

Supplementary Information

Figure S1. Evolution-associated difference spectra (EADS) estimated from global analysis of the data. The decay times are shown in the figure legends.

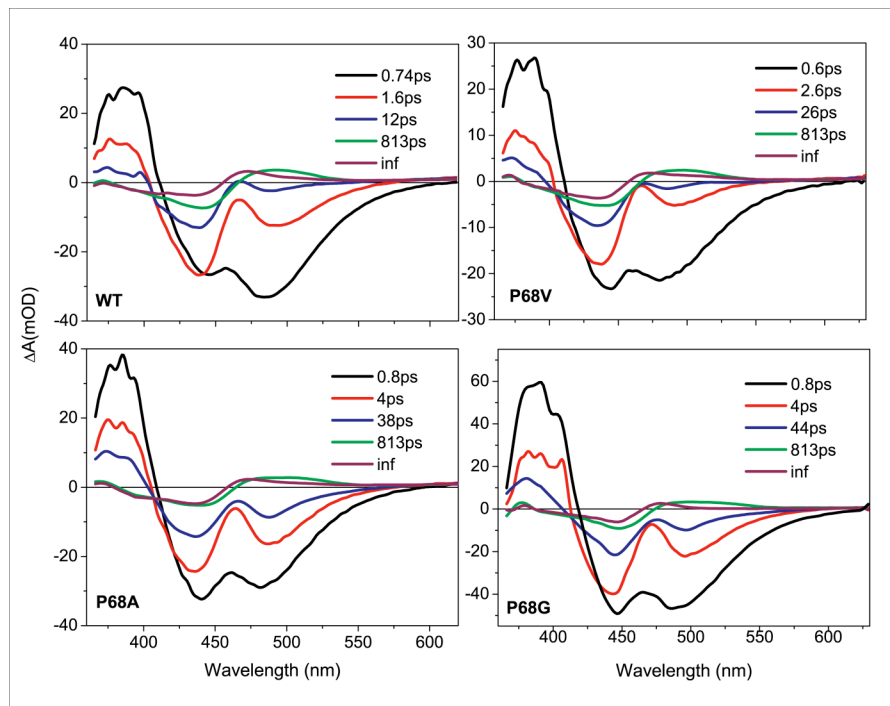


Figure S2. Time traces of the WT and the mutants together with the fit resulting from the target model depicted in the upper panel of Figure 3.

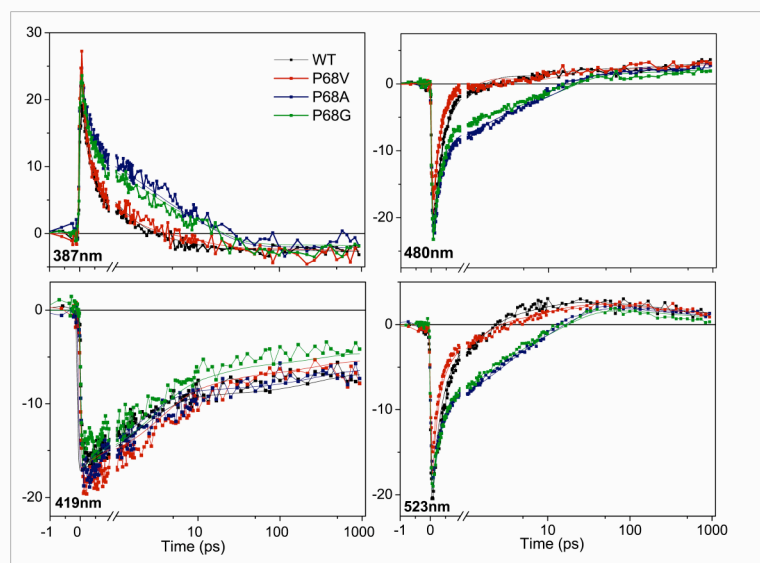
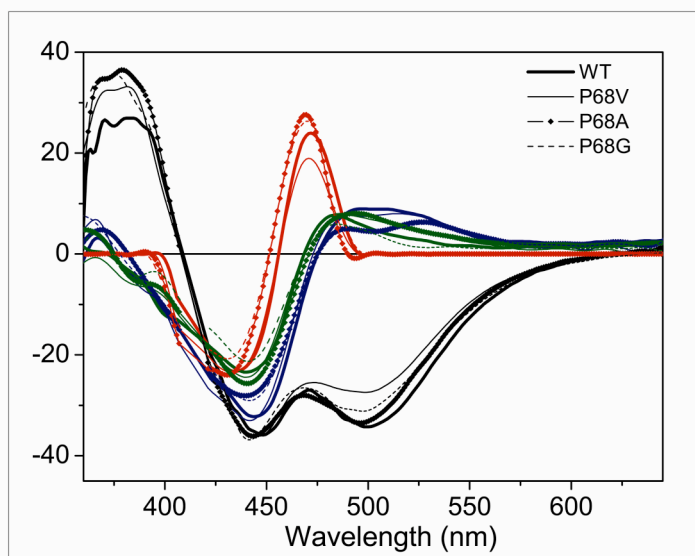


Figure S3. A) SADS derived from simultaneous target analysis of the four datasets without a constraint of equality of the spectra of the four samples. The color coding is: black – ES, red – GSI, blue – I₀, green - I₁.

