Supplementary Figures

Figure S1. (A) Two trials of time-resolved anisotropy experiments for H148G YFP at pH 7.0, with fluorescence detected at 527 nm following 400 nm excitation. (B) The difference in protein concentration in these two trials is illustrated in the corresponding absorption spectra.

Figure S2. (A) Excitation spectra (solid lines) for 527 nm fluorescence of YFP 10C at pH 6.0, 7.0, 8.0, and 9.0, all normalized to the intensity at 450 nm. To illustrate the contribution from excitation of the neutral chromophore, the spectrum at pH 9.0 when the anionic chromophore dominates is subtracted from the spectra at pH below 9.0 and the difference spectra are shown (dashed lines). (B) The corresponding anisotropy at pH 6.0 through 9.0.

Figure S3. Time-resolved anisotropy measured for wild-type GFP at pH 7.8 (λ_{em} = 508 nm) and YFP 10C at pH 6.0 (λ_{em} = 527 nm) with 800 nm two-photon excitation.

Figure S4. The absorption dichroism with plane-polarized light incident normal to the *ac* (red) and *bc* (blue) planes of GFP crystal calculated as a function of angle α (solid lines), using the coordinates in GFP crystal structure (PDB code: 1EMB (*1*)). The experimental value of 1.6 (*2*) measured for the neutral GFP chromophore is also shown (dashed line) for comparison.

References

1. Brejc, K., Sixma, T. K., Kitts, P. A., Kain, S. R., Tsien, R. Y., Ormo, M., and Remington, S. J. (1997) Structural basis for dual excitation and

photoisomerization of the Aequorea victoria green fluorescent protein, *Proc. Natl. Acad. Sci. U.S.A. 94*, 2306-2311.

2. Rosell, F. I., and Boxer, S. G. (2003) Polarized absorption spectra of green fluorescent protein single crystals: Transition dipole moment directions, *Biochemistry 42*, 177-183.

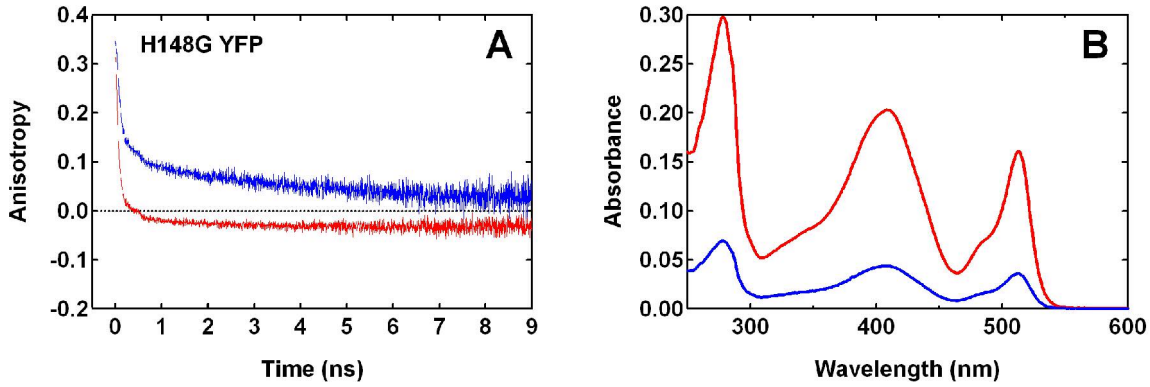

Figure S1

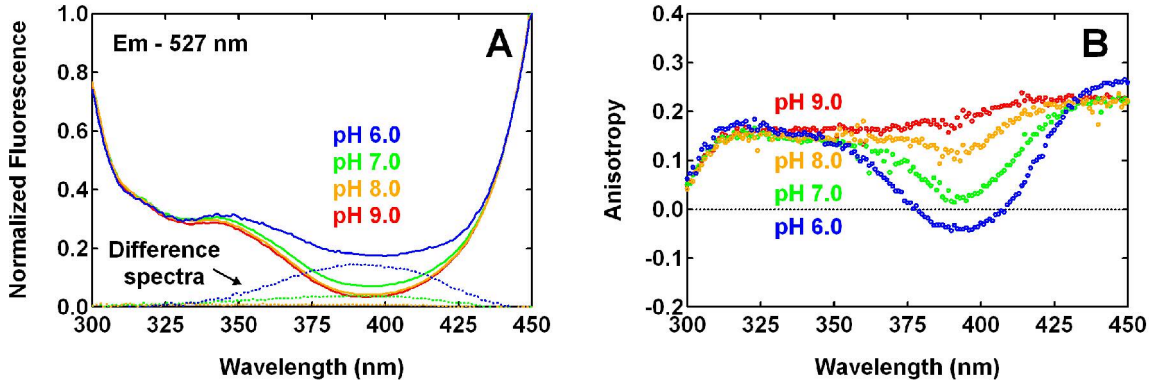

Figure S2

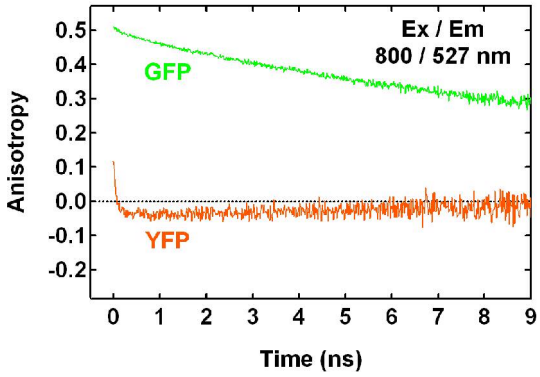

Figure S3

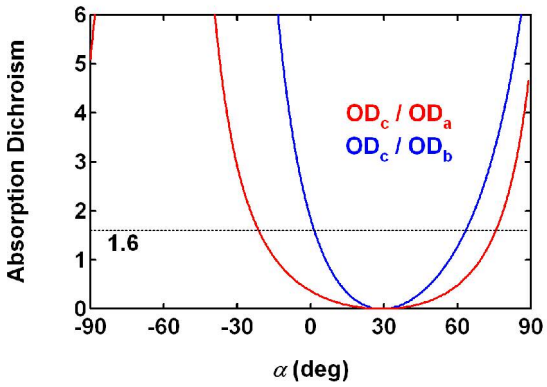
Absorption Dichroism
6
5
4
3
2
1
0
-90
-60
-30
0
30
60
90
α (deg)
1.6
OD_c / OD_a
OD_c / OD_b

Figure S4