Supporting Information for:

Light Activated Reassembly of Split GFP

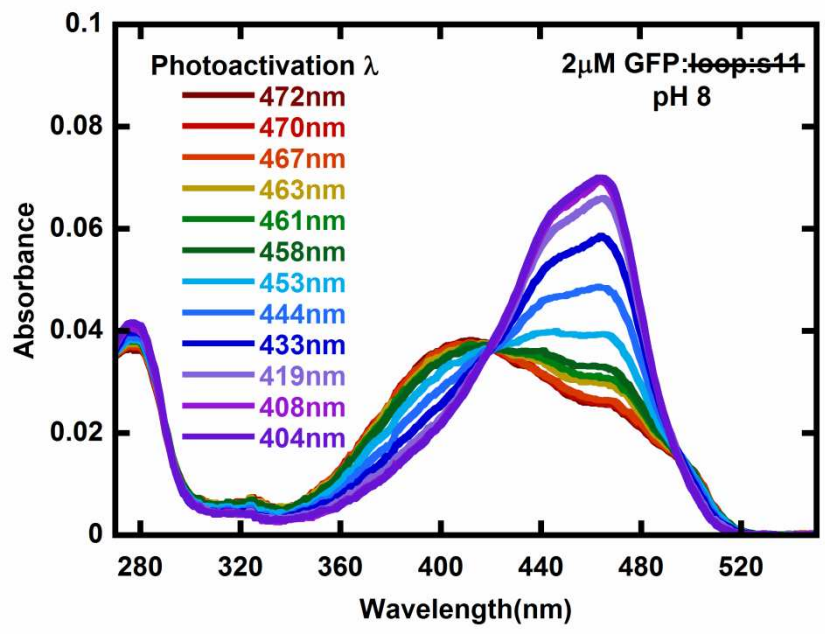
Kevin P. Kent and Steven G. Boxer*

Department of Chemistry, Stanford University, Stanford, California, 94305-5080

Contents:

Wavelength Dependence of Photostationary States

As shown in Supplementary Figure 1 there is a wavelength dependence to the ratios of concentrations in the photostationary states of *cis* and *trans* GFP:~~loop:s11~~. When excited at longer wavelengths (~460 nm), *trans* GFP:~~loop:s11~~ absorbs most of the light, so the spectrum shifts towards nearly all *cis* GFP:~~loop:s11~~ because the light activated rate of *trans* to *cis* isomerization has been increased. Conversely, at shorter wavelengths (400 nm) *cis* GFP:~~loop:s11~~ is absorbing most of the light so the spectrum shifts towards nearly all *trans* GFP:~~loop:s11~~.



Supplementary Figure 1. Absorbance spectra of the photostationary state of refolded GFP:~~loop:s11~~ light activated using different wavelengths. The spectra were obtained by illuminating *trans* GFP:~~loop:s11~~ with low intensity light (2mW), and taking absorbance spectra until the spectrum stopped changing in time, i.e. when the photostationary state was reached. Increasing the intensity of light to 10mW did not change the spectra, but does accelerate the rate at which the photostationary state is reached.

Quantum Yields of Photoisomerization

The quantum yields of photoisomerization determine the light activated rates of photoisomerization. The following equations describe a quantitative model to fit the observed light activated rates and obtain the quantum yields of *cis* to *trans* and *trans* to *cis* photoisomerization. The model was derived starting from a previous simple model used to determine reaction quantum efficiencies in systems with multicomponent inner filter absorbances[1f].

