

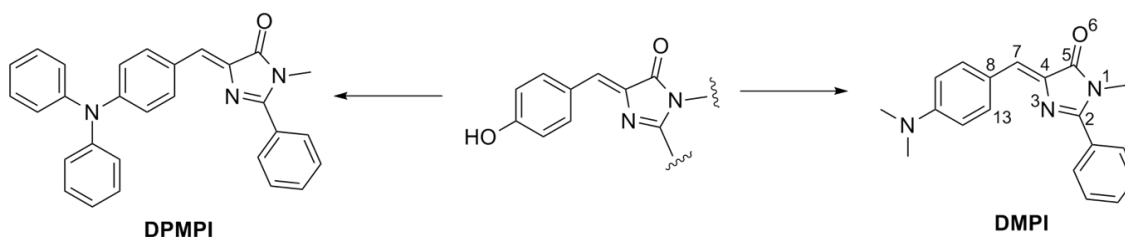
Why GFP Chromophore Analogs Weakly Fluorescent? A Spectroscopic and Theoretical Study

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Introduction

Green fluorescent protein is highly fluorescent ($\phi \sim 0.8$, $\tau_f = 3.3$ ns) in its native, however the denatured protein and most of the synthetic analogs are unexpectedly nonfluorescent ($\phi \sim 10^{-3}$, $\tau_f = 1$ ps) in bulk solutions, which renders it photophysically very important.¹⁻³ The most arguable debate is how the excited state relaxes, whether through a single bond rotation, rotation about exocyclic double bond or via the simultaneous rotation of both the bridging bonds known as “hula-twist”.

To understand the above phenomenon, two GFP luminophore analogs: (4Z)-4-(*N,N*-dimethylaminobenzylidene)-1-methyl-2-phenyl-1,4-dihydro-5*H*-imidazolin-5-one (DMPI) and (4Z)-4-(*N,N*-diphenylaminobenzylidene)-1-methyl-2-phenyl-1,4-dihydro-5*H*-imidazolin-5-one (DPMPI) were synthesized and their excited state dynamics was explored by fluorescence up-conversion spectroscopy and quantum mechanical calculations with the results being substantiated by HPLC and NMR.



Scheme 1. Molecular structure of GFP chromophore and two analog under consideration of present work.

Results and Discussions

Bi-exponential decay kinetics in solvents of varying viscosity establishes the viscosity dependent relaxation dynamics. Based on previous literature³ and observed viscosity dependence, the second time constant (τ_2) was assigned to internal conversion via a large amplitude motion, which negates the “hula-twist” based relaxation kinetics. Solvent polarity was found to have a monotonous

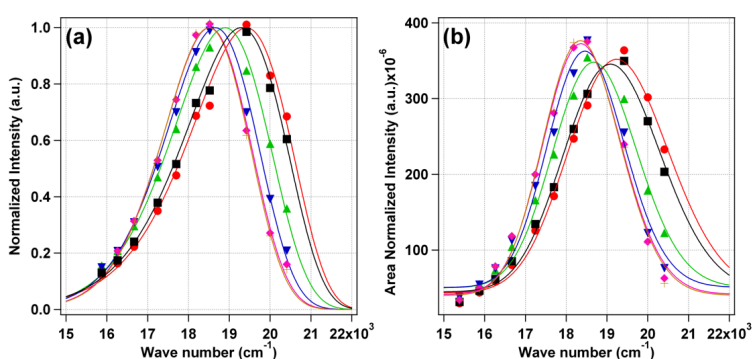


Fig. 1. (a) Intensity normalized time resolved emission spectra (TRES) of DMPI in acetonitrile, constructed from the decay transients obtained at ten different wavelengths. The time resolved emission spectra were plotted for 0 ps (•, Red), 0.1 ps (■, Black), 0.5 ps (▲, Green), 1 ps (▼, Blue), 3 ps (◆, Pink), and 5 ps (+, Orange). (b) Time-resolved area normalized emission spectra (TRANES) of DMPI in acetonitrile. The symbols have their meaning as mentioned for TRES. The TRANES reports the occurrence of an isoemissive point and hence infers the existence of two states in the excited state deactivation process.

effect on the first time component (τ_1), which fastens with increase in polarity and hence inferences towards the depletion of locally excited state to charge transfer state. Time resolved emission spectra (TRES) and time resolved area normalized emission spectra (TRANES) were constructed to authenticate the occurrence of excited state intramolecular charge transfer, which suggest that the charge transfer is

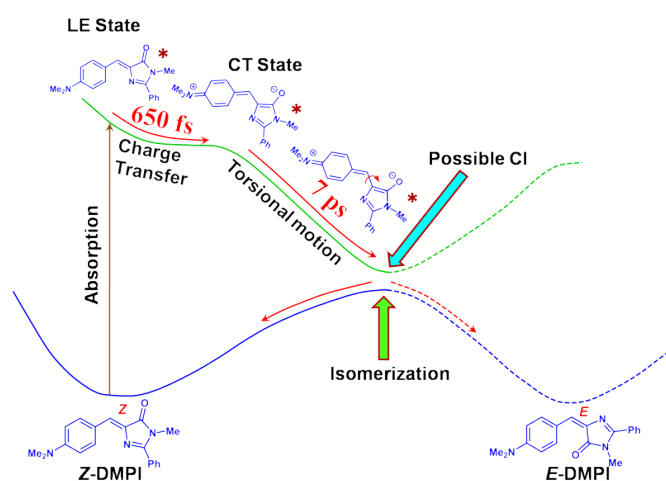


Fig. 2. Schematic excited state dynamics of DMPI in acetonitrile..

completed within 3-5 ps followed by IC via torsional motion. Quantum chemical calculations were used to map the potential energy surfaces of the S_0 and S_1 states, which divulge that the IC via torsional coordinate is only possible by a rotation of the exo-methylene double bond. A torsional rotation of the bridging single bond cannot lead to IC because of the high energy (S_0 - S_1) gap. Also, the rotational coordinates of both the bridging single and double bonds cannot lead to IC because of high activation barrier of twisting. Both the up-conversion measurements and the three dimensional energy maps anticipate an *E-Z* isomerization about the exo-methylene double bond. HPLC profiles authenticated the occurrence of two peaks corresponding to *E*- and *Z*- isomers after irradiating the *Z*-DMPI. Similarly, proton-NMR measurements of the irradiated analogs manifest the presence of additional peaks certifying the existence of both the isomers and hence reveal isomerization about the exo-methylene double bond. These all observations invalidate the role of “hula twist” motion and the torsion about single bond in the relaxation dynamics of the two GFP chromophore analogs under investigation. Present study ascertain the role of the β -barrel coat in the GFP to suppress the deactivating intramolecular charge transfer and internal conversion via the non-radiative pathway resulting in high quantum yields in the GFP.

References

1. Meech, S. R. *Chem. Soc. Rev.* **2009**, 38, 2922.
2. Tsuji, F. I. *et al. Proc. Natl. Acad. Sci. U. S. A.* **1996**, 93, 13617.
3. Huppert, D. *et al. J. Am. Chem. Soc.* **2006**, 128, 1540.

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