Supplementary information

Global target analysis method

Global target 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 ΔA (λ, t) is that the variable delay time t and wavelength λ are independent. That is, the measured ΔA (λ, t) is a superposition of several species components and can be separated as:

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

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 ΔA (λ, 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:

$$\frac{dC_i(t)}{dt}=-k_iC_i(t)+\sum_{j\neq i}\chi_{ji}k_jC_j(t) \tag{2}$$

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:

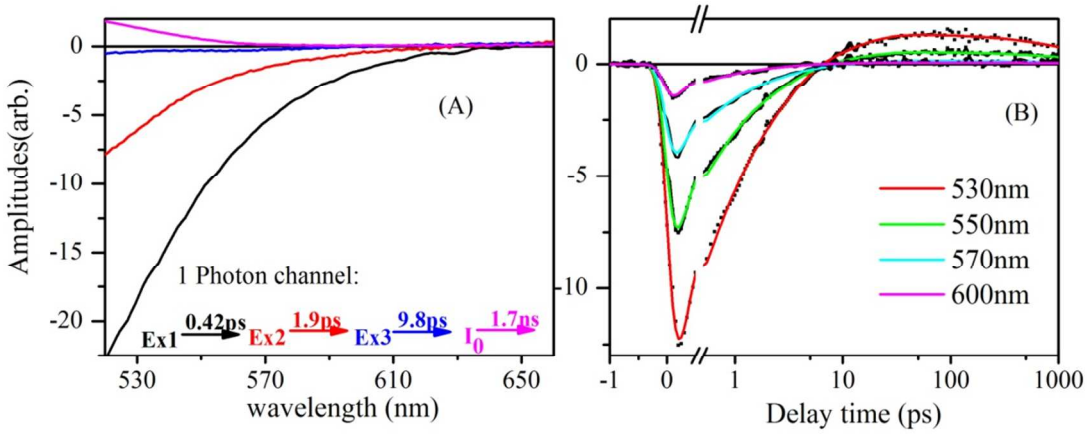
$$\sqrt{\left(\Delta A(\lambda,t)-\sum_{i=1}^{N}C_i(t)\Delta\varepsilon_i(\lambda)\right)^2} \tag{3}$$

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 to searching the minimum value of (3), a program developed by the author using computer language LabView.

Global fitted results of lower energy excitation data:

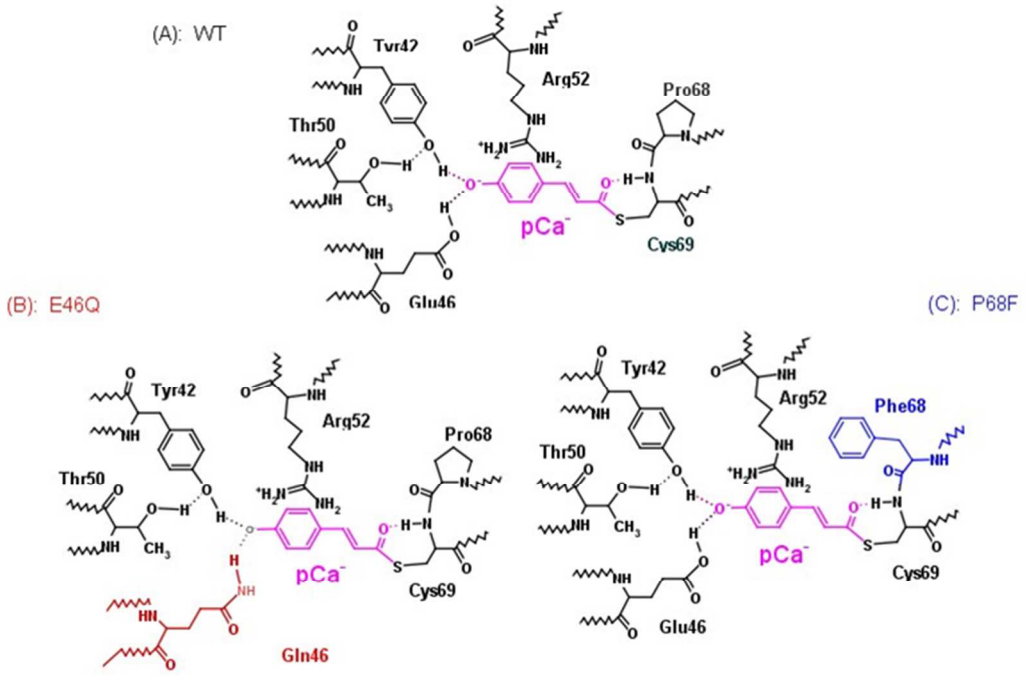
The successful separation of the one-photon channel and two-photon channel was performed by considering the consistence of the one photon excited state stimulation spectra and their life time constants with those observed in the visible region at lower excitation energy. Data with lower excitation intensity in the wavelength range from 520nm to 660nm were fitted to a sequential decay model of Ex1→Ex2→Ex3→I_0→, as shown in Figure S1 (A), where the Ex represent different stages of the one-photon excited state. At low excitation intensity, the small contribution of electron absorption can be ignored, and the obtained one-photon excited state lifetimes can be used as reference for the NIR window analysis at high excitation intensities. The fitted SADS in figure S1(A) perfectly represent excited state stimulated emission and I_0 absorption. The global fitted excited state life times were 0.42ps, 1.9ps, and 9.8ps, selected time decay traces and fits are shown in figure 4(B). It can be seen that the amplitude of the third SADS (9.8ps) of the one-photon excited state is only few percents of the other two, as shown in figure S1(A), especially in the wavelength region above 580nm. Therefore, this component can be ignored when a global model fitting with two reaction channels is performed for the higher intensity excitation data.



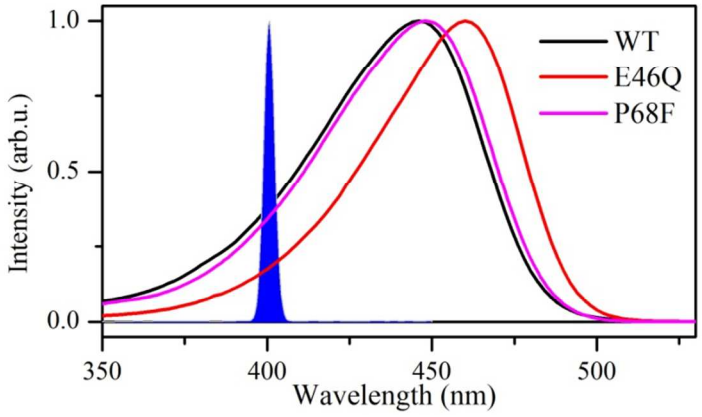
Supplementary figure 1: (A) SADS of the one-photon excitation channel extracted at low excitation intensity, ~0.4x10^{15}photons/cm^2, in the stimulated emission wavelength region. (B) representative time traces and the global fitted ones at different wavelengths.

WT and mutants PYP structures and ground state absorptions

Figure S2 shows the PYP chromophore structures at the active sites for wild type and mutants. In wild type PYP, the chromophore is buried inside the pocket, and at the active site, three hydrogen bonds are formed between the chromophore and the protein residue Tyr42, Glu46, and Pro68 respectively. In mutant E46Q, glutamic acid 46 residue is replaced by Glutamine, the hydrogen bond around this site became weaker, the absorption shifts to red by ~15nm, as shown in figure S3. In mutant P68F, the Proline 68 is replaced by a larger residue of Phenylalanine while the hydrogen bonds at the active sites has no change, the absorption has only a slight shift of ~2nm compare to wild type PYP (figure S3).



Supplementary figure 2: Schematic depiction of PYP structures at the active site. (A) wild type PYP; (B) mutant E46Q; (C) mutant P68F.



Supplementary figure 3: Steady state absorption of WT, E46Q and P68F. Blue is the excitation wavelength for transient measurements.