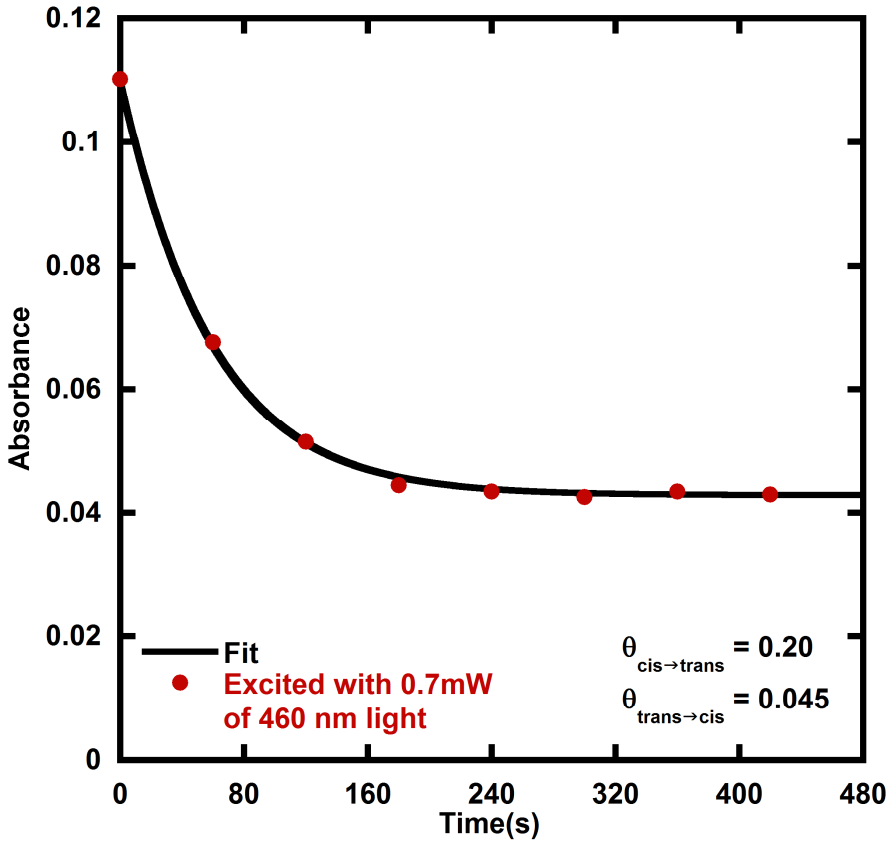
$$\frac{d[trans]}{dt} = \frac{P_0 * \left(1 - 10^{-A_t}\right) * \left(-\frac{\varepsilon_{trans} * l * [trans]}{A_t} * \varphi_{trans \rightarrow cis} + \frac{\varepsilon_{cis} * l * [cis]}{A_t} * \varphi_{cis \rightarrow trans}\right)}{V} \quad \text{S1}$$

$$\frac{d[cis]}{dt} = -\frac{d[trans]}{dt} \quad \text{S2}$$

Where P_o is the power measured before the beam hits the sample, A_t is the total measured absorbance, ε is the molar extinction coefficient of the indicated species, l is the path length of the beam through the sample, φ indicates the quantum yield of the indicated process, and V is the volume of solution. The term $P_o * (1 - 10^{-A_t})$ is the number of photons absorbed per time, the terms $\frac{\varepsilon_{trans} * l * [trans]}{A_t}$ and $\frac{\varepsilon_{cis} * l * [cis]}{A_t}$ give the fraction of photons absorbed by each species, and the quantum yields convert from photon absorbed by each species to photoisomerization of each species per time. Finally dividing the number of *trans* species depleted (first term in parentheses) or created (second term in parentheses) per time by the volume gives the changes in *trans* concentration per time. The change in *cis*

concentration per time is just the negative of the change in *trans* concentration per time. Equation 1 in the text can be derived by setting $\frac{d[trans]}{dt}$ to zero in equation S1 (as it should be at the photostationary state).

All of the variables in Equation S1 are either obtained from Figure 4 (ε_{trans} and ε_{cis}), measured directly (P_0, A_t, l, and V), or determined by using both (the concentrations) except for the quantum yields. The light driven kinetics experiment in Supplementary Figure 2 was achieved by directing a 0.7 mW of a 460 nm laser beam from above through a 3.2 mL solution of 2.2 μM *trans* GFP:~~loop:s11~~ (the path length was 3.2 cm). Absorbance measurements were taken every 60 seconds by blocking the laser for ~3 seconds and measuring the 1 cm path length absorbance at 460 nm. The fit was obtained by using equations S1 and S2 to numerically predict the changes in *trans* and *cis* concentration (0.01s intervals), then the extinction coefficients were used to predict the changes in 1 cm path length absorbance (8900 $M^{-1}cm^{-1}$ for *cis* and 49000 $M^{-1}cm^{-1}$ for *trans*). In Equation S1 the extinction coefficient of 3700 $M^{-1}cm^{-1}$ was used because only excitation of the protonated *cis* state leads to photoisomerization to the *trans* state (see text and the Absorbance Spectra of Photostationary States in PVA below).

