Supporting Information for "Excitation-Wavelength Dependent Photocycle Initiation Dynamics Resolve Heterogeneity in the Photoactive Yellow Protein from *Halorhodospira halophila*"

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The deactivation of a photoexcited pCA chromophore in PYP occurs via one of three general pathways: (1) photocycle initiation, (2) fluorescence and (3) non-radiative and non-photocycle decay. The quantum yields, the percent of excited protein molecules, which produce the measured parameter, for these processes are governed by the following relationship:

$$\Phi_f + \Phi_{ph} + \Phi_{nr} = 1$$

with Φ_f, Φ_{ph}, and Φ_{nr} representing the quantum yields of fluorescence, photocycle initiation and non-radiative decay, respectively. The effective (or apparent) excited-state rate constants observed directly from the experimental data can be decomposed into constituent microscopic time constants of the three pathways:

$$\mathrm{k}_{eff} = \mathrm{k}_f + \mathrm{k}_{ph} + \mathrm{k}_{nr}$$

with

$$\Phi_f = \frac{k_f}{k_f+k_{ph}+k_{nr}}, \Phi_{ph} = \frac{k_{ph}}{k_f+k_{ph}+k_{nr}}, \text{ and } \Phi_{nr} = \frac{k_{nr}}{k_f+k_{ph}+k_{nr}}$$

for the fluorescence, photocycle initiation and non-radiative pathway respectively. Photoisomerization reactions often exhibit a rough correlation between faster decay timescales ($1/\mathrm{k}_{eff}$) and higher Φ_{ph} values. For example, rhodopsin exhibits exceptionally rapid decay and high Φ_{ph} (τ =200 fs, Φ_{ph} = 70%)[1], while PYP (τ =2 ps, Φ_{Ph} = 33%)[2] and the forward reaction of the Cph1 phytochrome ($P_r \rightarrow P_{fr}$, τ =10 ps, Φ_{ph} = 16%)[3] are slower and less efficient. The loose relationship between decay rates and quantum yield was also observed in the set of 20 E46X mutants of PYP by Hoff and coworkers.[4] Although increased Φ_{ph} is often accompanied by a decreased Φ_F, the presence of the non-radiative channel, Φ_{nr}, precludes a simple one-to-one correspondence between Φ_f and Φ_{ph}. Hence, it is not uncommon for systems that exhibit near identical quenching kinetics to exhibit different Φ_{ph} values.[5, 6] For example, El-Sayed and co-workers demonstrated for bacteriorhodopsin that a 20-fold variation in reaction rate did not affect Φ_{ph}.[7]

Table S1: Known Photoactive studies on Hhal PYP WT organized by excitation wavelength, and depicted graphically in Figure 2.

