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Photoabsorption of green and red fluorescent protein chromophore anions *in vacuo*

Songbo Wan [a], Shasha Liu [b], Guangjiu Zhao [c], Maodu Chen [b,*], Keli Han [b,c], Mengtao Sun [a,*]

[a] *Beijing National Laboratory for Condensed Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing, 100080, PR China*
[b] *Department of Chemistry, and School of Physics and Optoelectronic Technology, Dalian University of Technology, Dalian 116024, PR China*
[c] *State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, PR China*

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Abstract

Photoabsorption properties of green and red fluorescent protein chromophore anions *in vacuo* were investigated theoretically, based on the experimental results in gas phase [Phys. Rev. Lett. 2001, 87, 228102; Phys. Rev. Lett. 2003, 90, 118103]. Their calculated transition energies in absorption with TD-DFT and ZINDO methods are directly compared to the experimental reports in gas phase, and the calculations with ZINDO method can correctly reproduce the absorption spectra. The orientation and strength of their transition dipole moments were revealed with transition density. We also showed the orientation and result of their intramolecular charge transfer with transition difference density. The calculated results show that with the increase of the extended conjugated system, the orientation of transition dipole moments and the orientation of charge transfer can be reversed. They are the linear responds with the external electric fields. These theoretical results reveal the insight understanding of the photoinduced dynamics of green and red fluorescent protein chromophore anions and cations *in vacuo.*

Keywords: Green and red flourescent protein chromophore; Transition dipole moment; Intramolecular charge transfer

1. Introduction

Since the first successful cloning of the gene encoding for green fluorescent protein (GFP) was discovered in the jellyfish *Aequorea victoria* [1,2], GFP has attracted great interest as a biological fluorescence labeling [3,4]. The GFP exhibits two absorption maxima at 395 and 477 nm, which are attributed to the neutral and anion forms in solution [5–7]. To improve sensitivity and make multicolor labeling, the red fluorescent protein (DsRed) is isolated from *Discosoma* corals [8,9]. The DsRed protein has an absorption maximum at about 559 nm, and there is a significant redshift of DsRed, compared to GFP, which is likely related to the electronic structure of the chromophore. The chromophore of GFP and DsRed has been studied experimentally in solution [10–12], and it is found that their spectroscopic characteristics are sensitive to the microenvironment of the chromophore. Polar perturbations and hydrogen binding in the protein have an influence on the absorption [13]. Theoretically, excited state properties of GFP have been studied *in vacuo* [6,14–16], but calculations of excited electronic states are nontrivial, and unfortunately there are large discrepancies between results obtained with different theoretical models. These calculations cannot be tested against solution-phase measurements because of strong solvent effects. Gas-phase experiments are therefore useful to elucidate the function of GFP, to validate or reject the reliability of calculations, and to better understand solvent perturbations [17–19].

It is a difficult challenge to perform gas-phase measurements because of low target densities, but they are crucial because external perturbations are avoided [17], and they can provide us a simpler picture about the photophysical and photochemical processes than ones in solution phase [20]. Recently, Pedersen et al. investigate the absorption properties of bare RFP and DsRed anions in gas phase, i.e., without external interactions of any kind [18–20]. They presented evidence that the exact location of the absorption band of the anionic form of the GFP chromophore is ascribed almost purely to the intrinsic chemical properties of the chromophores rather than to a result of interactions with amino acid side chains in its vicinity [18,21].

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* Corresponding authors.
E-mail address: mtsun@aphy.iphy.ac.cn (M. Sun).