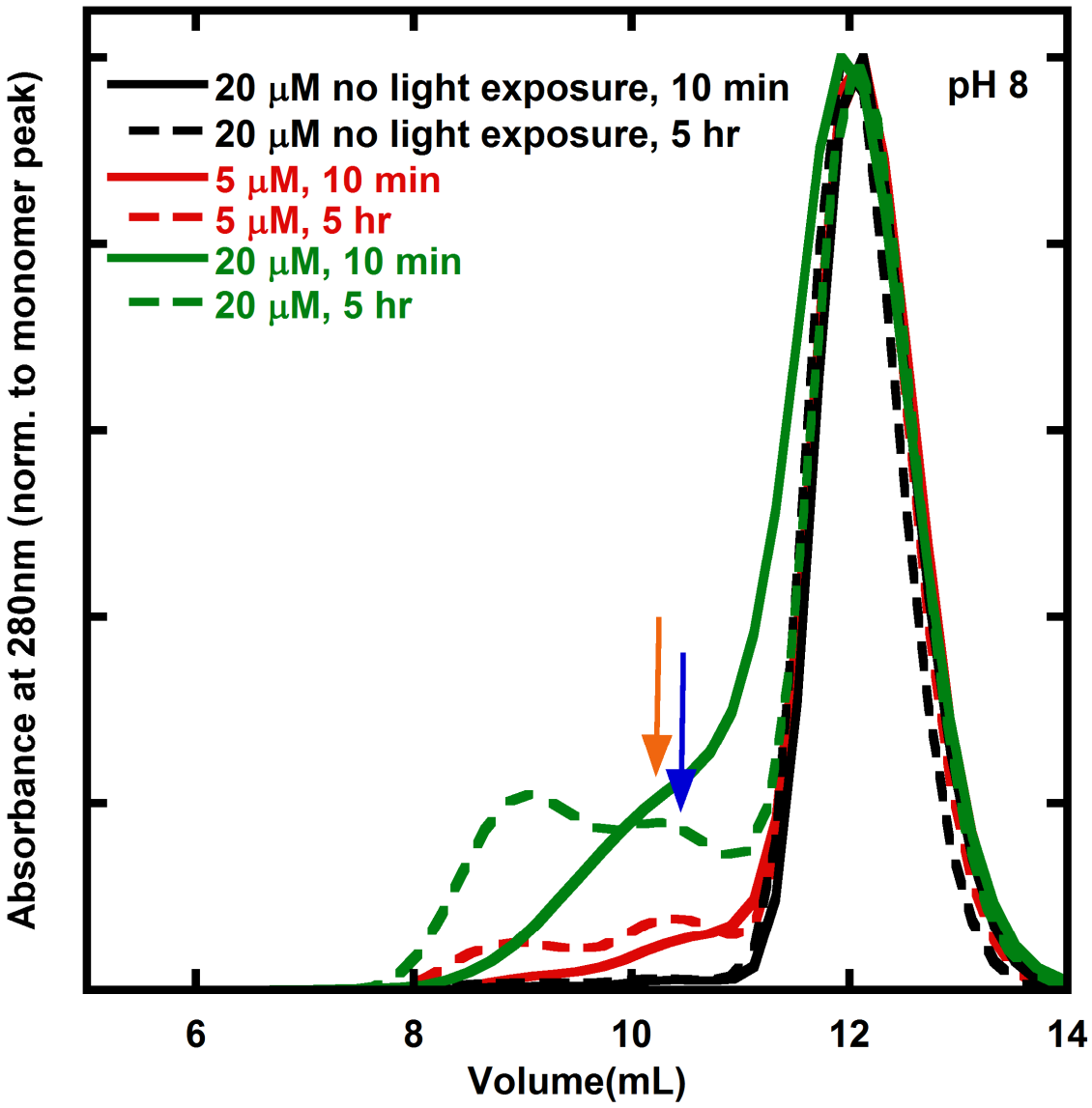


Supplementary Figure 2. The absorbance change at 460 nm when 2.2 μM *trans* GFP:~~loop:s11~~ is irradiated with 460 nm light (0.7 mW). The red dots show the 1 cm path length absorbance, which was measure every 60 seconds, and the black line is the fit obtained from equations S1 and S2. The absorbance stops changing at about 240 s when the sample has reached the photostationary state, which according to equation 1 in the text defines the ratio of quantum yields. The initial decay of absorbance to the absorbance at the photostationary state gives information about the magnitude of the quantum yields.