Excitation Wavelengths (nm)				Study Type*	Year	Corresponding Author
290	447			fsPP	2011	Delmar S. Larsen[8]
310	318	395		pCA	2005	Mikas Vengris[9]
335	365	446		pCA	2013	Delmar S. Larsen[10]
355	446	460	472	LIOAS	1995	K.J. Hellingwerf[2]
355	430			pCA	2006	Pascale Changenet-Barret[11]
390				fsPP	2001	Yasushi Imamoto[12]
390				Crys	2012	Philip A. Anfinrud[13]
390	400			Crys	2013	Hyotcherl Ihee[14]
395	460			fsPP	1999	G. Tolllin[15]
400	485			fsPP	2002	R. van Grondelle[16]
400				fsFL	2004	Delmar S. Larsen[17]
400				fsPP	2004	Delmar S. Larsen[18]
400				fsFL	2006	I.H.M. van Stokkum[19]
400				fsFL	2007	Ryosuke Nakamura[20]
400				fsFL	2007	Ryosuke Nakamura[21]
400	415			fsFL	2008	Ryosuke Nakamura[6]
400				fsPP	2011	Alisa B. Rupenyan[22]
400				fsPP	2013	Takayoshi Kobayashi[23]
400				fsPP	2013	Marie Louise Groot[24]
408	490			Crys	2008	Ulrich K. Genick[25]
410				fsFL	1998	Fumio Tokunaga[26]
410				psFL	2000	Fumio Tokunaga[27]
410				fsFL	2001	H. Hanada[28]
413				fsIR,FL	2004	Noboru Mataga[29]
429	450			Temp	1996	Fumio Tokunaga[30]
430	450			Temp	2001	Yasushi Imamoto[31]
431	475			Temp	2000	Mostafa A. El-Sayed[32]
435				CD	2005	Maarten P. Heyn[33]
435	475			fsPP	2017	*This Work*
440				fsFL	1997	Fumio Tokunaga[34]
440				fsFL	2002	Noboru Mataga[35]
440				fsFL	2003	Noboru Mataga[36]
440				fsPP	2016	Delmar S. Larsen[37]
441				Temp	1992	K.J. Hellingwerf[38]
445				fsIR	2005	Michael A. Cusanovich[39]
450				Crys	2014	Marius Schmidt[40]
450				Crys	2016	Marius Schmidt[41]
452				fsPP	1998	G.H. Atkinson[42]
455				fsPP	2015	Marie Louise Groot[43]
460				fsIR	2012	Ryosuke Nakamura[44]
475				fsIR	2003	Marie Louise Groot[45]
475				fsIR	2006	Marie Louise Groot[46]

485	Crys	2005	Hyotcherl Ihee[47]
485	Crys	2013	Marius Schmidt[48]
496	Crys	1997	Elizabeth D. Getzoff[49]

*Study Type Abbreviations are: CD – Circular Dichroism; Crys – Crystallography; fsFL – Femtosecond Fluorescence; fsIR – Femtosecond Infrared including Fourier Transform, Raman and Absorption; fsPP – Femtosecond Visible Pump Probe; LIOAS – Laser-Induced Optoacoustic Spectroscopy; pCA – Multiple techniques applied to only the pCA chromophore without the PYP protein matrix.

Table S2: Global Analysis Parameters of the DEWI datasets. These are optimized by target analysis to the model in Figure S1B for Hhal PYP after 435-nm and 475-nm excitation.

State	pG* 1_V	pG* 1_R	pG* 2_V	pG* 2_R	GSI 1	GSI 2	I_0	pR
Heterogeneous Vibrational Model								
Population (%)	**79**		**21**					
Lifetime (ps)	1.1	5.1	1.4	36	4.5	52	1200	∞
Branching	43 (GSI 1)	60 (GSI 1)	60 (GSI 1)	60 (GSI 1)	4.5 (GSI 2)	100 (pG)	73 (pR)	
Yields (%)	29 (I_0)	40 (I_0)	0 (I_0)				27 (pG)	
	28 (pG* $1_{R)}$		40 (pG* 2_R)					
pR Yield (%)	16	7	0	0			Total -	23

Relative error for each parameter is 10%.

