Modeling Photoabsorption of the asFP595 Chromophore

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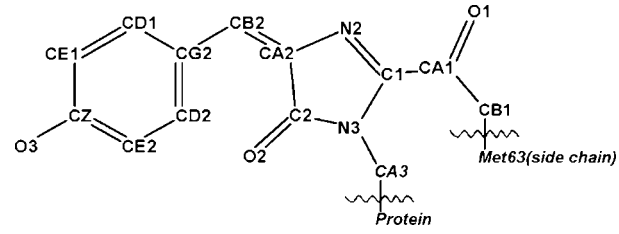
The fluorescent protein asFP595 is a promising photoswitchable biomarker for studying processes in living cells. We present the results of a high level theoretical study of photoabsorption properties of the model asFP595 chromophore molecule in biologically relevant protonation states: anionic, zwitterionic, and neutral. Ground state equilibrium geometry parameters are optimized in the PBE0/(aug)-cc-pVDZ density functional theory approximation. An augmented version of multiconfigurational quasidegenerate perturbation theory (aug-MCQDPT2) following the state-averaged CASSCF/(aug)-cc-pVDZ calculations is used to estimate the vertical S0−S1 excitation energies for all chromophore species. An accuracy of this approach is validated by comparing the computed estimates of the S0−S1 absorption maximum of the closely related chromophore from the DsRed protein to the known experimental value in the gas phase. An influence of the CASSCF active space on the aug-MCQDPT2 excitation energies is analyzed. The zwitterionic form of the asFP595 chromophore is found to be the most sensitive to a particular choice and amount of active orbitals. This observation is explained by the charge-transfer type of the S0−S1 transition involving the entire conjugated π-electron system for the zwitterionic protonation state. According to the calculation results, the anionic form in the trans conformation is found to possess the most red-shifted absorption band with the maximum located at 543 nm. The bands of the zwitterionic and neutral forms are considerably blue-shifted compared to those of the anionic form. These conclusions are at variance with the results obtained in the TDDFT approximation for the asFP595 chromophore. The absorption wavelengths computed in the aug-MCQDPT2/CASSCF theory are as follows: 543 (535), 470 (476), and 415 (417) nm for the anionic, zwitterionic, and neutral forms of the trans and cis (in parentheses) isomers of the asFP595 chromophore. These data can be used as a reference for further theoretical studies of the asFP595 chromophore in different media and for modeling photoabsorption properties of the asFP595 fluorescent protein.

Introduction

During the past decade the green fluorescent protein (GFP) and GFP-like proteins became widely used as fluorescent tags within living cells.[1−4] The protein asFP595 purified from *Anemonia sulcata* gained special attention because of its ability to be reversibly switched between nonfluorescent, off-state, and fluorescent, on-state.[5] The originally dark form of asFP595 with the absorption band maximum at 568 nm starts to emit at 595 nm upon intense irradiation with green light, the phenomenon known as a "kindling" effect.[6,7] The nonfluorescent state is eventually recovered from the kindled state with the half-life of 7 s at the room temperature.[5] The fluorescence can be also quenched by irradiation of the on-state of asFP595 with the blue light.[5−7]

Important data on the structure and the mechanism of photophysical conversion of the asFP595 protein are provided by X-ray analyses.[8−10] The chromophore group of the protein is formed upon cyclization of the amino acid residues Met63-Tyr64-Gly65 (MYG) and is extended by an additional double bond in comparison to the GFP chromophore. However, the X-ray crystallography cannot distinguish whether the imide or carbonyl fragment is attached to the imidazole ring formed upon cyclization.[8] The carbonyl type of the attached double bond was demonstrated only recently by the tandem mass spectrometry study of the denatured protein subjected to pepsin digestion.[11] The chemical structure of the asFP595 chromophore in its trans configuration with respect to the bridging CA2−CB2 double bond is depicted in Scheme 1.

SCHEME 1: Heavy Atom Representation of the asFP595 Chromophore (MYG)



The anionic, zwitterionic, and neutral states of MYG formed upon protonation/deprotonation of the O3 and N2 atoms were previously discussed as species involved in the photoswitching processes.[7,9,12−15] In line with initial suggestions by Chudakov et al.,[7] the X-ray studies[9] revealed that the chromophore of the

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A143S-mutated asFP595 protein undergoes cis−trans isomerization upon fluorescence kindling. Thus, MYG in the asFP595 dark state accepts the trans conformation,[8−10] while the chromophore of the A143S mutant in its on-state accepts the cis form.[9] However, despite previous efforts,[13−15] the absorbing and fluorescent species of the asFP595 protein are not yet unambiguously assigned to specific protonation states of the chromophore, and the mechanism of kindling is still unclear.