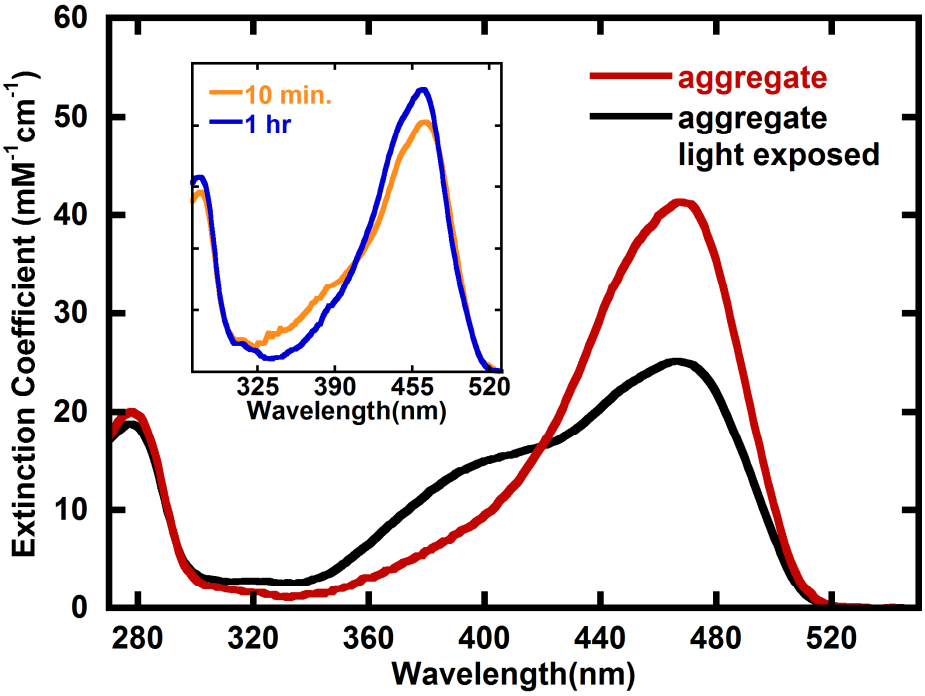
Aggregation of *cis* GFP:~~loop:s11~~

Upon light activation of *trans* GFP:~~loop:s11~~, a photostationary mixture of *cis* and *trans* GFP:~~loop:s11~~ is obtained (see text and Supplementary Figure 1 above). The stable *trans* form of GFP:~~loop:s11~~ appears to remain a monomer up to very high concentrations (1mM) based on size exclusion chromatography; however, after light activation there is a concentration dependent formation of larger aggregates, presumably due to aggregation of *cis* GFP:~~loop:s11~~ (Supplementary Figure 3). The

elution volume of the aggregates in Supplementary Figure 3 is consistent with dimers, and trimers of GFP:~~loop:s11~~, based on comparisons to proteins of similar molecular weight run on the same size exclusion column (Superdex 75 10/300 GL). The spectra of the aggregates show changes similar to those observed when the photostationary state is thermally relaxing back to all *trans* GFP:~~loop:s11~~ (inset of Supplementary Figure 4). After leaving the samples at 4°C overnight the spectra are very similar to that of *trans* GFP:~~loop:s11~~, and after light activation with 460 nm light what appears to be a photostationary state forms, very similar to the behavior of the monomers (Supplementary Figure 4). The aggregation was taken into account in the kinetic fits shown in Figure 5 of the text. The aggregation was modelled as only dimerization with a bimolecular rate constant. The aggregates were not taken into account in the kinetic fits shown in Figure 6 of the text because the fits did not change appreciably upon addition of the aggregate spectra to the fitting (see Quality of Absorbance Spectra Fits section below), presumably due to the difference in timescales at low micromolar concentrations of truncated GFP.