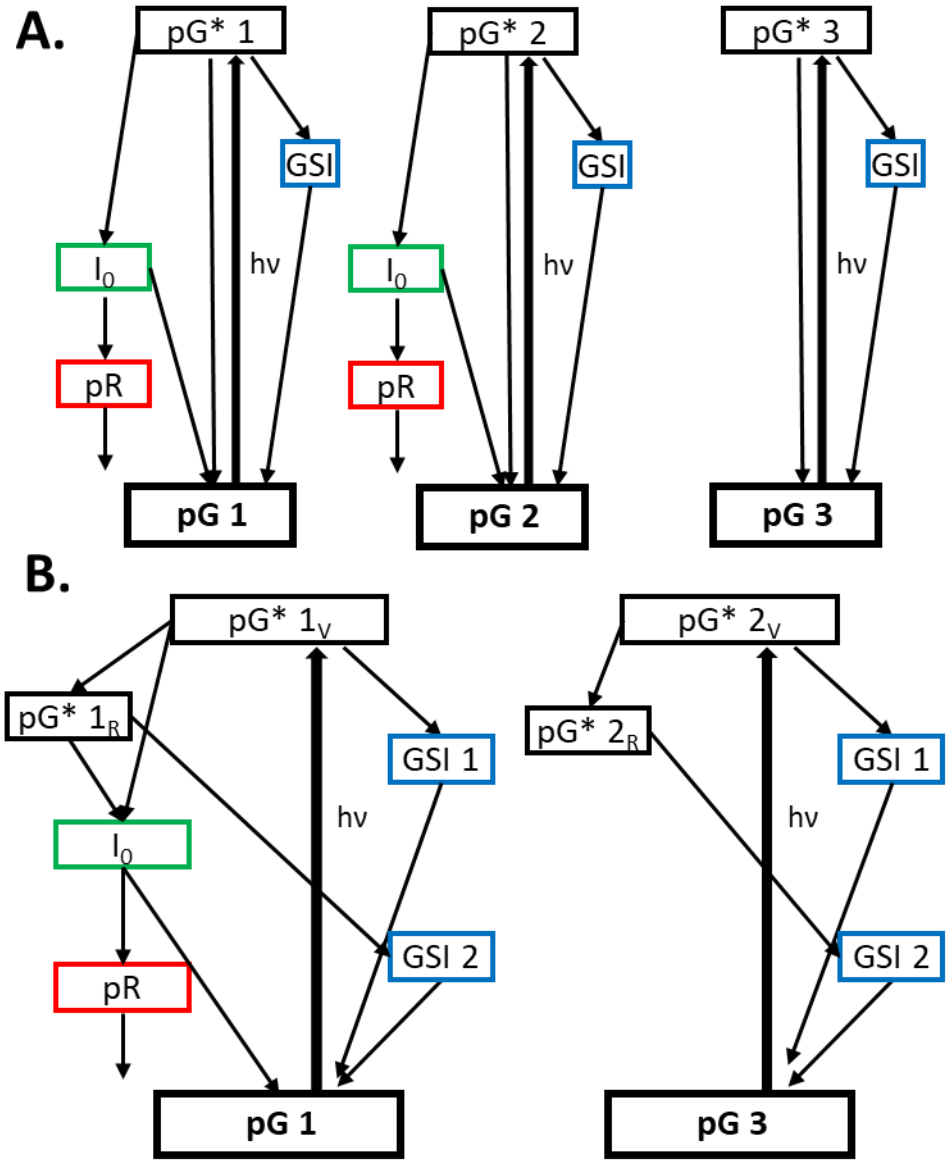
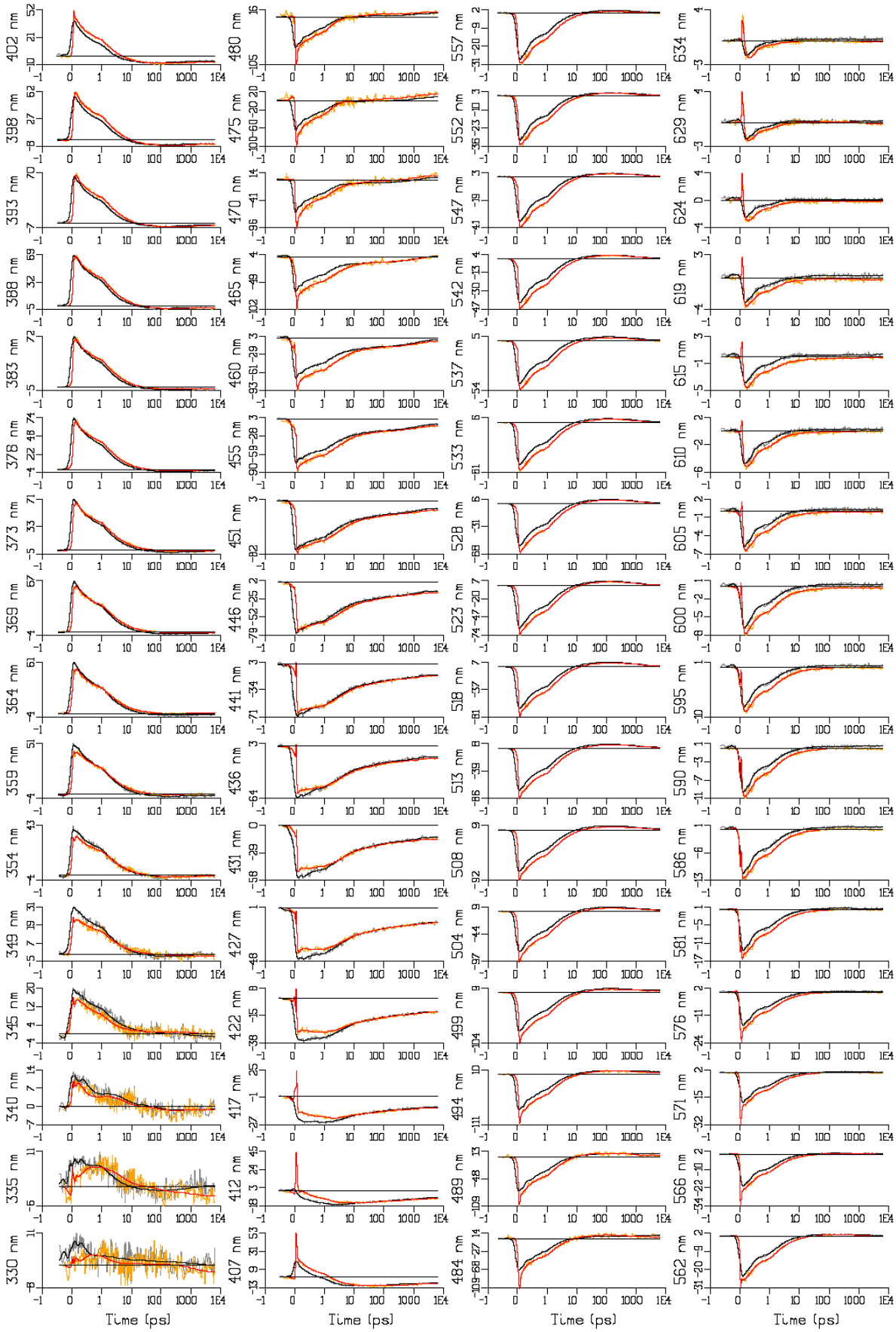