This work aims at the accurate theoretical description of the photoabsorption properties of the gas-phase asFP595 chromophore in various conformations and protonation states. These results should provide reference data for modeling effects of the protein or solvent environment on the chromophore photoabsorption in condensed phases.

Several theoretical studies of the asFP595 chromophore in the gas phase,[12,16,17] in solutions,[18] and in protein [13−15] were reported previously. Amat et al.[16] applied the time-dependent density functional theory (TDDFT) and the *N*-electron valence state perturbation theory, primarily in order to justify a particular cyclization scheme of the chromophore formation. Sun applied the TDDFT//DFT methodology for an analysis of charge transfer and transition density upon the S0−S1 excitation for the anionic and neutral forms of the cis isomer of the asFP595 chromophore.[17] Schäfer et al.[15] employed the SA-CASSCF approach within QM/MM molecular dynamics for studying the photoinduced dynamics of the asFP595 protein. It is worth noting that most theoretical studies of the asFP595 chromophore except refs 15 and 16 rely primarily on the results of the TDDFT approximation,[12−14,16−18] whereas the only studies based on multiconfigurational approaches employed CASSCF procedure with strongly reduced active spaces.[15,16]

Although the TDDFT method seems to be an inexpensive tool for excited-state calculations and performs efficiently for a large set of more than 100 organic dyes in solutions,[19] its applications to the GFP-related chromophores should be considered with caution.[20] First, asFP595 chromophore in anionic form absorbs in the long wavelength region of the visible spectra, where the errors of the TDDFT approach are expected to be higher.[19] Second, the role of charge-transfer excitations may be considerable for the charged or charge-separated systems (such as in anionic and zwitterionic forms of the asFP595 chromophore), and in this case the TDDFT approach is known to fail.[21−23] An alternative way for accurate calculations of excited states energies of medium-sized conjugated molecules is the use of the multireference (MR) approaches, in particular the MR perturbation theory.

In this work we employ an augmented version of the multiconfigurational quasidegenerate perturbation theory (aug-MCQDPT2) which is based on the MCQDPT2 formalism[24] to study the ground and first excited-state properties of the asFP595 chromophore. This approach[25] preserves all advantages of the original MCQDPT2 method in general implementation, in particular its ability to treat degenerate or nearly degenerate electronic states and electronic states with significant valence-Rydberg mixing at the CASSCF level.[24] Successful treatment of the latter cases with the MCQDPT2 approach is explained by the use of the effective Hamiltonian technique, which accounts for electronic state mixing under the influence of dynamic electron correlation through the effective Hamiltonian matrix. Moreover, the aug-MCQDPT2 technique takes into account additional dynamic electron correlation effects by considering a large number of interacting states. This technique was successfully applied previously for accurate calculations of vertical excitation energies of biological chromophores in different media and resulted in very close agreement with the experimental data on the positions of absorption band maxima.[25−27] To the best of our knowledge, it is the highest level of theory applied for modeling photoabsorption of the asFP595 chromophore.

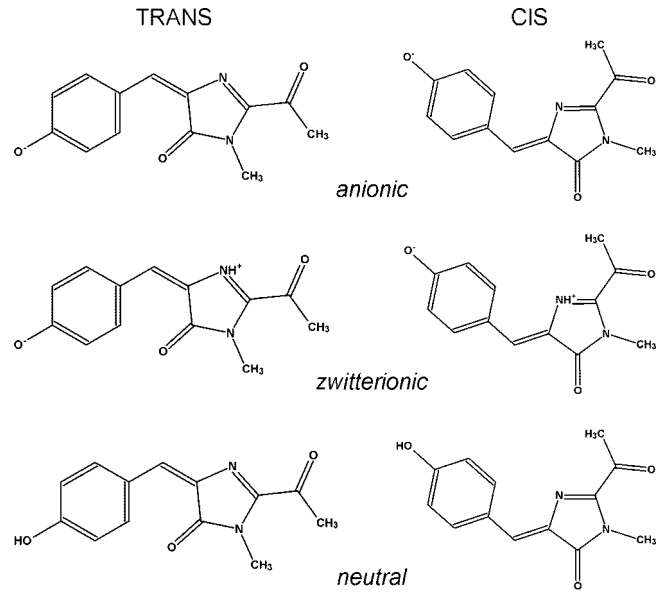

Figure 1. Model systems of the trans and cis MYG chromophore in its anionic, zwitterionic, and neutral forms.