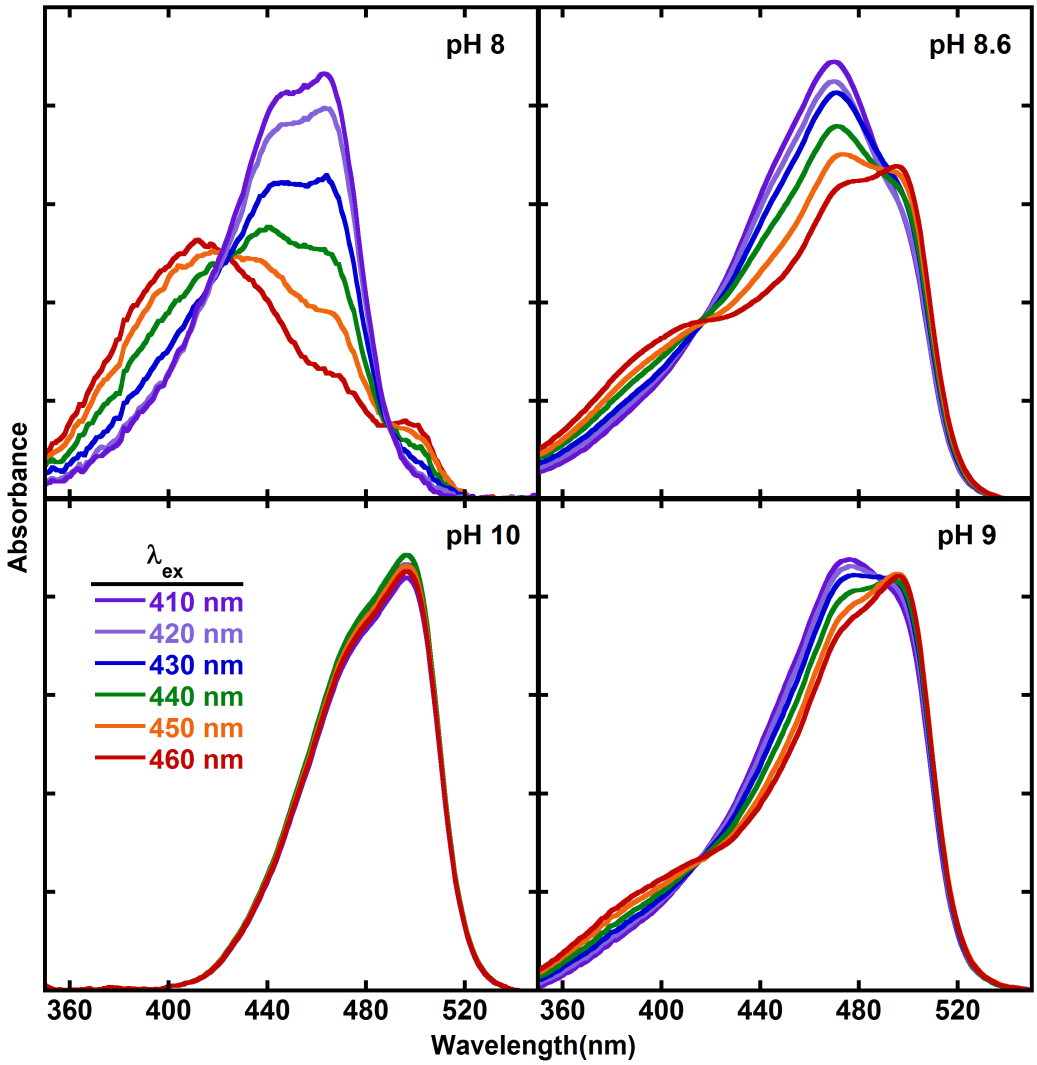
Supplementary Figure 3. Size exclusion absorbance chromatograms of *trans* GFP:~~loop:s11~~ with and without light exposure to 460 nm light at variable concentrations. The black traces are *trans* GFP:~~loop:s11~~ held at room temperature in the dark for 10 minutes (solid black line) and 5 hours (dashed black line) at a protein concnetration of 20μM in pH 8 buffer. The red and green traces show the photostationary state of GFP:~~loop:s11~~ after being held at room temperature for 10 minutes (solid lines) and 5 hours (dotted lines) at protein concentrations of 5 μM (red traces) and 20 μM (green traces) in pH 8 buffer. The *trans* GFP:~~loop:s11~~ remains a monomer throughout the incubation in the dark, but upon light activation with 460nm light there is a concentration dependent aggregation.



Supplementary Figure 4. Absorbance spectra of aggregates isolated from the size exclusion chromatorgraphy shown in Supplementary Figure 2. In the inset the orange spectrum corresponds to aggregate isolated from the elution volume indicated by the orange arrow on Suppelementary Figure 3 which was run on size exclusion 10 minutes after generation of the photostationary state. The blue spectrum corresponds to aggregate isolated from the elution volume indicated by the blue arrow on Supplementary Figure 3 which was run on size exclusion 1 hour after generation of the photostationary state. It appears that the aggregate shows similar absorbance changes as those observed when the photostationary state is converting back to all *trans* GFP:~~loop:s11~~. The red spectrum corresponds to aggregate isolated from the same elution volume as indicated by the arrows in Supplementary Figure 3, but it was left overnight at 4°C and concentrated to obtain the spectrum. The black solid line is the absorbance spectrum after irradiating the sample with the red solid spectrum with 460 nm light. After irradiation with 460 nm light the black spectrum slowly changes back to the red spectrum in the dark (data not shown). The red and black spectra were used in fitting the data shown in Figure 2 in the text. Aggregates isolated from ~9 mL in the size exclusion data in Supplementary Figure 3 show similar spectra and and changes in spectra with time and light (data not shown).