Figure S1: Three state heterogeneous global analysis model in panel A and two state vibrational relaxation model in panel B. Transient absorption dynamics from each excitation wavelength were fit using the same model with all the differences evident in the SADS spectra.

Figure S2 (next page). Transient DEWI-PP data (in units of mOD) of PYP at 64 selected wavelengths with fits from the three state heterogeneous model (Figure 7A). Key: 435 nm excitation (grey), 475 nm excitation (orange). Black and red lines indicate the simultaneous target analysis fit. Note that the time axis is linear until 1 ps and logarithmic thereafter. Note also that each panel is scaled to its maximum.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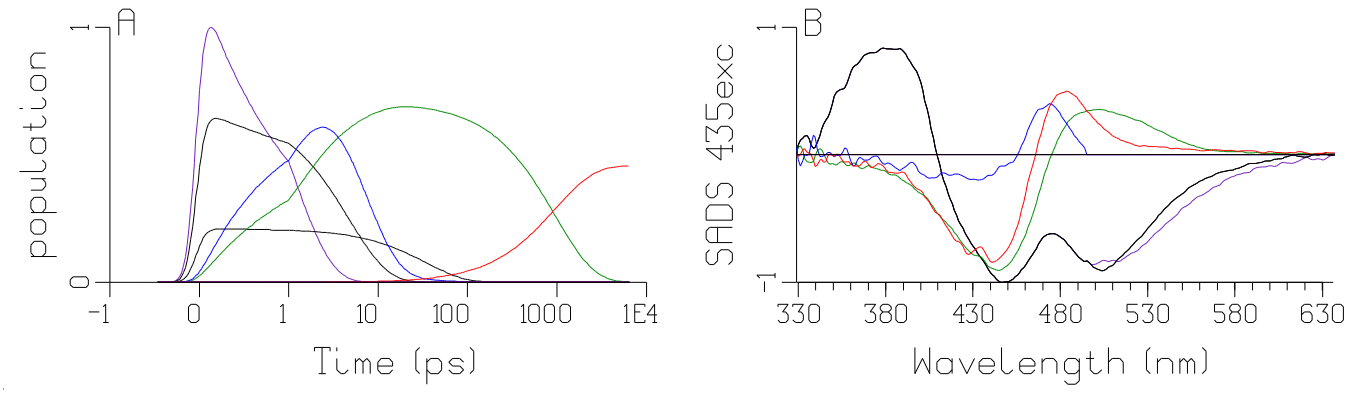