The described computational scheme is used to model photoabsorption properties of cis and trans isomers of the MYG chromophore with the Met63 side chain and protein linkage being replaced by hydrogen atoms (Figure 1) in the anionic, zwitterionic, and neutral forms. We believe that these data are essential for understanding the photophysical properties of the asFP595 protein. We pay special attention to sensitivity of the predicted photoabsorption wavelengths to the active space variations within the multistate multireference perturbation theory approach.

Computational Details

The computations are performed by using the PC GAMESS program package[28] which is an Intel-specific version of the GAMESS(US) quantum chemistry program.[29]

Density functional theory approximation with the hybrid PBE0 functional and the correlation-consistent basis set augmented with diffuse functions on oxygen atoms, (aug)-cc-pVDZ, is employed for ground-state geometry optimization of all model systems shown in Figure 1. It should be noted that DFT with the hybrid-type B3LYP functional was previously demonstrated to provide geometry configurations of conjugated organic molecules, e.g. the retinal chromophore, close to that obtained with the CASPT2 approach.[30,31] Therefore, one can expect a similar accuracy obtained with the PBE0. We have verified that the computed stationary points refer to the true minima on the S0 ground-state potential energy surfaces with no imaginary frequencies. The atomic coordinates are presented in the Supporting Information.

The multistate multireference perturbation theory approach based on the state-averaged complete active space SCF (SA-CASSCF) solutions is used to estimate the vertical S0−S1 excitation energies. The full conjugated π-electron system of the asFP595 chromophore implies consideration of the CASSCF active space in which 18 electrons are distributed over 16 active orbitals. Such a computational scheme is not feasible, and therefore selection of the reduced active space is a subject of

primary concern in this work. Two types of reduced active spaces are considered: 16 electrons distributed over 14 orbitals (16/14) and 14 electrons distributed over 12 orbitals (14/12). We use natural orbitals obtained in preliminary calculations as starting orbitals for the SA-CASSCF computations. Specifically, we use the configuration interaction singles (CIS), perturbation theory MP2, and multiconfigurational SCF (MCSCF) (18/16) with only single and double excitations. Accordingly, 14 or 12 natural orbitals with the highest (for the vacant orbitals) and the lowest (for the occupied orbitals) occupancy values are set as active for the SA-CASSCF(16/14) or SA-CASSCF(14/12) computations, respectively. The images of orbitals included into the active spaces are presented in Supporting Information. Averaging of electron density in the SA-CASSCF procedure is performed over two states for the anionic form, over four states for the neutral form and over six and seven states for the trans and cis zwitterionic forms. Fifteen CASCI states obtained by using the described procedure comprise the reference space for the multistate multiconfigurational perturbation theory.

Application of multireference perturbation theories (MRPT) is a general way to account for dynamic electron correlation starting from the CASSCF solution. MRPTs are now widely used to modeling excited states of different biochromophores; see, for example, refs 25−27, 32−36. The state-specific MRPT approaches, such as the multireference Möller−Plesset perturbation theory (MRMP2) [37,38] and CASPT2,[39,40] correlate CASSCF states independently. The MRMP2 approach provides generalization of the MP2 formalism for the multiconfigurational reference states. Further extension can be achieved by using multistate multireference perturbation theories, e.g. MCQDPT2[24] and MS-CASPT2.[41] In the latter approaches, multidimensional reference space spanned by CASSCF wave functions and construction of an effective Hamiltonian matrix that couples reference states under the influence of dynamic electron correlation are used. The diagonalization of the effective Hamiltonian matrix provides corrected energies and wave functions which are now linear combinations of original reference states. MCQDPT2 with a single CASSCF wave function used as a reference state is very similar to the MRMP2 model. Since the CASSCF approximation results in the wrong ordering of the excited states for almost all species considered here, and the root flipping occurs upon addition of perturbation theory corrections, we use the MCQDPT2 approach.[24] The aug-MCQDPT2 technique implies construction of the effective Hamiltonian matrices with sequentially augmented dimensions of the reference space. Then the excitation energies obtained upon diagonalization of the effective Hamiltonian are plotted versus the dimension of the reference space. When a saturated solution with respect to the number of reference states is achieved, the excitation energy reaches a plateau, and the resulting value is claimed here as the aug-MCQDPT2 excitation energy. The typical plots for the trans isomers of anionic, zwitterionic, and neutral forms of the asFP595 chromophore computed here in the aug-MCQDPT2/CASSCF(16/14)//PBE0/(aug)-cc-pVDZ approach are presented in Figure 2. This technique and its application to computation of vertical excitation energies of various biological chromophores were described in refs 25−27. The S0−S1 transition dipole moments are computed for perturbation-modified CAS wave functions, i.e. a linear combination of CASCI reference states. Obtained values of transition dipole moments and excitation energies computed with aug-MCQDPT2 technique are used for oscillator strengths estimations.