Absorbance Spectra of Photostationary States Generated in PVA

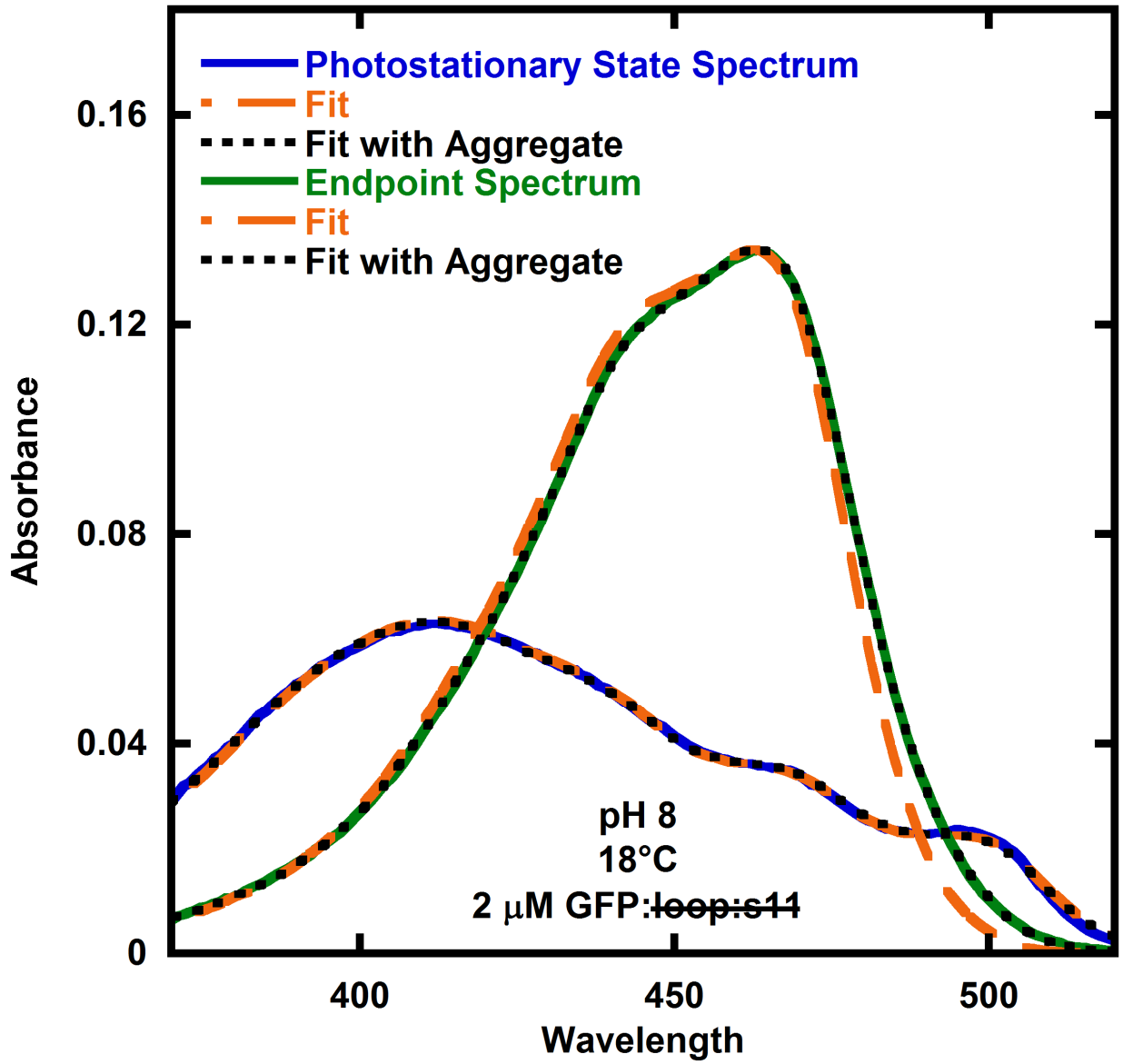
In order to avoid aggregation while observing the light induced changes in the absorbance spectrum of *trans* GFP:~~loop:s11~~, the protein was encased in polyvinyl alcohol. The polymer films were made by adding *trans* GFP:~~loop:s11~~ to 10% PVA solutions of pH 8, 8.6, 9, and 10, and allowing the solution to evaporate water and become a film over a period of days (see main text). Photostationary states were generated with light ranging from 410 nm to 460 nm at each pH (Supplementary Figure 5). The pH was altered in order to observe changes in the *cis* GFP:~~loop:s11~~ spectrum with pH. The spectrum of *trans* GFP:~~loop:s11~~ remains the same from pH 8 to pH 10, so the changes in the photostationary states shown in Supplementary Figure 5 due to changes in pH are due to changes in the spectrum of *cis* GFP:~~loop:s11~~, consistent with titration from a protonated chromophore at lower pH to a deprotonated chromophore at higher pH (see Figure 8 in the text). At pH 10 there is no wavelength dependence of the photostationary state, suggesting that there is complete conversion of *trans* GFP:~~loop:s11~~ to *cis* GFP:~~loop:s11~~. At the lower pHs there are wavelength dependent changes, and the changes increase in magnitude as the pH is decreased. These spectra are the basis for the hypothesis in the text that the deprotonated state of *cis* GFP:~~loop:s11~~ has no appreciable quantum yield of isomerization from the *cis* to *trans* configuration.