Figure S3A: 435-nm excitation target analysis results (A) Populations of heterogeneous model with three excited states (Figure S1A); SADs for the corresponding populations (B) Key: pG*1,2 (purple and black), GSI (blue), I_0 (dark green), pR (red). The pG*3 and pG*2 SADS are identical.

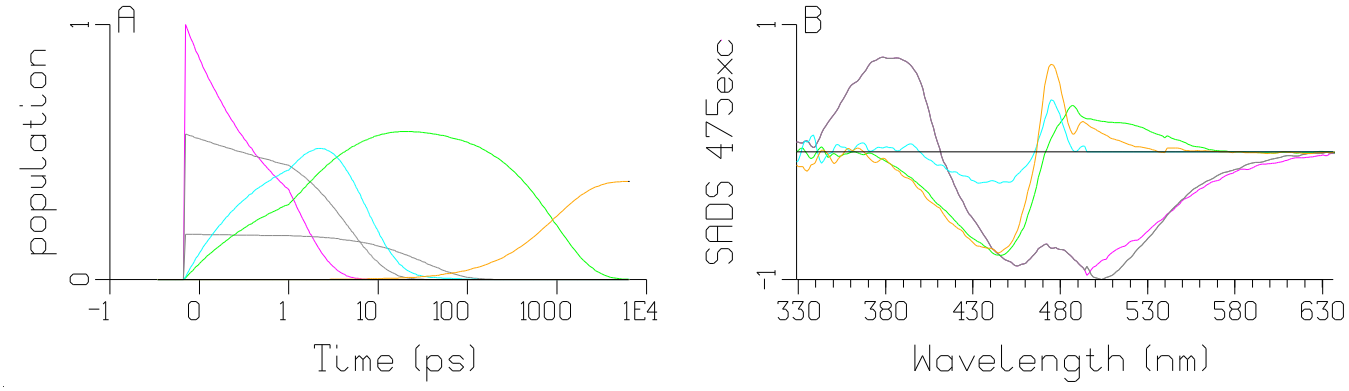


Figure S3B: 475-nm excitation target analysis results (A) Populations of heterogeneous model with three excited states branch; SADs for the corresponding populations (B) Key: pG*1,2 (magenta and grey), GSI (cyan), I0 (green), pR (red). The pG*3 and pG*2 SADS are identical.

