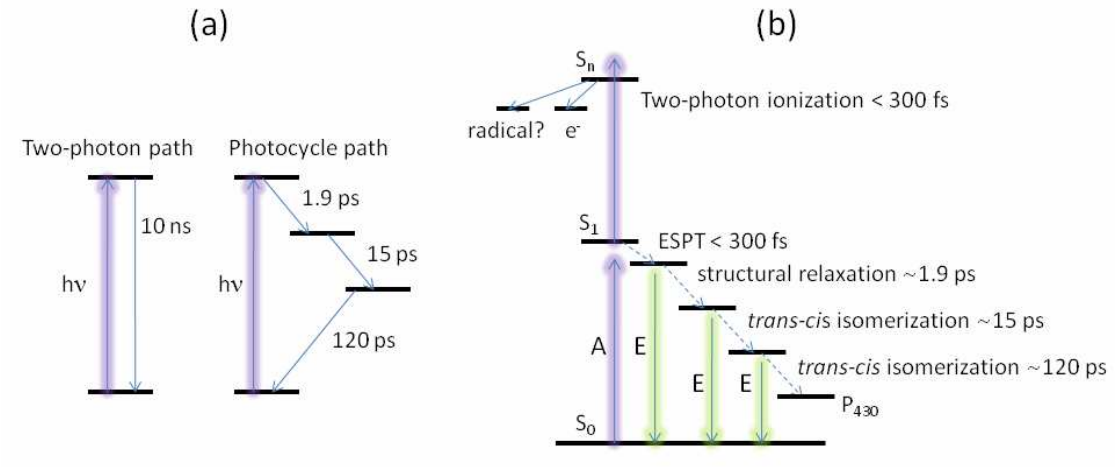

Figure S1. (a) State model used for global analysis. (b) Proposed Jablonski diagram. A= absorption; E=emission; ESPT = excited state proton transfer.

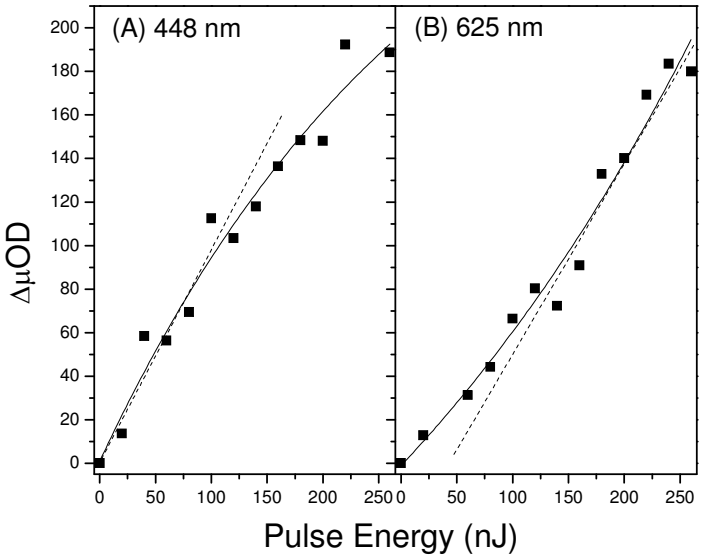

Figure S2. Power dependence in terminal transient spectrum following excitation of the pB state in M100A PYP.

