Short Hydrogen Bonds and Negative Charge in Photoactive Yellow Protein Promote Fast Isomerisation but not High Quantum Yield.

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Supporting Information

Global Fitting analysis method

Model based Global fitting analysis can provide an integral and more compact description of the system dynamics than a single wavelength time trace fitting. The physics and mathematics basis for global target analysis of the measured data matrix $\Delta A(\lambda, t)$ is that the variable delay time t and wavelength λ are independent. That is, the measured $\Delta A(\lambda, t)$ is a superposition of several species components and can be separated as:

$$\Delta A(\lambda,t) = \sum_{i=1}^{N} C_i(t)\Delta\varepsilon_i(\lambda) \qquad (1)$$

where $C_i(t)$ and $\Delta\varepsilon_i(\lambda)$ are the concentration and extinction coefficient of the generalized species component N_i respectively. A parametric dependent kinetic model based on the first order reaction was resorted to $C_i(t)$ and by globally fitting the data $\Delta A(\lambda, t)$ to extract the spectral components of $\Delta\varepsilon_i(\lambda)$, which generally is the species extinction coefficient and has different

nomination according the specific kinetic models used. Based on the kinetic model, $C_i(t)$ was constructed and resolved by the differential equation:

$$\frac{dC_i(t)}{dt} = -k_i C_i(t) + \sum_{j \neq i} \chi_{ji} k_j C_j(t) \quad (2)$$

where k_i represent the total decay of state species i and χ_{ji} is the branching ratio of species j to i. The reconstructed $C_i(t)$ were convoluted with the instrument response function (IRF, normally a Gaussian shape) to globally fit the data by minimizing:

$$\sqrt{\left(\Delta A(\lambda, t) - \sum_{i=1}^{N} C_i(t) \Delta \varepsilon_i(\lambda) \right)^2} \quad (3)$$

The initial guess for $\Delta\varepsilon_i(\lambda)$ were given by multiplying the pseudoinverse of matrix $C_i(t)$ with matrix ΔA (λ, t). The fitting leads to a set of rate constants and branch ratios and the corresponding species spectra $\Delta\varepsilon_i(\lambda)$ simultaneously. This global fitting process was realized by using genetic evolution method by searching the minimum value of (3), a program developed by the author using computer language LabView.

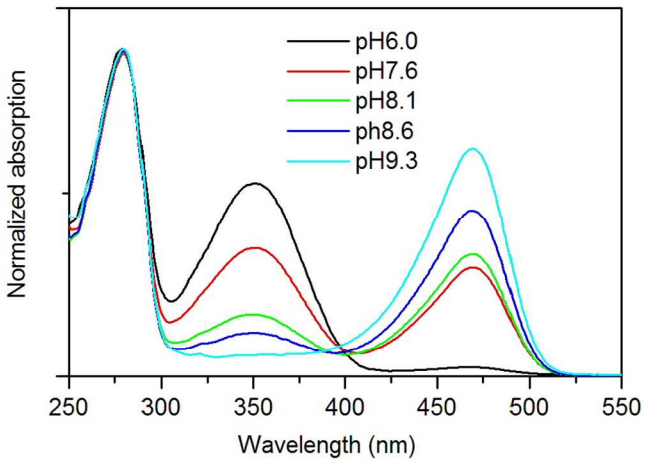

Figure S1: pH dependent absorption spectra of PYP mutant E46A.