To test the performance of the aug-MCQDPT2 technique with the reduced active spaces, we also consider the anionic form of the model chromophore DsRed (1) studied experimentally in refs 42 and 43. We use precisely the same approach for the ground-state geometry optimization and for estimates of vertical excitation energies as for the asFP595 chromophore.

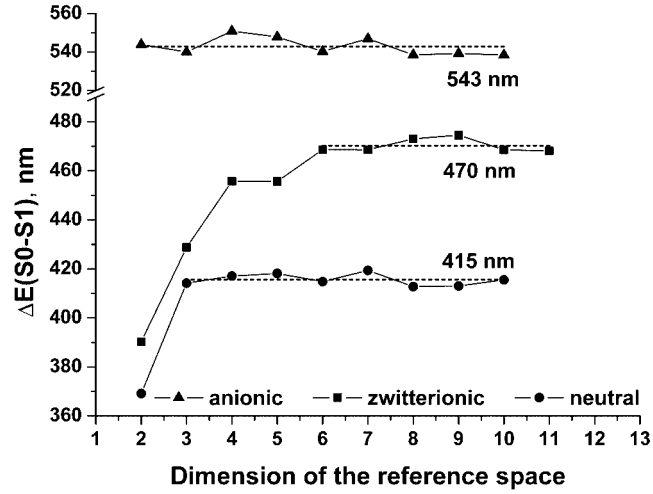

Figure 2. Dependence of the computed S0−S1 transition energies on the dimension of the reference space in the aug-MCQDPT2 technique for the trans isomers of anionic, zwitterionic, and neutral forms of the asFP595 chromophore.

Results and Discussion

Ground-State Properties. According to the results of the PBE0/(aug)-cc-pVDZ geometry optimization, the cis isomers of anionic, zwitterionic, and neutral forms are found to possess lower energies than the corresponding trans isomers. These conclusions are in line with the previous computational studies of the asFP595 chromophore[16,18] and closely related red fluorescent protein (RFP) chromophore in the anionic form.[44] The neutral and anionic MYG chromophores are planar in both cis and trans conformations, with the cis isomers being lower in energy by 2.7 and 2.3 kcal/mol, respectively. The asFP595 chromophore in the zwitterionic form is characterized by a less pronounced energy difference of 0.75 kcal/mol between the cis and trans isomers than those in the anionic and neutral forms. This is caused by nonplanarity of the cis isomer and therefore by a partial disruption of conjugation in the molecule. In turn, the nonplanarity is due to steric repulsion of hydrogen atoms bound to the N2 atom from the imidazolinone ring and to the CD2 carbon atom of the phenoxy ring (Scheme 1).

Another feature of the ground-state geometry configuration and of the electron density distribution that affects optical properties of the chromophore is the bond order alternation in the conjugated π-system. The computed bond orders for the ground electronic state of the chromophore in the anionic, zwitterionic, and neutral protonation states in the trans and cis conformations are presented in Figure 3. Both cis and trans isomers are found to have similar single/double bond distribution in the ground state. The different forms of the chromophore can be characterized by two most pronounced features in the bond order pattern: the phenoxy/quionone-type resonance and the degree of conjugation in the bridging region. The phenoxy ring accepts the quinone-like form in case of both anionic and zwitterionic forms, which is consistent with a superposition of three possible resonance forms with the negative charge located at one of three oxygen atoms of the conjugated system (Scheme 1). The similar single/double bond pattern was earlier described for the RFP chromophore in the anionic form on the basis of