B) Time traces together with the fit resulting from simultaneous target analysis of the four datasets without a constraint of equality of the spectra of the four samples

A)



B.

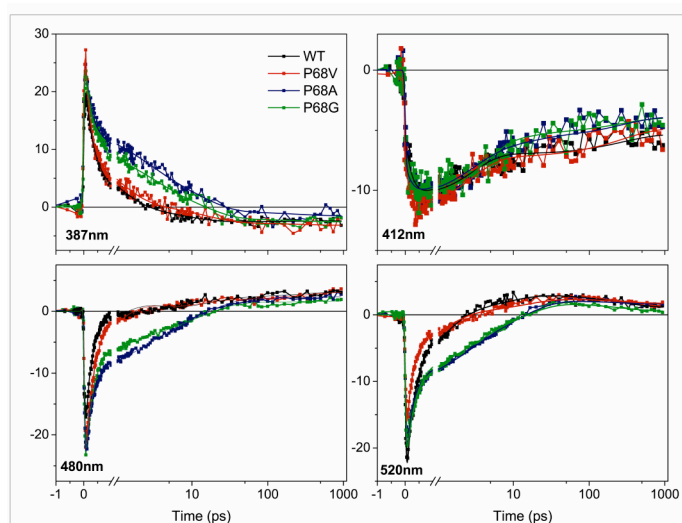


Figure S4. Absorption of WT, P68V, P68A, and P68G in the pG state.

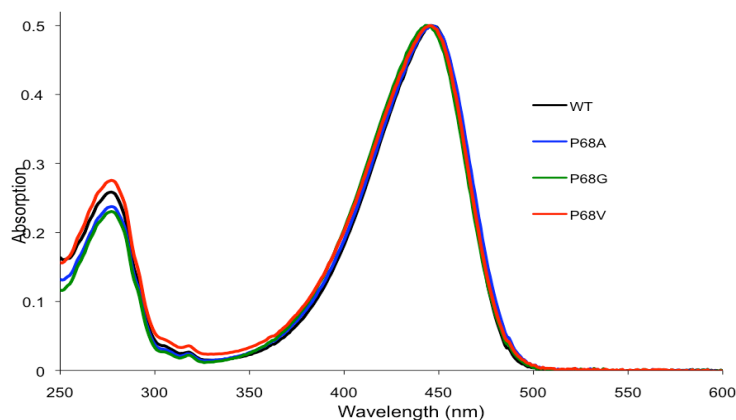


Table S1. Fractions of initial population and accompanying rate constants for all transitions in ps^{-1} (relative error 10%)

	Initial population			$k_{I_0} : S_1 \rightarrow I_0$ (1/ps)			$k_{GSI} : S_1 \rightarrow GSI$ (1/ps)			$k_{S_0} : S_1 \rightarrow S_0$ (1/ps)			k_{GS}	k_{I1}
	ES1	ES2	ES3	1	2	3	1	2	3	1	2	3	GSI	I_0
WT	50%	40%	10%	0.7	0.09	0.02	0.55	0.08	0.01	0.2	0.18	0.02	0.15	0.0012
P68V	55%	35%	10%	0.8	0.1	0.019	0.9	0.1	0.0005	0.4	0.19	0.0111	0.15	0.0012
P68A	50%	25%	25%	0.65	0.02	0.011	0.52	0.116	0.019	0.45	0.05	0.001	0.15	0.0013
P68G	55%	23%	22%	0.4	0.014	0.007	0.56	0.074	0.014	0.35	0.07	0.0006	0.15	0.0012

Table S2. Lifetimes of all the intermediates in ps

	ES1	ES2	ES3	I_0	GSI	I_1
WT	0.7 (50%)	2.8 (40%)	20 (10%)	840	6.6	inf
P68V	0.5 (55%)	2.5 (35%)	32 (10%)	833	6.6	inf
P68A	0.6 (50%)	5.9 (25%)	40 (25%)	769	6.6	inf
P68G	0.75(55%)	6.2 (23%)	44 (22%)	840	6.6	inf

Table S3. Recovery rate constants (k_1 , k_2), fraction of the predominant rate constant k_1 (frk_1) of the four proteins, and standart deviations estimated from three measurements

	k₁	k₂	frk₁	stdev_k₁	stdev_k₂
WT	3.20	0.24	0.92	0.04	0.03
P68V	2.37	0.69	0.49	0.13	0.02
P68A	2.86	0.55	0.95	0.05	0.14
P68G	0.99	0.23	0.95	0.003	0.03