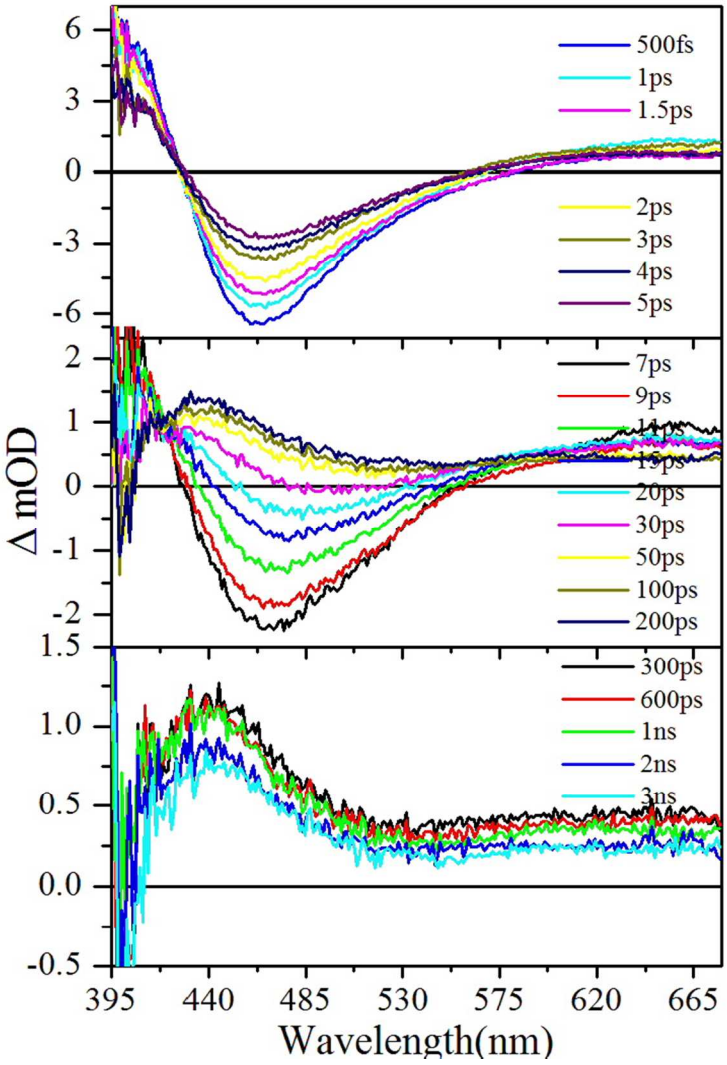

Figure S2: Dispersion-corrected transient spectra pE46A in pH6.0, at different probing delay time. Excitation wavelength is 330nm.

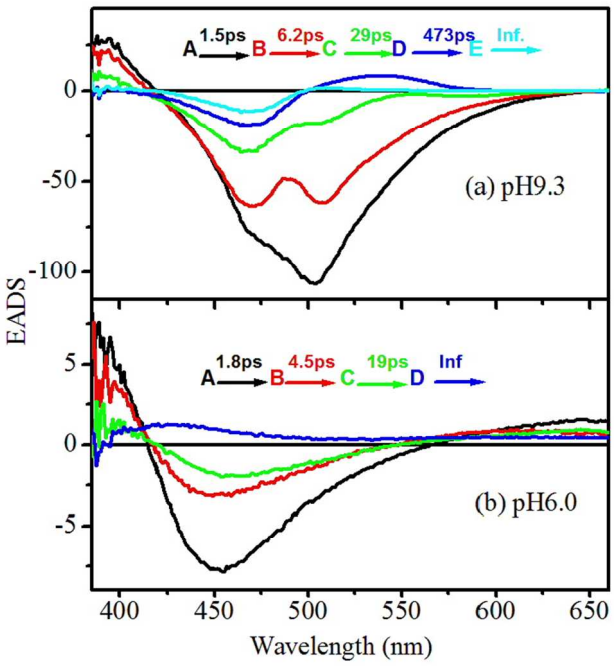

Figure S3: EADS and decay time constants of E46As from experiments at different buffer with pH values of 9.3 and 6.0.