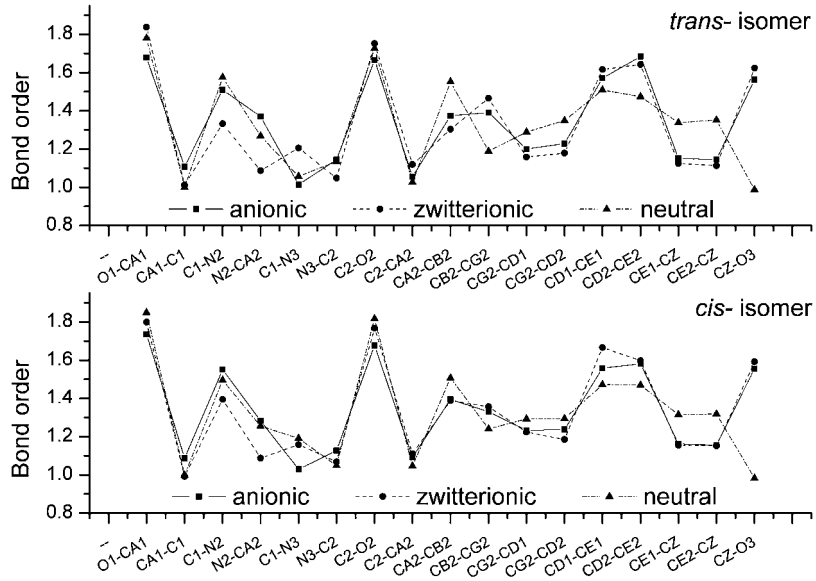

Figure 3. The bond orders of the trans and cis isomers of the asFP595 chromophore in the anionic, zwitterionic, and neutral forms according to the SA-CASSCF(16/14)//PBE0/(aug)-cc-pVDZ calculations.

CASSCF bond length alternation, and three resonance forms of the chromophore were also discussed.[44] The bond orders of two bridging C−C bonds are almost the same in the case of the anionic chromophore. The CA2−CB2 bond possesses larger single bond character while the CB2−CG2 bond assumes a larger double bond character for the zwitterionic form. This region of the chromophore is of particular interest in regard to photoisomerization processes that occur inside the protein matrix.[7,9] Protonation of the N2 atom in the case of zwitterions leads to an overall decrease in bond orders in the imidazolinone ring. The neutral form of the chromophore is characterized by a similar distribution of bond orders in the imidazolinone ring compared to that of the anionic form; however, it assumes a phenolic-like structure in the phenoxy region. The overall trends resemble those found previously for the B3LYP/6−31+G(d) optimized geometries of the cis isomer of the asFP595 chromophore in neutral and anionic forms.[17]

Vertical Excitation Energies. According to the preliminary CIS results, the S0−S1 excitation corresponds to the $\pi-\pi^*$ electron transition for all forms of the asFP595 chromophore. Therefore, the ground and excited state wave functions are obtained with the π-bonding and π^*-antibonding orbitals considered as active in the CASSCF calculations. The aug-MCQDPT2 performance was earlier tested for the related chromophore in which the terminal C(O)−CH_3 fragment was replaced by the methyl group (see Figure 1).[25] In this case the complete set of π and π^* orbitals was included into the CASSCF active space. The aug-MCQDPT2/CASSCF(16/14) approximation allowed us to obtain the vertical excitation energies for this model GFP chromophore very close to the observed absorption maxima: 489 and 398 nm as compared to the experimental values of 479 and 406 nm for the isolated anionic and cationic forms, respectively.[45]

For larger conjugated systems, as in case of the asFP595 chromophore, we have to consider the reduced active spaces at the CASSCF level. Therefore, a detailed analysis of the performance of the CASSCF-based aug-MCQDPT2 approach with different choices of reduced active spaces should be carried out. We present here the estimates of the S0−S1 vertical excitation energies with the aug-MCQDPT2 technique for the (16/14) and (14/12) distributions (Table 1).