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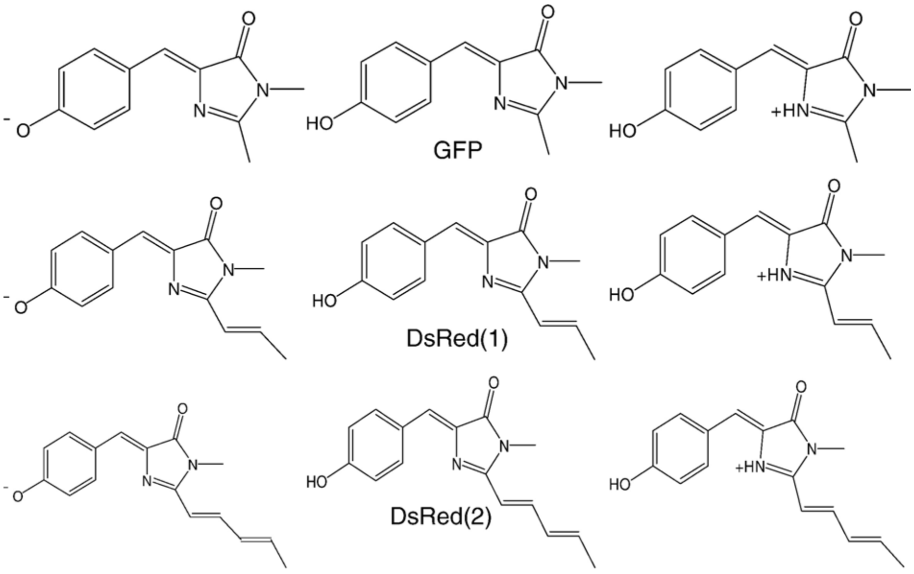

Fig. 1. The chemical structure of neutral, cationic and anionic GFP, DsRed(1) and DsRed(2).

They also demonstrated that the redshift of DsRed compared to GFP is a direct consequence of the extended conjugated system in the DsRed chromophore [19]. The experimental results of optical properties of GFP, DsRed(1) and DsRed (2) cations were reported in Ref. [22].

In this paper, the excited state properties of bare neutral, anionic and cationic RFP and DsRed (Fig. 1) are investigated theoretically *in vacuo*. The calculated transition energies in absorption are directly compared to the experimental reports. The orientation and strength of their transition dipole moments are revealed with transition density [23,24]. The orientation and result of their intramolecular charge transfer are shown with charge difference density [25,26].

2. Methods

The ground state geometries of neutral, anionic and cationic GFP and DsRed(1) and DsRed(2) were optimized with density functional theory (DFT) [27], B3LYP functional [28] and 6-31G basis set. Their optimized geometries with DFT are of the planar structures. With the optimized geometries, the frequency analysis were done, and there is no imaginary frequency, so the optimized geometries are true energy minima [6,22] The electronic structures were calculated with time-dependent DFT (TD-DFT) [29] method, and the same functional and basis set. The effect of the basis set to the gas-phase excitation energy were also examined with TD-B3LYP/6-311++G(D)//DFT-B3LYP/6-311++G(D). For comparison, their electronic structures were also calculated with ZINDO method [30], with the optimized ground states by the method of DFT-B3LYP/6-31G and DFT-B3LYP/6-311++G(D), respectively. The gas-phase excitation energy calculated with the semiempirical ZINDO is perfectly agreed with the experimental results, whereas gas-phase excitation energy calculated with the TD-DFT is significantly overestimated [6,15,18]. Their permanent dipole moments at the excited state were calculated with ZINDO method and with $\mu_{11} = \mu_{00} + \Delta\mu_{10}$, where the $\Delta\mu_{10}$ is the difference of permanent dipole moments between ground and excited state, which can be fitted by [24,31]

$$E_{exc}(F) = E_{exc}(0) - \Delta\mu F - \frac{1}{2}\Delta\alpha F^2 \tag{1}$$

where F is the external static electric field F, $E_{\mathrm{exc}}(0)$ is the excitation energy at zero field, $\Delta\alpha$ is the change in polarizability. All the calculations were done with Gaussian 03 suite [32].

The transition density, charge difference density and transition density matrix were employed to study their photoinduced excited state properties, which reveal the orientation and strength of transition dipole moments, the orientation and result of intramolecular charge transfer, and the electron-hole coherences on the excitation, respectively.

3. Results and discussion

3.1. Ground and excited state properties of GFP, DsRed(1) and DsRed (2) anions

Their optimized geometries at the ground state reveal that they are of planar structure, which are consistent with other's theoretical reports [6,21]. The influence of the extended conjugated system to ground state properties can be seen from

Table 1
The calculated permanent dipole moments at the ground and excited states for GFP, DsRed (1) and DsRed (2), and the difference of permanent dipole moments between ground and excited states

	μ_{00}			$\Delta\mu_{10}$		μ_{11}
	X(D)	Y(D)	Z(D)	X(au)	X(D)	X(D)
GFP	10.540	1.6938	0.0016	0.92	2.337	12.877
DsRed (1)	11.451	0.2848	−0.0002	1.52	3.061	14.512
DsRed (2)	12.624	1.4036	0.0002	2.52	6.401	19.025