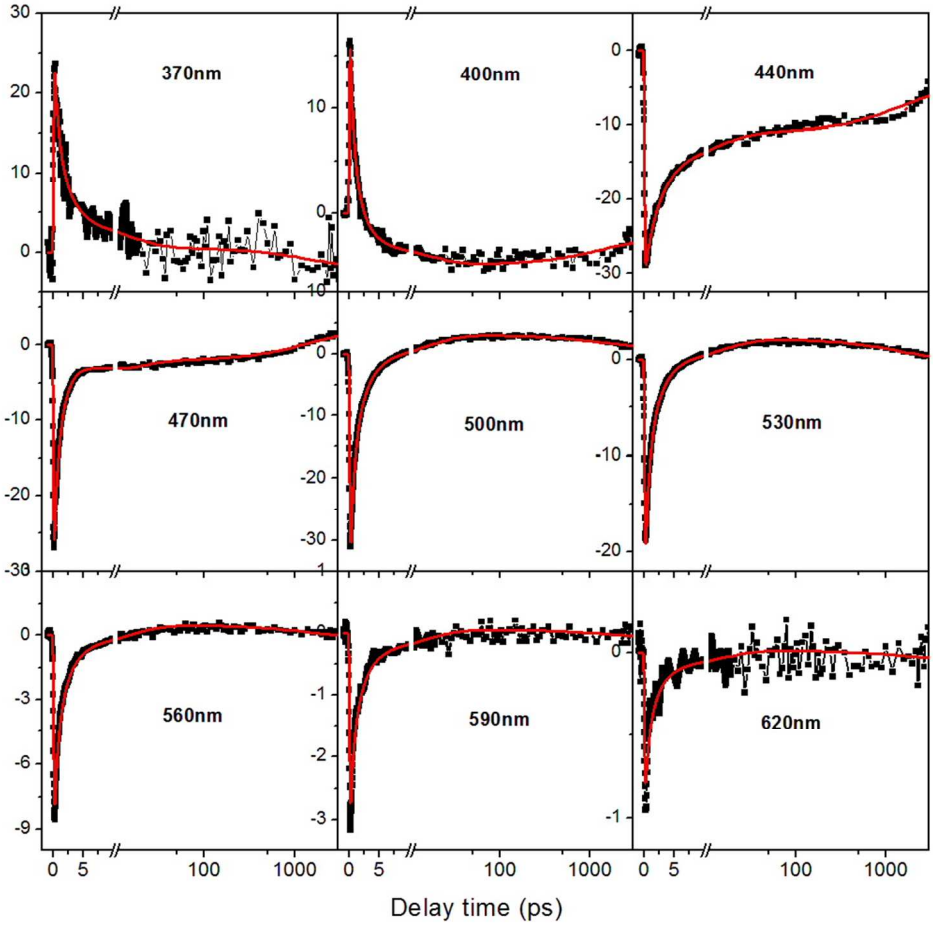

Figure S4: Time decay traces at representative wavelengths wtPYP data (black) and fitted (red) according to the target model as in Figure 7.