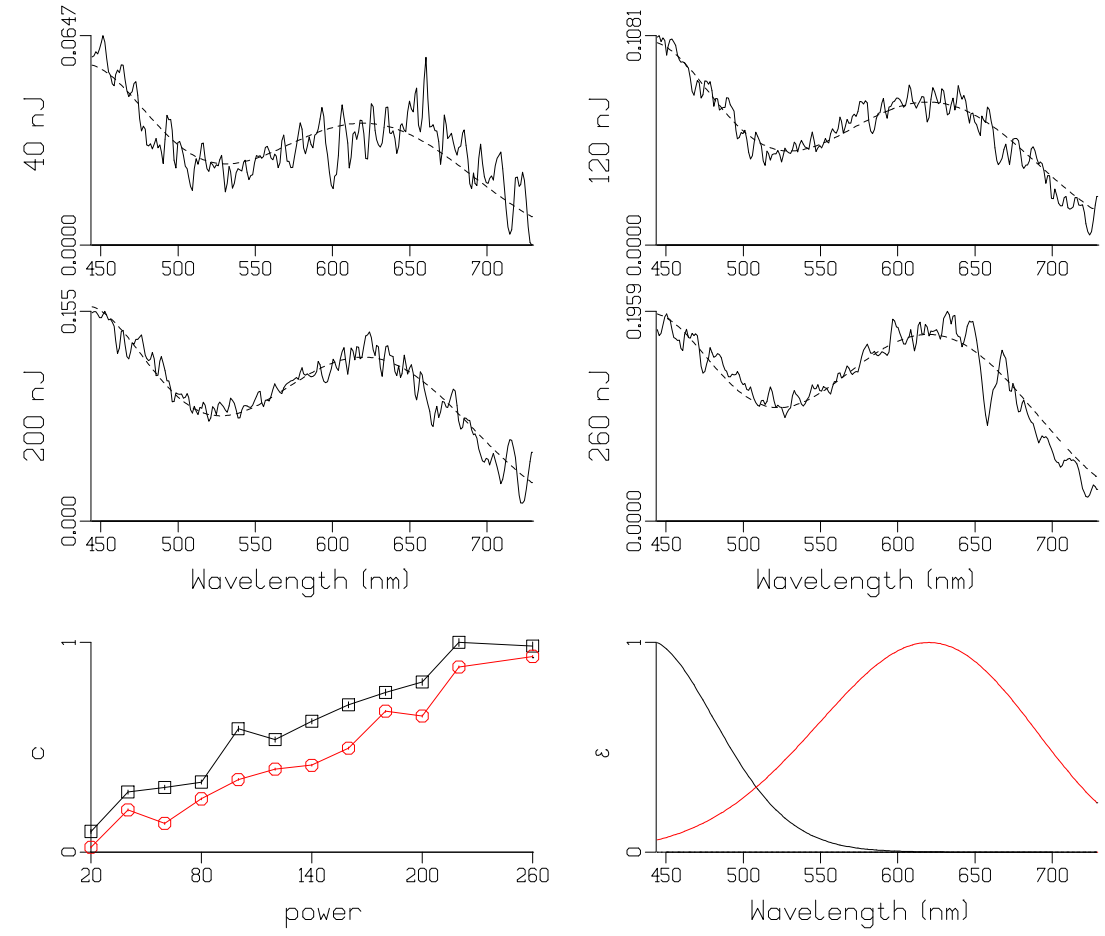

Figure S3. Global analysis of power dependence in terminal transient spectrum following excitation of the pB state in M100A PYP.

In **Fig. 3** of the main text, the 15-ps Evolution Associated Difference Spectrum (EADS) from M100A pB is compared to a Species Associated Difference Spectrum (SADS) obtained from photoexcitation of the pG state of wild type PYP.[1] Both EADS and SADS are composite spectra that describe a common kinetics feature, generally linked to a process like protonation or excited

state decay.[2] Spectra associated with excited state decay processes always contain a negative contribution from the bleached ground state absorption (GSA) of the starting species (e.g., pG or pB), and often also contain a negative contribution from stimulated emission (SE) and a positive contribution from excited state absorption (ESA). The associated difference spectra are then described by the following general relations:

(Eq. 1a) $$EADS_{pB} = (ESA + SE)_{pB} - GSA_{pB}$$

(Eq. 1b) $$SADS_{pG} = (ESA + SE)_{pG} - GSA_{pG}$$

(Eq. 2) $$\Delta\Delta = EADS_{pB} - SADS_{pG}$$

$$= (ESA + SE)_{pB} - (ESA + SE)_{pG} + GSA_{pG} - GSA_{pB}$$

The difference spectrum ΔΔ in Fig. 3 not only shows no difference in the stimulated emission from the *wt* pG and M100A pB states, but also closely resembles the pG – pB difference spectrum. The observation that (ΔΔ ≈ pG – pB) requires that the first two terms in Eq. 2 are equivalent, which suggests that the excited-state signals in the M100A pB (after exciation with 335-nm light) and wild-type pG (after excitation with 440-nm light) are *indistinguishable*. Considering the large effect protonation has on the absorption and emission properties of *p*CA, identical excited state optical properties can only be explained by a rapid excited-state deprotonation of the *p*CA chromophore in the pB state.

References

(1) Larsen, D. S.; van Stokkum, I. H.; Vengris, M.; van Der Horst, M. A.; de Weerd, F. L.; Hellingwerf, K. J.; van Grondelle, R. *Biophys J* **2004**, *87*, 1858-72.

(2) van Stokkum, I. H. M.; Larsen, D. S.; van Grondelle, R. *Biochim. Biophys. Acta* **2004**, *1657*, 82-104.