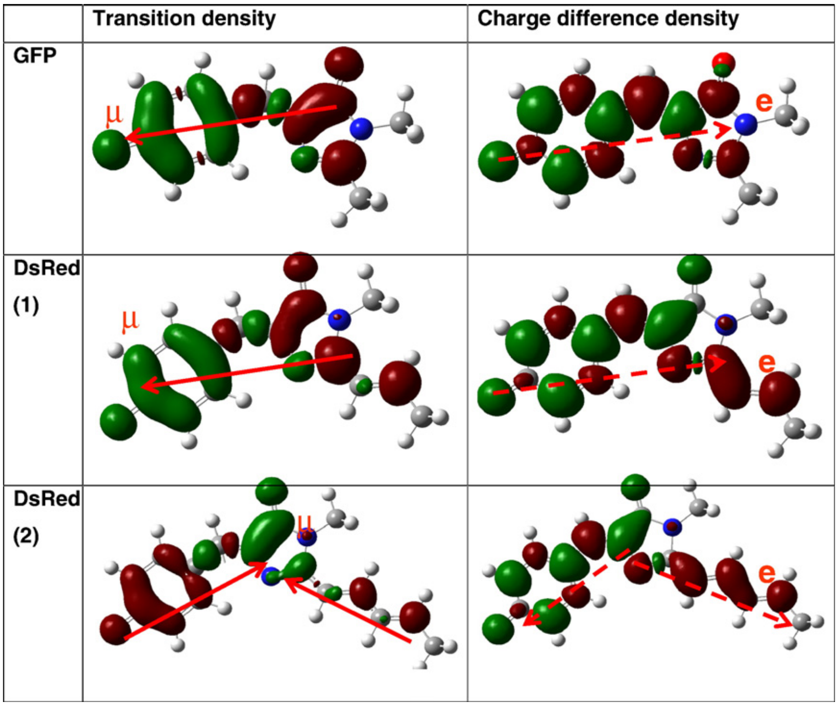

Fig. 2. The transition density and charge difference density for the anionic GFP, DsRed(1) and DsRed(2).

Table 1. From Table 1, with the increase of the extended conjugated system, the static (permanent) dipole moments are also increased along the molecular backbone, and the orientation of the permanent dipole moments of DsRed(2) is reversed, compared to those of GFP and DsRed(1), according to the orientation of their Cartesian coordinates (see Fig. 2) and the data in Table 1.

Their calculated transition energies (and oscillator strengths) and transition dipole moments were calculated with TD-DFT and ZINDO methods, respectively, which were listed in Tables 2 and 3. The gas-phase excitation energy calculated with the semiempirical ZINDO is perfectly agreed with the experimental results [18,19], whereas gas-phase excitation energy calculated with the TD-DFT is significantly overestimated. The effect of the basis set to the gas-phase excitation energy also be examined with TD-B3LYP/6-31++G(D)/DFT-B3LYP/6-31++G(D), which also listed in Table 2. It can be seen that the effect of the basis set to the gas-phase excitation energy (with strong

Table 2
The calculated transition energies (in nm) and oscillator strength (*f*) of anionic GFP, DsRed (1) and DsRed (2) with different methods

		Experimental results	ZINDO [a]		ZINDO [b]		TD-B3LYP-6-31G [c]		TD-B3LYP-6-31++G(D) [d]	
		nm	nm	*f*	nm	*f*	nm	*f*	nm	*f*
GFP	S_1	479	481.46	1.2257	477.00	1.2203	426.20	0.0000	481.88	0.0003
	S_2		373.18	0.0000	369.68	0.0000	389.73	0.9976	405.49	0.9607
DsRed (1)	S_1	521	532.95	1.2731			451.39	0.0000		
	S_2		382.69	0.2781			449.26	0.9896		
DsRed (2)	S_1	549	586.31	1.3744			508.40	1.1201		
	S_2		407.92	0.3984			475.74	0.0000		

[a] With the optimized ground state calculated with DFT/B3LYP/6-31G method.

[b] With the optimized ground state calculated with DFT/B3LYP/6-311++G(D) method.

[c] With the optimized ground state calculated with DFT/B3LYP/6-31G method.

[d] With the optimized ground state calculated with DFT/B3LYP/6-311++G(D) method.