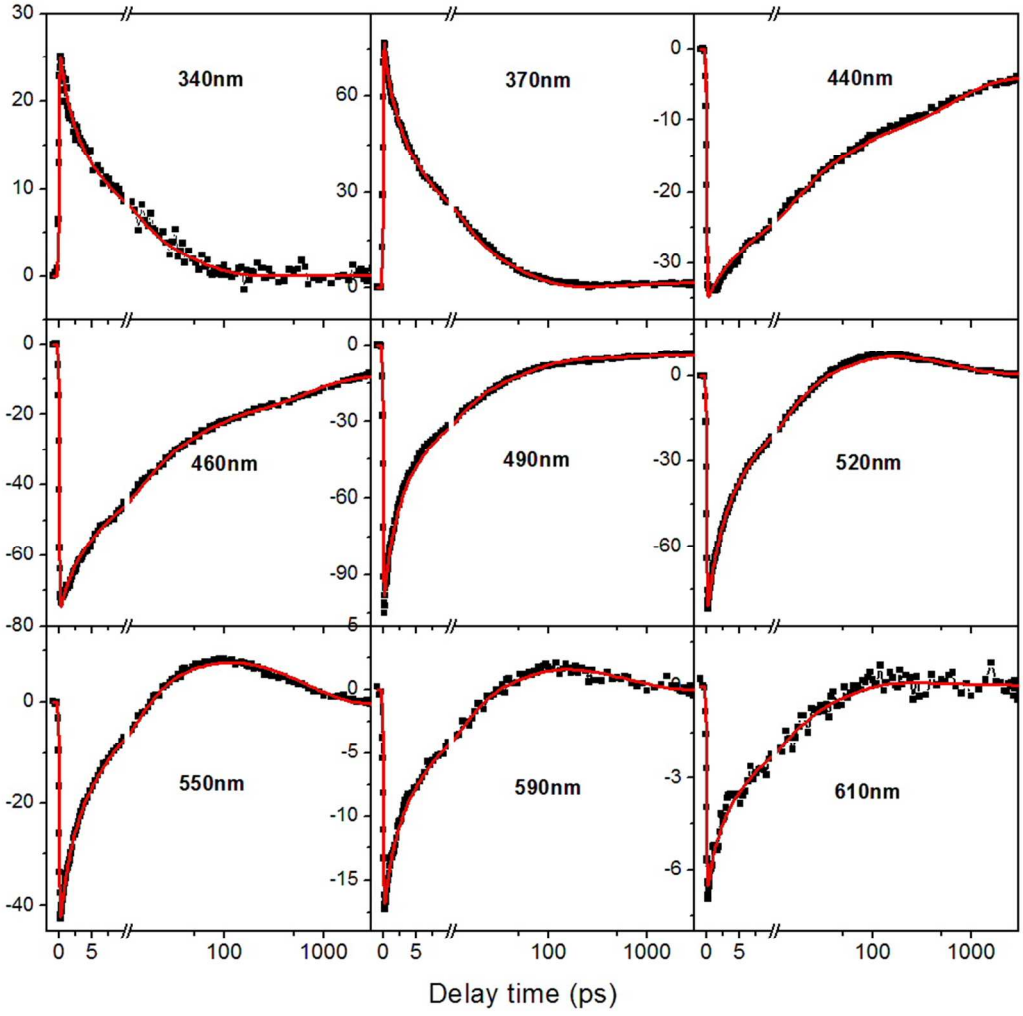

Figure S5: Time decay traces at representative wavelengths dpE46A data (black) and fitted (red) according to the target model as in Figure 7.