TABLE 1: Comparison of S0−S1 Vertical Excitation Energies Computed in the aug-MCQDPT2/CASSCF//PBE0/(aug)-cc-pVDZ Approximation with Different Active Spaces to the Earlier Theoretical Estimates for the Anionic, Zwitterionic, and Neutral Forms of the MYG Chromophore in Cis and Trans Conformations

		TDDFT (B3LYP)	PC-NEVPT2[e]	aug-MCQDPT2/CASSCF (14/12)[f]	aug-MCQDPT2/CASSCF (16/14)[f]
anion	cis	484,[a] 480,[b] 485,[c] 479[d]	486	574	535
	trans	452[a]	571	537	543
zwitterion	cis	521[a]	-	504	476
	trans	486[d]	-	481	470
neutral	cis	429,[a] 413,[b] 406,[c]	372	403	417
	trans	-	366	394	415

[a] B3LYP/6−311++G(2df,p)//B3LYP/6−31+G(d,p), from ref 18. [b] B3LYP/6−31+G(d)//B3LYP/6−31+G(d), from ref 17. [c] B3LYP/SV(P)//B3LYP/6−31+G(d), from ref 17. [d] B3LYP/6−31+G(d)//B3LYP/6−31+G(d), from ref 13. [e] *N*-electron valence state perturbation theory (NEVPT2(8/7))/6−31G(d)//B3LYP/6−31G(d), from ref 16. [f] This work.

Although no direct reference experimental data on the absorption bands for any of the asFP595 chromophore forms in the gas phase are available, one can verify the performance of the aug-MCQDPT2/CASSCF(16/14)//PBE0/(aug)-cc-pVDZ methodology by comparing the computed S0−S1 vertical excitation energy for the closely related model DsRed chromophore to the observed absorption maxima wavelength in the gas phase.[42,43] The π-system of the latter compound closely resembles that of the asFP595 chromophore with the only exception: the C2−O2 double bond is replaced by the C−C double bond for DsRed model chromophore. Although the direct comparison with the experiment is possible only for the anionic form of the chromophore, we believe that the accuracy obtained for other protonation states should be the same. The performance of the aug-MCQDPT2 technique applied for modeling neutral forms of biochromophores was demonstrated in our earlier studies.[26] Also, it is known from the literature that the multistate CASPT2 approach, which is close in accuracy to MCQDPT2, allows a description of electron spectra of zwitterionic species

of diamino-*m*-quinonoid molecules.[46] The observed position of the absorption maximum for the cis isomer of DsRed chromophore in the anionic form corresponds to the wavelength of 521 nm,[42,43] which is close to the aug-MCQDPT2/CASSCF(16/14) value 540 nm. Because of the chemical similarity of these two chromophores, the same accuracy of the aug-MCQDPT2/CASSCF(16/14) approach is expected for the asFP595 chromophore. Therefore, the technique is able to provide quantitative estimation of the asFP595 chromophore absorption maxima position within about 20 nm.

The relative positions of the absorption maxima of the biologically relevant anionic and zwitterionic forms of the asFP595 chromophore are discussed in several theoretical studies.[13−15,18] The main absorption band of the asFP595 protein at 568 nm and a shoulder at 550 nm in the experimental spectra is ascribed to absorption of the zwitterionic and anionic forms respectively by Schäfer et al.[14] This assignment is made on the basis of the TDDFT modeling of absorption spectra of the asFP595 protein within the QM/MM framework. The TDDFT method applied for estimates of vertical excitation energies of the gas-phase chromophore also results in the vertical excitation energy for the zwitterionic form of MYG that was red-shifted in regard to that of the anionic species [13,18] (Table 1). Therefore, the TDDFT results are in strong variance to the present aug-MCQDPT2 conclusions since the inverse relative position of the absorption bands of anionic and zwitterionic chromophores is obtained with TDDFT (Table 1). Although the shift of the absorption maxima between the anionic and zwitterionic forms of the chromophore at the TDDFT level of theory is quite small [13,18] (Table 1), the wrong relative positioning can cause misleading interpretation of the asFP595 experimental spectra. The higher vertical excitation energy value for the zwitterionic form of the chromophore in comparison to the anionic form can be speculatively explained by an analysis of charge distribution along the conjugated system for the ground and the first excited states. To illustrate the charge localization in the chromophore, the latter may be subdivided into two parts: one contains the phenoxy ring with the bridging CB2 atom (part I) and the other includes the imidazole ring (part II) (Figure 4). Part II accommodates most of the negative charge (−0.74) in the ground electronic state whereas only −0.56 is located at this moiety in the first excited state for the anionic form of the chromophore. Therefore, addition of the positively charged hydrogen atom at the imidazole ring (zwitterionic form) would stronger stabilize the ground state than the first excited state, thus causing an increase of the vertical excitation energy. The presented considerations provide additional arguments in favor of the aug-MCQDPT2 results. Consequently, an accuracy of the TDDFT approach in this case does not allow one to distinguish between the anionic and zwitterionic forms and correctly assign the experimental absorption maxima when relying only on the TDDFT computations.[13] The failure of TDDFT to provide correct ordering the excitation energies of the anionic and zwitterionic forms of the isolated asFP595 chromophore makes questionable an assignment of the main observed absorption band for the asFP595 protein to the zwitterionic form of the chromophore as formulated in ref 14 following the TDDFT-based QM/MM modeling of the protein absorption spectra. There are two possible reasons why the TDDFT approach significantly underestimates the S0−S1 excitation energy for the zwitterionic chromophore. First of all, as it follows from Figure 5, the S0−S1 excitation of the zwitterion is accompanied by a strong electron density transfer from the phenoxy ring to imidazolinone moiety, and therefore