Table 3
Transition dipole moments of neutral, cationic and anionic for GFP, DsRed (1) and DsRed (2), respectively

	Anionic			Cationic			Neutral		
	X(D)	*Y*(D)	*Z*(D)	*X*(D)	*Y*(D)	*Z*(D)	*X*(au)	*X*(D)	*X*(D)
GFP	−4.3765	0.5222	0.0001	3.9849	7.3786	0.0001	1.0976	−4.2354	0.0002
DsRed (1)	−4.6883	0.5978	−0.0001	4.9717	6.3554	−0.0000	2.3190	4.2260	−0.0001
DsRed (2)	5.0682	0.9175	0.0000	6.2052	−5.3954	0.0001	3.8247	−3.9130	−0.0001

oscillator strength) is little. With the increase of the extended conjugated system, the gas-phase excitation energies are red shifted, which shows that the origin of the redshift of DsRed is related to the structure of the chromophore; i.e., it is not a result solely of the protein environment [18]. Also, with the increase of the extended conjugated system, their transition dipole moments are also increased. It should be noted that the orientation of transition dipole moment of DsRed (2) along the *x* is opposite to those of GFP and DsRed (1) along the *x* axis (see data in Table 3). So, when the extended conjugated system is large enough, the orientation of transition dipole moment can be changed.

It is very important to study the orientation of their transition dipole moments, since photoinduced reaction, such as intramolecular charge transfer and electron-hole coherence on excitation, depend on it. Here, we show the orientation of the transition dipole moment with transition density. From Fig. 2, the orientation of transition dipole moment of DsRed (2) is opposite to those of GFP and DsRed (1), due to the increase of the extended conjugated system. Noted that the orientation of transition dipole moment is from the electrons (red color) to holes (green color). Acceding to the orientation of transition dipole moment of GFP and DsRed (1), the electrons transfer from *p*-hydroxybenzylidene to imidazolidone and the extended conjugated system. For DsRed(2), according to the orientations of the subunit of transition dipole moments, the electron transfer from imidazolidone to *p*-hydroxybenzylidene and to the extended conjugated system, respectively. So, with the increase of the extended conjugated system, the orientation of transition dipole moment and the orientation of charge transfer can be strongly influenced.

Linear and non-linear optical respond of biological system to the external environments is very important [24]. So, we checked the optical responds of GFP, DsRed(1) and DsRed (2) in gas phase to the external electric fields. According to Eq. (1), we firstly fitted the difference between the permanent dipole moment at the excited state and that at the ground state, which are listed in Table 1. We secondly calculated their permanent dipole moments at excited state, acceding to $\mu_{11}=\mu_{00}+\triangle\mu_{10}$. The calculated results in Table 1, μ_{11} and $\triangle\mu_{10}$ increase with the increase of the extended conjugated system, so polar perturbations and hydrogen binding in the protein environment should have stronger influence to the absorptions of DsRed (1) and DsRed (2). From the fitted results in Fig. 3, they are almost the linear optical respond to the external electric fields (to the external protein environment), since $\triangle\alpha\approx 0$ in Eq. (1).