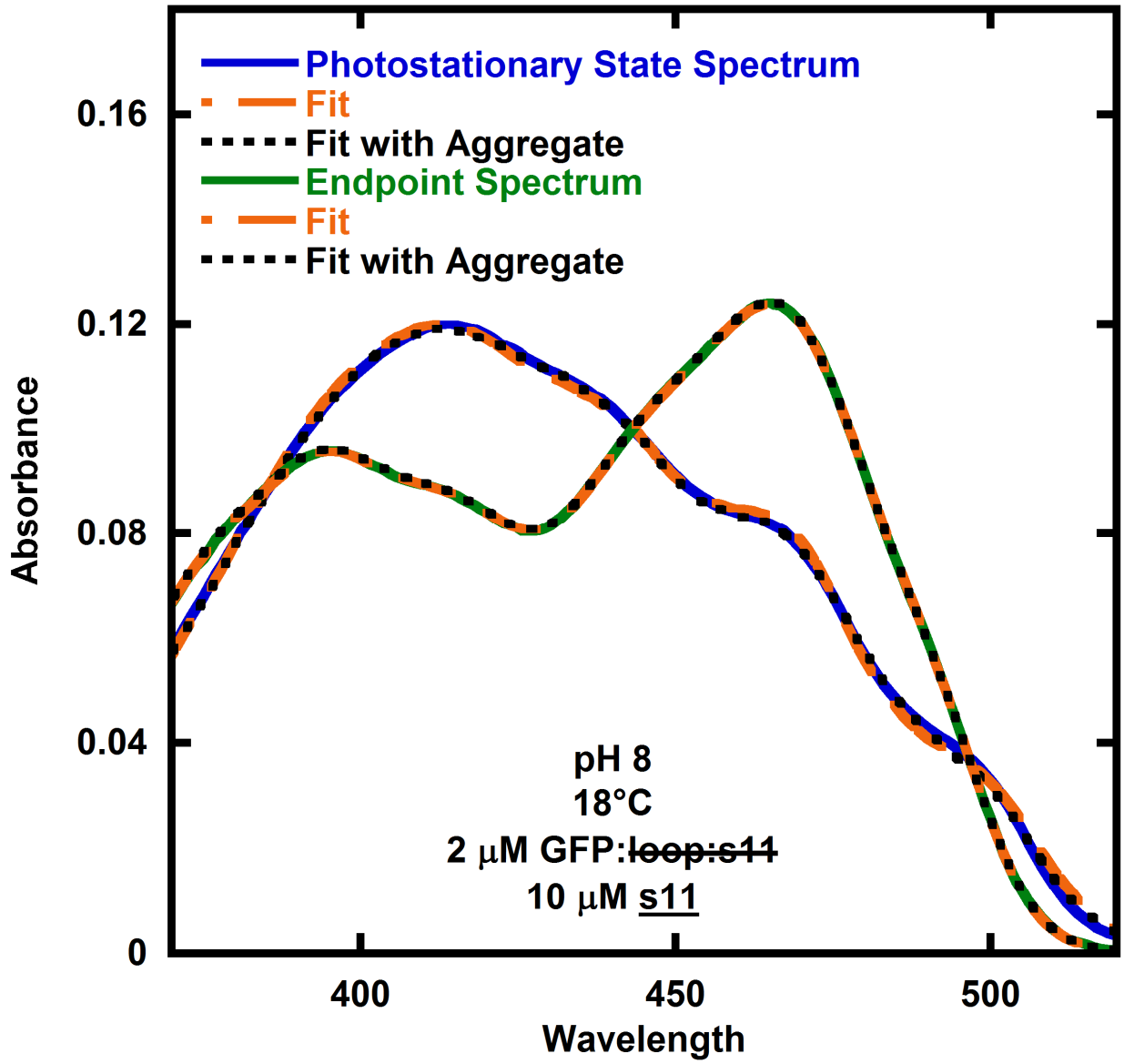
Supplementary Figure 5. Absorption spectra of light activated GFP:~~loop:s11~~ encased in polyvinyl alcohol (PVA) at different pHs. The GFP:~~loop:s11~~ was light activated at wavelengths from 470 nm (dark red spectrum) to 405 nm (dark purple spectrum). In all cases the spectra shown are different from the original trans GFP:~~loop:s11~~ spectrum. At pH 10 the light activated spectra are nearly identical regardless of the wavelength of light used to generate the photostationary state, and at all other pHs the spectra at each wavelength are different.

