GFP: a model system for understanding proton transfer, photoisomerization and optogenetics

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Green fluorescent protein (GFP) is the most widely used genetically encoded fluorescent probe. Since our early discovery that the GFP chromophore is a photoacid and does excited state proton transfer [1] and the observation that the isolated GFP chromophore is essentially non-fluorescent due to rapid isomerization in the excited state, this system has provided many opportunities to explore complex interactions of light and biological matter. Our lab has recently focused on three inter-related aspects of these complex interactions.

(1) The energetics of short hydrogen bonds [2]. Short hydrogen bonds and specifically low-barrier hydrogen bonds (LBHBs) have been the focus of much attention and controversy for their possible role in enzymatic catalysis. The GFP mutant S65T, H148D has been found to form a very short hydrogen bond between Asp148 and the chromophore resulting in significant spectral perturbations. Leveraging the unique autocatalytically formed chromophore and its sensitivity to this interaction we explored the consequences of proton affinity matching across this putative LBHB. We utilized spectral isotope effects, isotope fractionation factors, and a simple 1D model of the hydrogen bond coordinate in order to gain insight into the potential energy surface and particularly the role that proton delocalization may play in this putative short hydrogen bond. These GFP variants are now combined with electric field perturbations (electronic Stark effects) to explore the role of proton polarizability in biological systems.

(2) We discovered that "split" GFP's, in which a loop between adjacent β-strands is cut, photodissociate, albeit with low quantum yield. We elucidated the mechanism of strand photodissociation by measuring the dependence of its rate on light intensity and point mutations [3]. The results show that strand photodissociation is a two-step process involving light-activated *cis-trans* isomerization of the chromophore followed by light-independent strand dissociation. The dependence of the rate on temperature was then used to establish a potential energy surface diagram along the photodissociation reaction coordinate. The resulting energetics-function model reveals the rate-limiting process to be the transition from the electronic excited state to the ground state PES accompanying *cis-trans* isomerization.

(3) In order to better understand the relative contributions of steric and electrostatic effects to the efficiency of photoisomerization, we modified the GFP chromophore systematically by amber suppression using a wide range of different Tyr derivatives. Both steric and electronic effects are found to be at work.

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[2] L. M. Oltrogge, S. G. Boxer, *ACS Central Science,* **1**, 148-156 (2015).
[3] C-Y Lin, J. Both, K. Do, S. G. Boxer, *PNAS,* **114**, E2146-E2155 (2017).