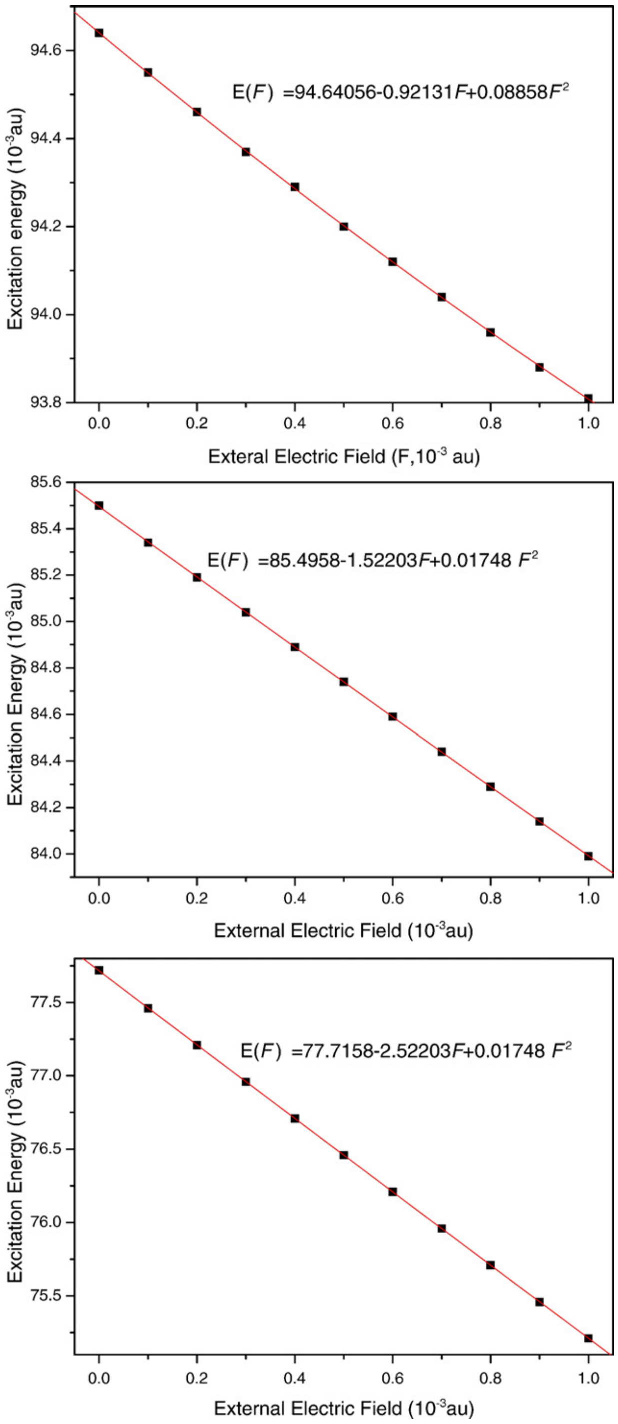

Fig. 3. The external electric field dependent excitation energies anionic GFP, DsRed(1) and DsRed(2).

3.2. Ground and excited state properties of neutral and cationic GFP, DsRed(1) and DsRed (2)

The experimental results of optical properties of GFP, DsRed (1) and DsRed (2) cations were reported in Ref. [22]. To our knowledge, the optical properties of neutral GFP, DsRed(1) and DsRed (2) in gas phase haven't been reported yet, while the optical properties of neutral GFP in different solutions has been reported experimentally [33]. To give some useful information for the possible such experiment in gas phase, and to compare the excited state properties of anionic and cationic GFP with those of neutral one, we also studied theoretically the ground and excited state properties of neutral GFP, DsRed(1) and DsRed (2).

From the calculated permanent dipole moments of neutral and cationic GFP, DsRed(1) and DsRed (2), there are two important information (see data in Table 4). One is with the increase of the extended conjugated system, the static (permanent) dipole

Table 4
The calculated transition energies (in nm) and oscillator strength (*f*) of neutral and cationic GFP, DsRed (1) and DsRed (2) with different methods

	Cationic			Neutral	
	Exp	Cal (ZINDO)		Cal (TD-DFT)	
	nm	*nm*	*f*	*nm*	*f*
GFP	406	420.07	0.8170	362.43	0.9513
DsRed(1)	441	445.05	0.8431	389.78	1.1104
DsRed(2)	448	461.13	1.1534	409.26	1.3791

moments are also increased along the molecular backbone, while the orientation of the permanent dipole moments of DsRed(2) is reversed, compared to those of GFP and DsRed(1). The other is that the permanent dipole moments of neutral and cationic GFP, DsRed(1) and DsRed (2) along the *y* axis is significant larger than those of GFP, DsRed(1) and DsRed (2) anions. The optimized geometries of neutral and cationic GFP, DsRed(1) and DsRed (2) are of the planar structures. The calculated transition energies of GFP, DsRed(1) and DsRed (2) cations in gas phase are consistent with the experimental results (see data in Table 4). The transition density and charge difference density of cationic and neutral GFP, DsRed(1) and DsRed(2) are listed in Fig. 4.