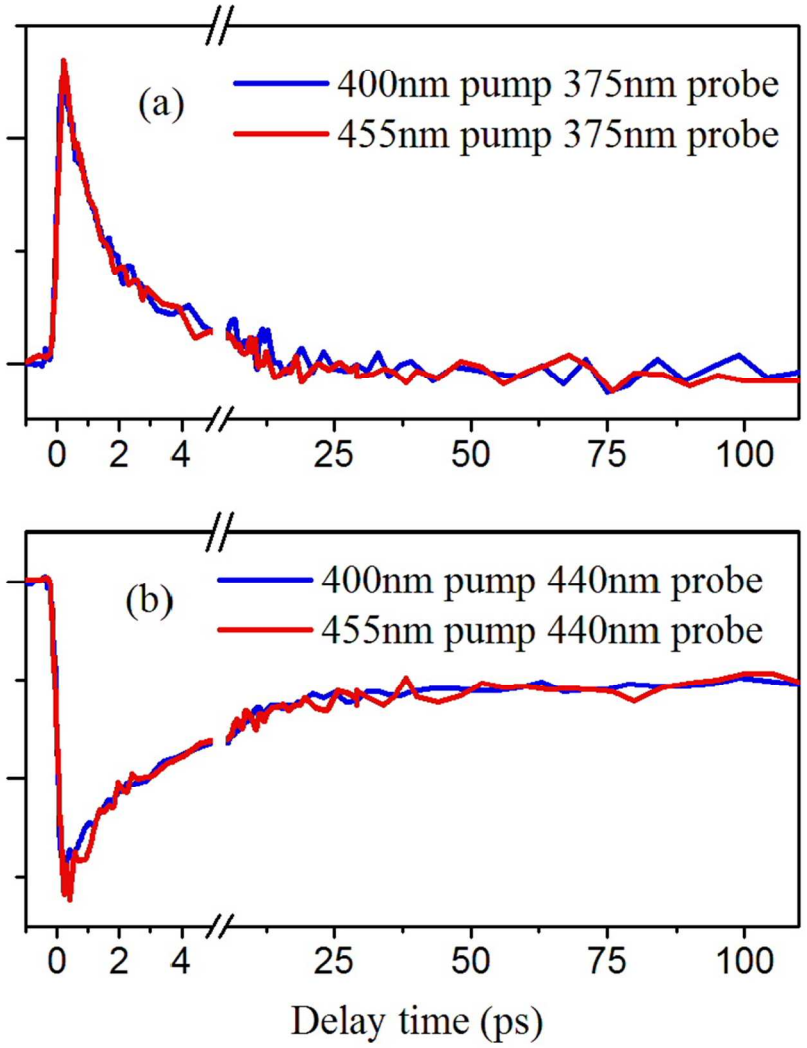

Figure S6: Excited decay dynamics at 375nm and bleach recover dynamics at 440nm for wtPYP excited with different wavelength at 400nm and 455nm respectively. For these two wavelength excitation, dynamics is the same, indicating that no significant ground or excited state heterogeneity exists in PYPs.

Estimation of quantum yield without target analysis

A rough estimate of the quantum yield of photoisomerization can be made from the transient spectra at 40 ps for wtPYP, 150 ps for dpE46A and 200 ps for pE46A, selected in figure 4 to show the I_0 product state at its respective maximum. If the amplitude of the ground state bleach can be well resolved, i.e. without a serious overlap by other bands, the amplitude at the peak can be viewed as the population of a photo-induced species, which has not decayed to the ground state. Thus, the bleach ratios of these maximum amplitudes over those at early delay time (0.2ps) can provide a rough estimate of the photoisomerization quantum yield. The bleach ratio at the two delay times of 0.2 ps and 40 ps in wtPYP is around $11/33 \approx 0.33$, which means that at the delay time of 40 ps, 33% of the initially excited state has converted to the product state. This value matches well to previously measured quantum yields of wtPYP [4-7]. Similarly, in dpE46A, the estimated quantum yield is $23/94 \approx 0.25$, consistent with the reported quantum yield of its pB product state on the millisecond time scale of 0.2 ± 0.1 [63]. For pE46A, as we lack the early-time bleach signal, a direct evaluation of the quantum yield is not possible. We can, however, use the magnitude of the stimulated emission band for this estimate, under the assumption that the maximum bleach and the stimulated emission signals have almost the same amplitude in pE46A. This assumption generally makes sense, since both of these two bands are induced by the transition from $S_0 \rightarrow S_1$, and the transition dipole moment μ_{01} is generally equal to μ_{10}. This assumption indeed holds for PYP samples, as can be seen from the early transient spectra of wtPYP and dpE46A, reported here and in other mutants [7]. However, as can be seen from figure 4(c), even the maximum peak value of the stimulated emission band in pE46A, located around 455 nm, is largely eliminated by the strong excited state absorption. Nevertheless, the peak value can be extracted by using the steady state emission information from figure 2 (b). In figure 2(b),

the emission amplitude ratio at 455 nm and at 475 nm is 5/4, thus the emission peak value at 455 nm in transient spectra would be around 1.25*5.9 ≈ 7.4. Based on this, the product quantum yield in pE46A was estimated to be 2.1/7.4 ≈ 0.28, more or less the same as in wtPYP.