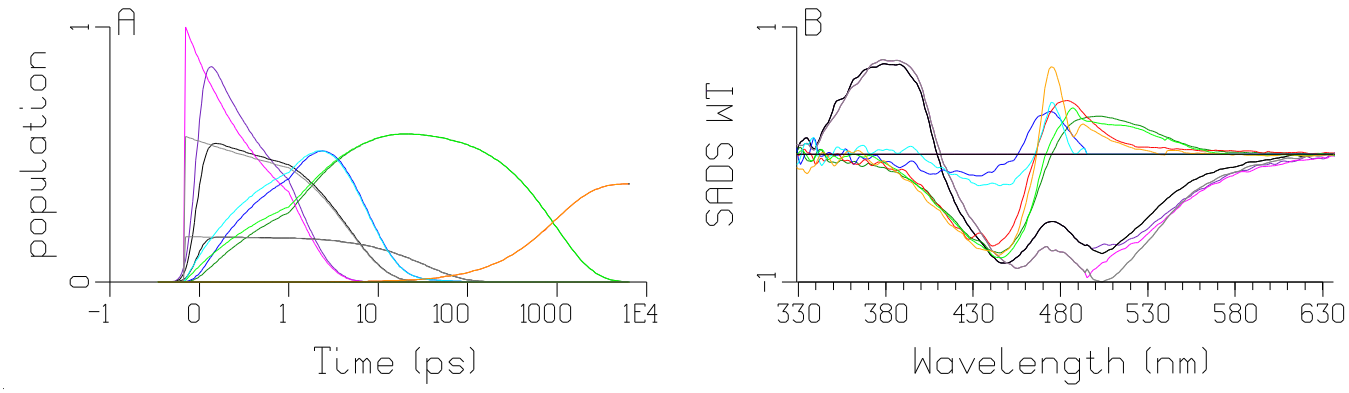
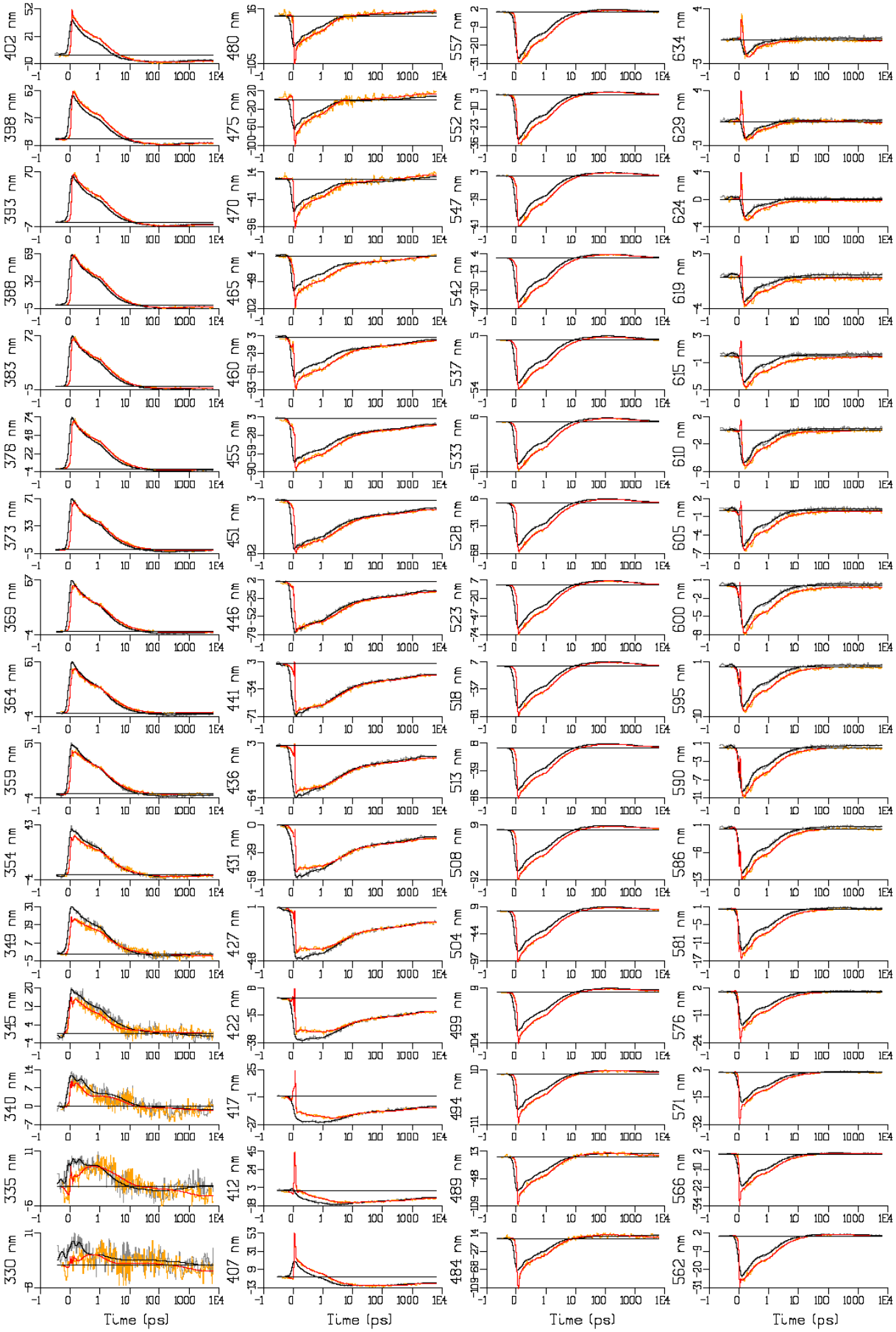