4. Conclusion

Photoabsorption properties of green and red fluorescent protein chromophore anions *in vacuo* were investigated theoretically. The calculated results have shown that with the increase of the extended conjugated system, the permanent

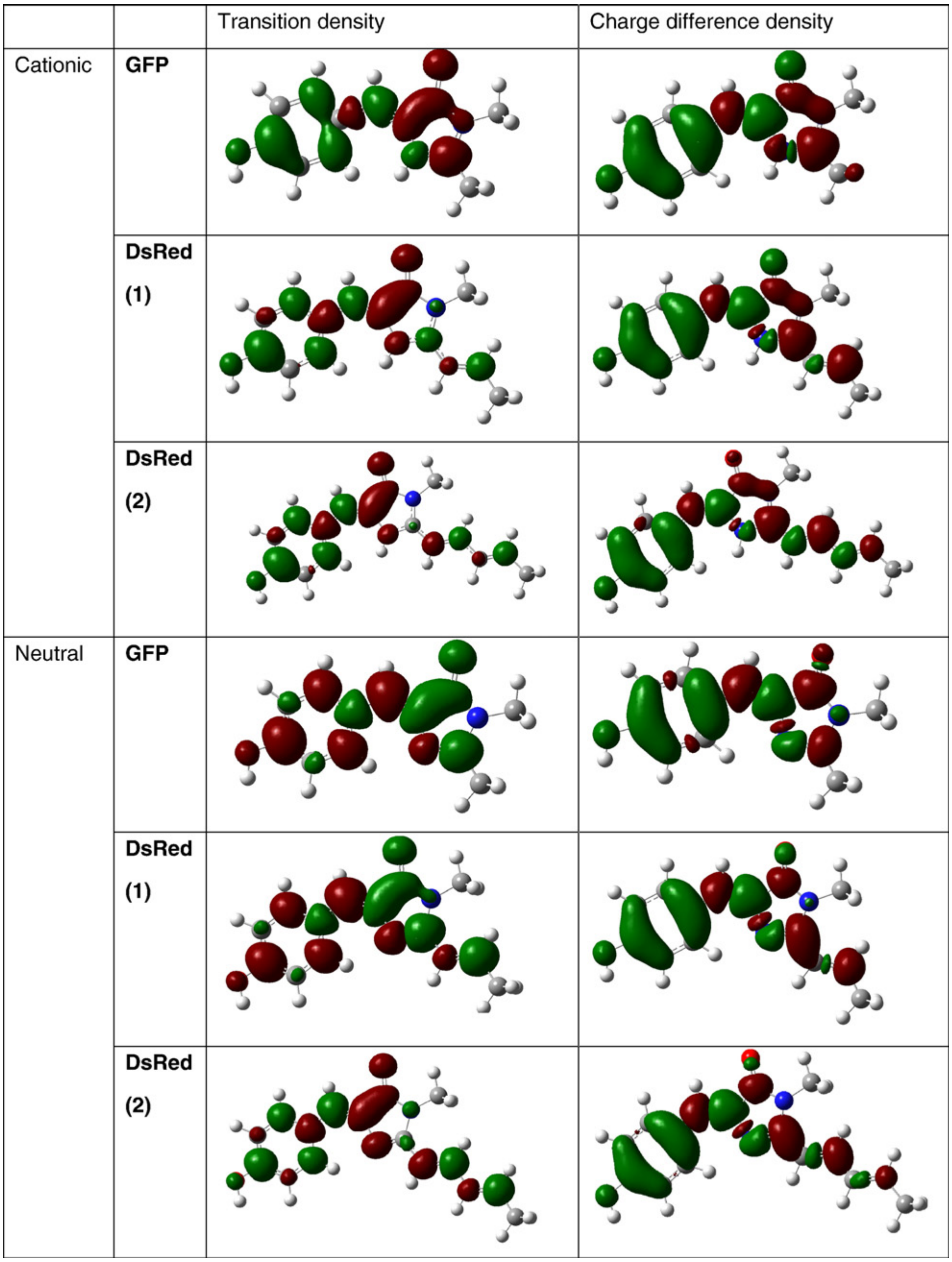

Fig. 4. The transition density and charge difference density for the cationic and neutral GFP, DsRed(1) and DsRed(2).

dipole moments at the ground and the excited states are also increased, and the bond lengths of C(10)–N(11) and N(11)–C(12) are strongly affected. The calculated results have also shown that with the increase of the extended conjugated system, the strength of the transition dipole moments are also increased, but the orientation of the transition dipole moment can be reversed when the extended conjugated system is large enough for DsRed(2), correspondingly, the orientation of electrons transfer also be changed. For comparison, the excited state properties of neutral and cationic GFP, DsRed(1) and DsRed(2) were also briefly studied.

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