



# Insights into the unusual dual fluorescence of the ortho-amino analogue of green fluorescent protein chromophore

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## ABSTRACT

Even though both *p*-aminobenzonitrile (*p*-ABN) and *o*-ABDI [*o*-amino analogue of green fluorescent protein (GFP)] have the same electron-donating amino group, *o*-ABDI displays dual fluorescence while *p*-ABN exhibits single fluorescence. Even though both *o*-AABDI (*o*-acetamido analogue of GFP) and *o*-ABDI have the similar intramolecular hydrogen bonding, *o*-ABDI displays dual fluorescence while *o*-AABDI shows single fluorescence. To explore the unusual phenomenon of dual fluorescence for *o*-ABDI, we used the time-dependent density functional theory (TD-DFT) method with the polarizable continuum model (PCM) to study the  $S_1$  excited state of *o*-ABDI in acetonitrile. What we found is that *o*-ABDI in acetonitrile has two isomeric conformations (minima) in the  $S_1$  excited state, the twisted intramolecular charge transfer (TICT) and the localized excited (LE) states. The TICT state involves a large dipole moment change during fluorescence emission, while the LE state with a flat structure involves only a slight dipole moment change without a charge transfer during fluorescence emission. Even though both *o*-DMABDI (*o*-dimethylamino analogue of GFP) and *o*-ABDI have the TICT excited state, *o*-DMABDI does not have the stabilized LE  $S_1$  state while *o*-ABDI does. It is because the intramolecular hydrogen bonding of *o*-ABDI makes its LE  $S_1$  state highly stabilized, and that leads its LE  $S_1$  state to become a global minimum. That is why *o*-ABDI displays dual fluorescence.

## 1. Introduction

According to Kasha's rule, fluorescent molecules usually exhibit single fluorescence [1]. A molecule that displays dual fluorescence usually involves a mixture of excited states or a conformational equilibrium in the excited state or the ground state [2]. For example, fluorescent molecules in which the electron donor and the electron acceptor moieties are linked by a single bond might exhibit dual fluorescence [3–8]. The dual fluorescence may be caused by the presence of two different conformations of the same molecule in the first singlet excited state ( $S_1$ ). One of popular examples is that two conformations in  $S_1$  excited state involve locally excited (LE) state and twisted intramolecular charge transfer (TICT) excited state [3]. A typical example of this type of fluorescent molecules with dual fluorescence is *p*-*N,N*-dimethylaminobenzonitrile (*p*-DMABN) with a tertiary amine [9–12]. It is interesting to know that *p*-aminobenzonitrile (*p*-ABN) with a primary amine displays single fluorescence [3,10].

In the last two decades, green fluorescent protein (GFP) and its mutants have attracted interest as fluorescent biological labels [13,14]. The wild-type GFP (*wt*GFP) is a globular protein of 238 amino acids that

are folded into an 11-stranded  $\beta$ -barrel [15–17]. At the center of the *wt*GFP, there is a *p*-hydroxybenzylidene-imidazolinone (*p*-HBDI) chromophore, which is biosynthesized from Ser(65)-Tyr(66)-Gly(67) [15–17]. To explore or tune the photophysical properties of *wt*GFP, chemists prepared various analogues of its chromophore, such as *p*-DMABDI [18], *o*-DMABDI [18], *o*-ABDI [18], *o*-AABDI [19], and so on. The *p*-DMABDI, *o*-DMABDI and *o*-AABDI were reported to exhibit single fluorescence, while *o*-ABDI displays dual fluorescence (Fig. 1)

Even though *p*-DMABN, *p*-DMABDI and *o*-DMABDI have the same electron-donating *N,N*-dimethylamino group, both *p*-DMABDI and *o*-DMABDI display single fluorescence while *p*-DMABN shows dual fluorescence. The time-dependent density functional theory (TD-DFT) method successfully explained the interesting phenomenon [20]. The  $S_1$  excited states of *p*-DMABDI and *o*-DMABDI are  $^1(\pi, \pi^*)$  charge transfer (CT) excited states with twisted structures, where the *N,N*-dimethylaminobenzene moiety functions as an electron donor, the methyleneimidazolone moiety serves as an electron acceptor and the electron donor is linked with the electron acceptor by the C-C single bond (P-bond) [20]. Their TICT states are more stable than their respective LE states that are not a global minimum. That is why both *p*-DMABDI and

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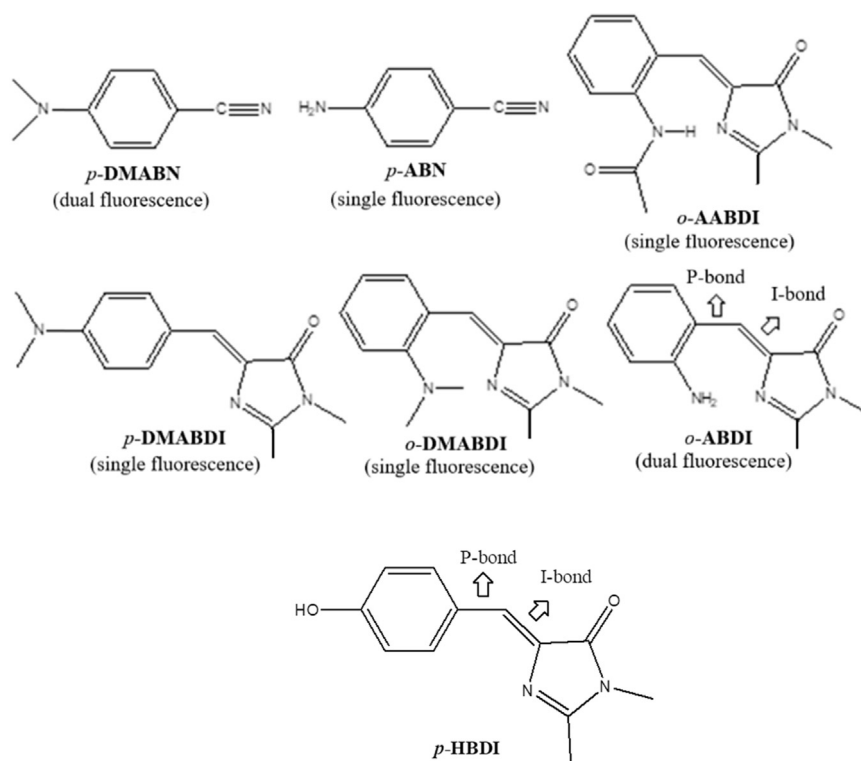