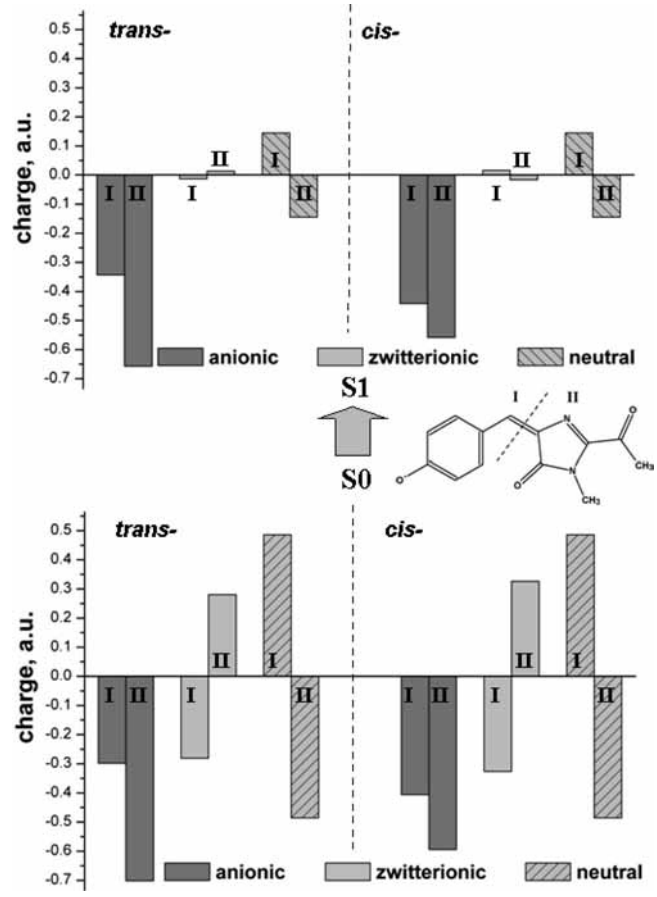

Figure 4. Atomic charges distribution for the ground S0 and the first excited S1 state for anionic, zwitterionic, and neutral forms of the chromophore obtained with the aug-MCQDPT2/CASSCF(16/14) zero-order electron density.

this is the case when the TDDFT is known to fail.[21−23] Another reason for an inadequate performance of TDDFT for zwitterionic species can be caused by a nearly degeneracy of the S1 and S2 excited states. We illustrate the latter statement by the results of MRMP2 calculations presented in the Supporting Information.

Although the aug-MCQDPT2 results with the 16/14 distribution provide an accurate description of the absorption band maximum for the asFP595 chromophore in the gas phase, the computational cost of this methodology is rather high. Hence, we tried to test the sensitivity of the obtained results to the further decrease of the active space in the aug-MCQDPT2 procedure. For this purpose, the aug-MCQDPT2/CASSCF(14/12) computations were carried out, and the best estimates of absorption band maxima for all considered forms of the chromophore are presented in Table 1. Although vertical excitation energies computed with the aug-MCQDPT2/CASSCF(14/12) technique qualitatively reproduce the order of absorption band maxima for the anionic, zwitterionic, and neutral forms, these results do not provide quantitative estimates. Indeed, one can compare the vertical excitation energies obtained for the (16/14) and (14/12) active spaces for the anionic cis isomer in which the discrepancy is as much as 40 nm. When this paper was under review, the results of MS-CASPT2 study on the absorption of the isolated asFP595 chromophore in anionic form were published.[47] The MS-CASPT2/CASSCF(4/3) S0−S1 vertical excitation energies of 534 and 514 nm[47] for cis and trans isomers, respectively, are quite close to values obtained in this work (Table 1). The discrepancy in the excitation energies for the trans isomer can be explained by the smaller CASSCF active space used by Olsen and Smith.[47]

