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Investigating spectral red shift mechanism of fluorescent chromophores using time-dependent density functional theory

Pei li Zhao[a], Huan Lou[a], JingBin Xu[b], and Jian Song[a,*]

[a] *College of Physics and Electronic Engineering, HeNan Normal University, Xin Xiang, 453007, China*
[b] *Mudan Jiang Medical University, Mudan Jiang, 157011, China*

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Abstract. Several fluorescent protein fluorophores with regular substitution were investigated in gas phase using TDDFT with long range corrected functional. Absorption and emission of both neutral and anionic chromophore states were calculated. The spectral shift amplitudes of calculation are in good agreement with experiment. The further intramolecular charge transfer process analysis show that conjugated area, charge transfer number/distance and transfer efficiency can affect spectral shift. Specially, the "N" atom with lone pair electrons of conjugated ring has an important influence on charge transfer process, and the conjugated length between hydroxyl and bridge bond only impact the anionic spectral shift. Our results about fluorescent chromophore spectral red shift mechanism do provide positive clues on new experimental far-red fluorescent protein designing.

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Key words: Fluorescent protein; spectral red shift; TDDFT; Intramolecular Charge Transfer.

1 Introduction

Green fluorescent protein (GFP), which was first discovered in the jelly-fish *Aequorea victoria* [1, 2], has been widely adopted as an internal fluorescent label in the field of cellular and molecular biology[3, 4]. GFP is particularly useful for tagging and observing gene expression, protein localization, and cell development [5, 6, 7]. The GFPs possess a barrel-like structure, in which the chromophore locates in the center and links with the surrounding α-helices [2]. The measured optical absorption spectrum of the wild type

*Corresponding author. *Email address:* songjian@htu.cn (J. Song)

(wt) GFP has a strong peak at 395nm and a minor one at 475nm, which have been attributed to the neutral and the negatively charged (anionic) states, respectively [8]. The neutral and anionic chromophores give emission spectral peaks around 503-508 nm [9].

The wild type fluorescent protein (FP) can be engineered into the mutants with the emission frequencies covering the full range of the visible electromagnetic spectrum [10, 11, 12, 13, 14]. These molecules, especially the red fluorescent proteins (RFPs) and far-red fluorescent proteins (f-RFPs) with the emission frequency above 600 nm, provide the powerful tools for biochemical and biomedical research in living cells [15, 16, 17]. A suitable RFP is compatible with the existing confocal and wide-field microscopes and thus increases the capacity to image the entire animal. The far-red fluorescent proteins whose emission maxima reach 650 nm are particularly high demanding for multicolor labeling and whole-mount labeling because long wavelength can penetrate tissue more deeply and easily.

The designing of mutated FPs, however, still mostly depends on experimental researcher's scientific intuition, which significantly retards the discovery of new functional mutants, since the mutagenesis of the FPs is a costing and tedious task. A better understanding of molecular details of the FP fluorescence transitions, by the theoretical modeling, can help rationalize the FP designing and summarize the possible general rules. Additionally, modeling and analyzing the fluorescence mechanism of a series of FP molecules can potentially propose new FP mutants with better performances. To achieve this purpose, however, the modeling techniques adopted should have a balance between the computational cost and the accuracy, since large amount of computation is needed. The multiconfigurational self-consistent field (MCSCF) theory [18] based methods such as CASSCF/CASPT2 [19] can provide accurate descriptions of the small molecular systems. Applications of these methods to the larger systems, however, often encounter problems due to their considerable computational cost.

Another commonly adopted option for the fluorescence frequency calculation is the time-dependent density functional theory (TDDFT) with long-range corrected (LRC) exchange-correlation functional. TDDFT can greatly reduce computational cost compared with the MCSCF techniques. At the same time, LRC functional can deal with the long range electron correlation better, which is common in π-conjugated fluorescent chromophore transition process, than conventional hybrid functional such as B3LYP [20]. Taking Filippi's work for example, LRC functional CAM-B3LYP [21] and LC-BLYP [22] present overall good agreement with the extrapolation of solution experiments to vacuum conditions for wild-type GFP as well as wave function methods [23].

In Wang's fluorescent protein mutant work [24], a new hydroxyquinoline ring was introduced to substitute phenol of the fluorophore in diverse fluorescent proteins and significantly red-shifted excitation and emission spectra were achieved. Inspired by this, here we select and design a series of fluorophores with different substitution as study objects. TDDFT with LRC functional method has been employed to investigate the structure related regularity of fluorescent protein spectral red shift and clarify the underlying