Fig. 1. Molecular structures of *p*-DMABN, *p*-ABN, *o*-AABDI, *p*-DMABDI, *o*-DMABDI, *o*-ABDI and *p*-HBDI.

*o*-DMABDI display single fluorescence [20].

Even though both *p*-ABN and *o*-ABDI have the same electron-donating amino group, *o*-ABDI displays dual fluorescence while *p*-ABN exhibits single fluorescence. In addition to that, even though both *o*-AABDI [19] and *o*-ABDI [18] have the similar intramolecular hydrogen bonding, *o*-ABDI displays dual fluorescence while *o*-AABDI shows single fluorescence. To explain why *o*-ABDI displays dual fluorescence, we used both the TD-DFT [21–26] and the coulomb-attenuating method (CAM)-TD-DFT methods [27] with the polarizable continuum model (PCM) [28–30] to study the  $S_1$  excited state of *o*-ABDI in acetonitrile.

## 2. Material and methods

### 2.1. Computational details

All the calculations were performed with the Gaussian09 program [31]. Geometry optimization of the  $S_1$  excited state of *o*-ABDI in  $\text{CH}_3\text{CN}$  and simulation of its electronic transition were carried out without any symmetry restriction by using the time-dependent hybrid functional B3LYP method [21–26] and the coulomb-attenuating method (CAM)-TD-B3LYP method [27] with the PCM model [28–30] and the correlation-consistent polarized valence double-zeta basis set of Dunning (cc-pVDZ) [32]. Geometry optimization for the ground state of *o*-ABDI in  $\text{CH}_3\text{CN}$  was carried out without any symmetry restriction at the B3LYP-PCM/cc-pVDZ level. Simulation of its electronic transition ( $S_0 \rightarrow S_1$ ) was performed at both the TD-B3LYP-PCM/cc-pVDZ and the CAM-TD-B3LYP-PCM/cc-pVDZ levels. Optimization convergence criteria were the standard criteria employed in Gaussian09. To investigate whether electronic absorption involves a charge transfer or not, the natural transition orbital technique was used [33]. This technique uses the corresponding orbital transformation of Amos and Hall and makes a dramatic simplification in the qualitative description of electron excitations become possible. The GaussView was utilized to visualize the molecular orbitals. The  $S_1$  excited-state and the  $S_0$  ground-state potential energy surfaces (PESS) of *o*-ABDI were calculated at the level of TD-B3LYP-PCM/cc-pVDZ.

## 3. Results and discussion

### 3.1. Photoexcitation

The TD-B3LYP/cc-pVDZ level predicts the electronic absorption ( $S_0 \rightarrow S_1$ ) of *o*-ABDI occurs at 430 nm (2.88 eV, 66.5 kcal/mol) with oscillator strength of 0.37, which is quite close to the experimental electronic absorption at 422 nm (2.93 eV) with oscillator strength of 0.11. (Table 1) [18] Similarly, the CAM-TD-B3LYP/cc-pVDZ level predicts the electronic absorption occurs at 392 nm (3.17 eV) with oscillator strength of 0.51, which is also close to the experimental electronic absorption [18].

The dominant natural transition orbital pair for the electronic excitations to the  $S_1$  excited state of *o*-ABDI in acetonitrile was calculated at the TD-B3LYP-PCM/cc-pVDZ level. (Fig. 2) Both the orbital, in which the excited particle can be found, and the orbital, in which the empty hole is located, are almost equally distributed around the whole molecule and their orbital coefficients look similar. Hence, the photoexcitation ( $S_0 \rightarrow S_1$ ) of *o*-ABDI in acetonitrile does not involve a CT. This result is consistent with the experimental result that the lowest-energy electronic absorption ( $S_0 \rightarrow S_1$ ) of *o*-ABDI is not regularly shifted with

Table 1

Electronic vertical transition energies  $\Delta E$  (in eV) and oscillator strengths  $f$  for the electronic absorption ( $S_0 \rightarrow S_1$ ) of *o*-ABDI in acetonitrile at the TD-B3LYP-PCM/cc-pVDZ (method 1) and the CAM-TD-B3LYP-PCM/cc-pVDZ (method 2) levels.

method 1 $\Delta E$ ( $f$ )	method 2 $\Delta E$ ( $f$ )	Exp. $\Delta E$ ( $\lambda$ , $f$ ) <sup>a</sup> .
2.88 <sup>b</sup> (0.37)	3.17 <sup>c</sup> (0.51)	2.93 (422 nm, 0.11)

<sup>a</sup> . Experimental electronic absorption energy ( $\Delta E$ ), wavelength ( $\lambda$ ) and oscillator strength ( $f$ ) of *o*-ABDI in acetonitrile.

<sup>b</sup> . The excited state is described mostly by 0.70[MO 57  $\rightarrow$  MO 58] – 0.12[MO 56  $\rightarrow$  MO 58].

<sup>c</sup> . The excited state is described mostly by 0.70[MO 57  $\rightarrow$  MO 58].

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