Quality of Absorbance Spectra Fits

All of the concentrations in Figures 5 and 6 in the text were determined by fitting absorbance spectra with basis spectra for the relevant species in solution. The absorbance spectra fits are shown in Supplementary Figures 6 and 7 for the decay of the photostationary state back to the trans configuration, and for mixing s11 with the photostationary state mixture, respectively. Both figures show the resulting fits obtained with and without the aggregates included in the fitting procedure in order to justify inclusion or exclusion, respectively, of the aggregate spectra in the basis spectra. In Supplementary Figure 5 at early time (photostationary state mixture) the fits with or without the aggregate spectra included are very similar. The similarity of the fits with or without the aggregate at early time is expected because the amount of aggregate is minimal. At late time the fit without aggregate does not fit as well as the fit with aggregate included, especially at longer wavelengths. While this difference in the fits is small, it leads to an overestimation of the concentration of *cis* GFP:~~loop:s11~~ remaining, which causes large problems in the kinetic modeling. In Supplementary Figure 6 both fits with and without the aggregate spectra present fit the data at early and late time well. The light activated split GFP reassembly experiment in Supplementary Figure 6 takes place at a much faster time scale at low micromolar concentrations, so there is no appreciable aggregation.



Supplementary Figure 5. Absorbance spectra of a photostationary state mixture of GFP:~~loop:s11~~ and the endpoint of absorbance changes after leaving the photostationary state in the dark, as well as fits with basis spectra from Figure 4 and Supplementary Figure 3. The photostationary state (blue line) was generated by excitation of trans GFP:~~loop:s11~~ with 460 nm light, and the endpoint (green line) is a spectrum obtained after leaving the photostationary mixture in the dark for 5 hours. The fits with only the *cis* and *trans* GFP:~~loop:s11~~ spectra from Figure 4 (dashed orange lines) are similar to the fits that also include the aggregate spectra from Supplementary Figure 3 (dotted black lines) at early time, but at late time there are significant differences. The concentrations shown in Figure 5 were obtained by fitting with the aggregate spectra present in the basis spectra.



Supplementary Figure 6. Absorbance spectra of a photostationary state of GFP:~~loop:s11~~ and the endpoint of absorbance changes after mixing the photostationary state with s11 in the dark, as well as fits with basis spectra from Figure 4 and Supplementary Figure 3. The photostationary state (blue line) was generated by excitation of *trans* GFP:~~loop:s11~~ with 460 nm light, and the endpoint (green line) is a spectrum obtained after leaving the photostationary mixture with s11 in the dark for 20 minutes. The fits with (dotted black lines) or without (dashed orange lines) the aggregate spectra from Supplementary Figure 3 are nearly identical. The spectra shown in Supplementary Figure 3 were not used when fitting to obtain the concentrations shown in Figure 6.

Amino Acid Sequences

GFP:loop:s11 and GFP:~~loop:s11~~ (trypsin cut site indicated by the '▼')

MGSSHHHHHHSSGLVPGGSHMGGTSSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFISTTGKLPV

PWPTLVTTLSYGVQAFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKED

GNILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDP

NEK▼GTRGSGSIEGRHSGSGSRDHMVLHEYVNAAGITHGMDELYKGSGGT

s11

RDHMVLHEYVNAAGIT

Reference:

1) Lees, A.J. *Anal. Chem.* 1996, *68*, 226-9.