Figure S3C: Overlay of 435 and 475 nm excitation target analysis results of heterogeneous model with three excited states. Note that with the common kinetics the SADS estimated with 435 and 475 nm excitation are comparable, thus indicating that the yields are practically identical. There are some differences in the pG* SADS above 400 nm: the isosbestic point is shifted to the red by ≈2 nm due to photoselection, the relative amplitude of the SE band is larger with 475 nm excitation, although the 380 nm ESA band is nearly identical.

Figure S4 (next page). Transient DEWI-PP data (in units of mOD) of PYP at 64 selected wavelengths with the two state heterogeneous vibrational model (Figure 7B). Key: 435 nm excitation (grey), 475 nm excitation (orange). Black and red lines indicate the simultaneous target analysis fit. Note that the time axis is linear until 1 ps and logarithmic thereafter. Note also that each panel is scaled to its maximum.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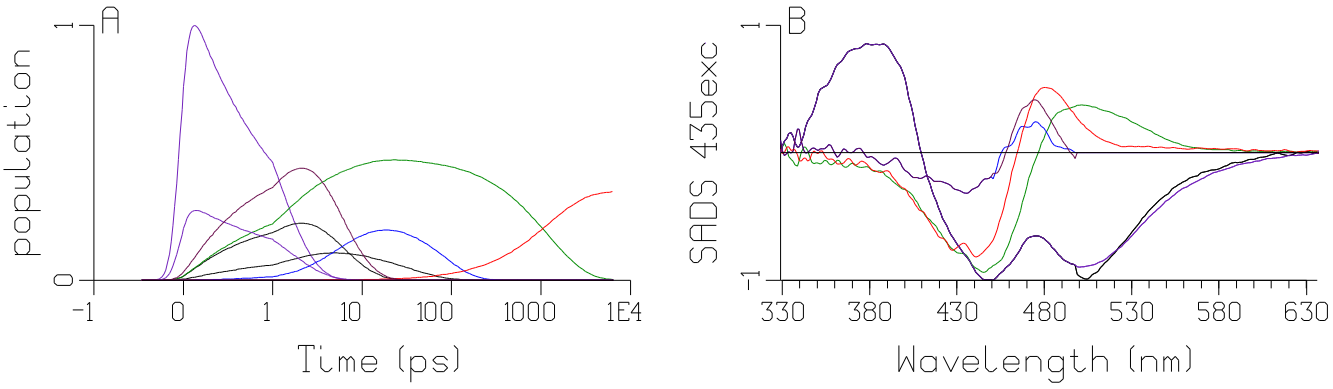

Figure S5A: 435 nm excitation target analysis results (A) Populations of heterogeneous model with vibrational relaxation (Figure 7B); SADs for the corresponding populations (B) Key: pG* 1_V, 2_V (purple and black), pG* 1_R, 2_R (light purple and grey), GSI 1,2 (maroon, blue), I_0 (dark green), pR (red)

