Green Fluorescent Protein and Its 10th Strand

Controlling Protein-Peptide Interactions with Light

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Truncated green fluorescent protein (GFP) that is refolded after removing the 10th β-strand (s10) can readily bind to a synthetic strand to recover the absorbance and the fluorescence of the whole protein. This allows rigorous experimental determination of thermodynamic and kinetic parameters of the split system including the equilibrium and the association/dissociation rate constants, which enables residue-specific analysis of peptide-protein interactions. The equilibrium constant calculated from the ratio of the two rate constants agrees with that directly estimated from the binding isotherm, which supports the one-to-one binding model. In measuring dissociation of s10, it was discovered that the strand can be photodissociated, where the dissociation rate is greatly increased by light irradiation.

We found that in the overall photodissociation process, a thermal step follows light-activation of the molecule, and the thermal activation barrier was determined through an Arrhenius plot of the rate constants acquired at a saturating light intensity. The thermal step could be selectively affected by mutating residue 209 on s10 and by changing the solvent viscosity, which suggests that the thermal step is the actual dissociation of s10. In the process, we found that the quantum yield of photodissociation can be enhanced by increasing the temperature or lowering the viscosity of the solvent, or by introducing mutations such as K209Q or Y203T. For the light-activation step, *cis-trans* isomerization of the chromophore could be suggested as the underlying mechanism referring to the previous work on truncated GFP with strand 11 removed (Kent and Boxer, 2011).

Finally, GFP variants carrying one extra s10 were created and characterized, and their possible applications were explored. These proteins can fold with either one or the other s10, and the ratio of the two folded forms, unambiguously distinguished by their resulting colors, can be systematically modulated by mutating the residues on s10 or by changing the lengths of the two inserted linker sequences that connect each s10 to the rest of the protein. Exploiting studies on photodissociation, ratiometric protease sensors were designed from the construct by engineering a specific protease cleavage site into one of the inserted loops, where the bound s10 is replaced by the other strand upon protease cleavage and irradiation with light to switch its color. Since the conversion involves a large spectral shift, these genetically encoded sensors display very high ratiometric dynamic range. Further engineering of this class of proteins guided by mechanistic understanding of the light-driven process will enable interesting and useful applications of the protein.