

Fluorescence Mechanism of GFP: Electronic States and Dynamics

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Aequorea green fluorescent protein (GFP) and GFP variants are essential as fluorescent markers in living cells in the field of cell and molecular biology. However, studies of fluorescence mechanism and dynamics of fluorescent proteins are limited to only *Aequorea* GFP. Here we present recent results on fluorescence mechanism of various fluorescent proteins by our newly developed spectroscopy.

Competing energy and proton transfer processes of a tetrameric fluorescent protein ¹

We applied femtosecond time-resolved fluorescence spectroscopy to a tetrameric fluorescent protein, red Kaede to examine a relationship between the excited-state dynamics and a quaternary structure. Observed ultrafast dynamics with a time constant of 13 ps was attributed to fluorescence resonance energy transfer (FRET). The excited state proton transfer (ESPT) proceeds with a time constant of 300 ps, which is much slower than that of *Aequorea* GFP (3 ps, monomeric protein). The difference arises from their different hydrogen bond network structure of the chromophore.

Hidden electronic excited state of enhanced green fluorescent protein ²

Fluorescence imaging by two-photon excitation has attracted attention as a new imaging technique. However, fluorescence mechanism of two-photon excited fluorescent proteins is completely unknown. We measured a two-photon absorption spectrum of enhanced GFP (eGFP), which is the most important marker, by a multiplex two-photon absorption spectroscopy to clarify the fluorescence mechanism. The result indicates the existence of a "hidden" excited state in the vicinity of the lowest excited singlet state. We conclude that this is the origin of the discrepancy between the one-photon and two-photon excitation spectra of eGFP, which is well known in the field of biology.

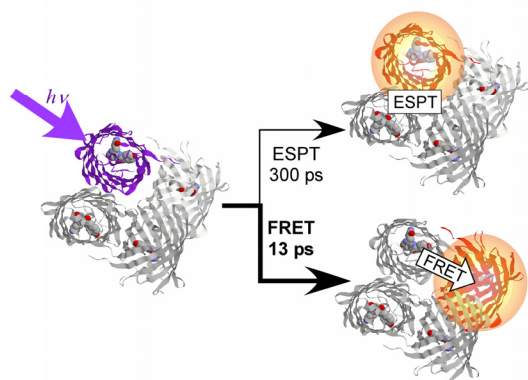


Fig. 1 Competing energy and proton transfer processes of red Kaede.

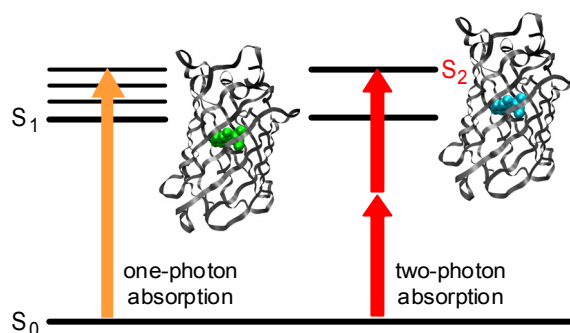


Fig. 2 Schematic diagram of one- and two-photon absorption of eGFP.

1. H. Hosoi, H. Mizuno, A. Miyawaki, and T. Tahara, *J. Phys. Chem. B*, **110**, 22853-22860 (2006).
2. H. Hosoi, S. Yamaguchi, H. Mizuno, A. Miyawaki, and T. Tahara, *J. Phys. Chem. B*, **112**, 2761-2763 (2008).