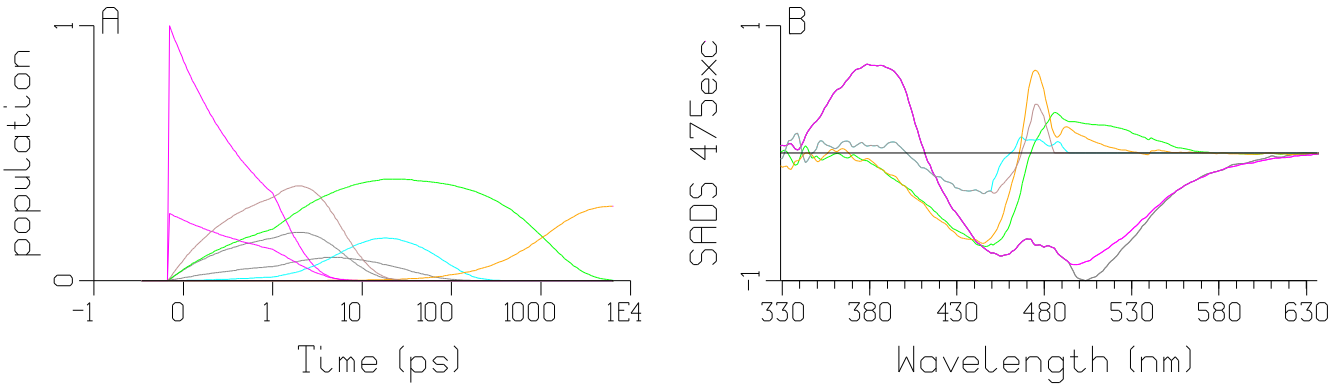

Figure S5B: 475 nm excitation target analysis results (A) Populations of heterogeneous model with two excited states in each branch; SADs for the corresponding populations (B) Key: pG* 1_V, 2_V (magenta and grey), pG* 1_R, 2_R (light magenta and light grey, 20%), GSI 1,2 (brown, cyan), I_0 (green), pR (orange)

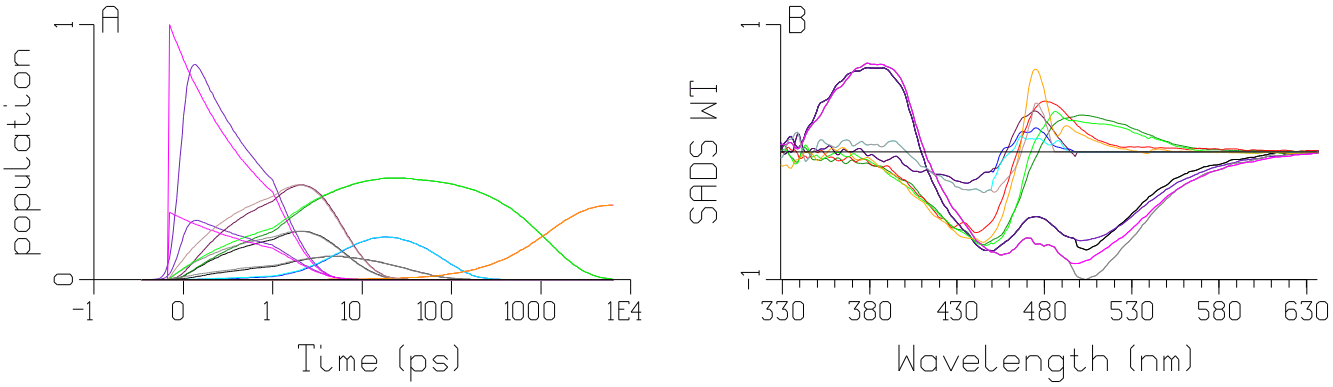

Figure S5C: Overlay of 435 and 475 nm excitation target analysis results. Note that with the common kinetics the SADS estimated with 435 and 475 nm excitation are comparable indicating that the yields are practically identical. There are some differences in the ESI SADS above 400 nm: the isosbestic point is shifted to the red by ≈2 nm due to photoselection, the relative amplitude of the SE band is larger with 475 nm excitation, although the 380 nm ESA band is almost identical.

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