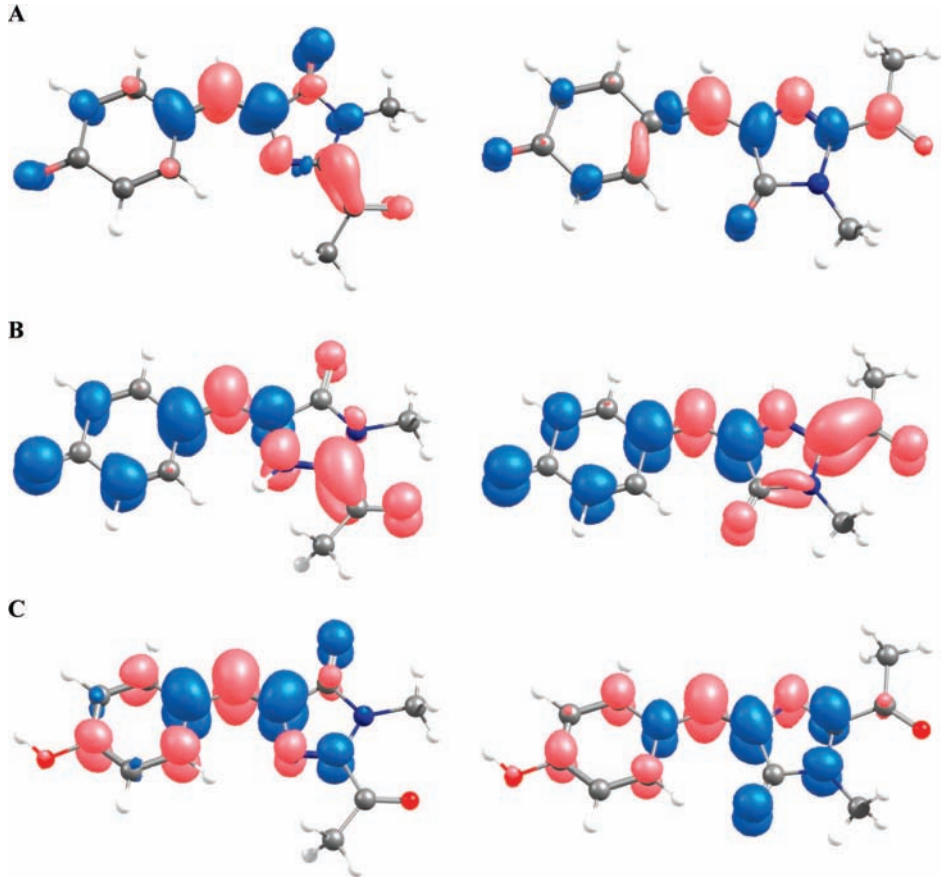

Figure 5. Differential aug-MCQDPT2/CASSCF(16/14) electron density for S1 and S0 state of anionic (A), zwitterionic (B), and neutral (C) protonation states of the asFP595 chromophore, where red and blue colors refer to the positive and negative sign, respectively. The left panel corresponds to the chromophore in cis conformation; the right panel corresponds to the trans conformation of the chromophore.

The zwitterionic form of the asFP595 chromophore is found to be the most sensitive to the particular choice of the active orbitals: the vertical excitation energies obtained for different active orbitals within the aug-MCQDPT2/CASSCF(14/12) approach varied from 406 to 504 nm. Such a strong influence from orbital changes within the active space for the zwitterionic form can be explained by an analysis of the electron density redistribution upon the S0−S1 excitation obtained as a difference of the S1 and S0 aug-MCQDPT2/CASSCF(16/14) zero-order densities (Figure 5). The anionic form of the chromophore is characterized by an electron density transfer from the phenoxy ring to the imidazolinone ring. However, the dominant redistribution occurs in the bridging region. In the case of the zwitterionic chromophore, the electron density transfer to the imidazolinone ring is strongly increased and is extended to the CA1═O1 double bond of the chromophore in comparison to the anionic form. The neutral asFP595 chromophore is characterized by electron density transfer in the opposite direction compared to the anionic and zwitterionic species in contrast to the results presented by Sun.[17] In this case, the redistribution is localized in the central part of the conjugated system and does not involve the terminal CA1−O1 double bond and the phenoxy oxygen atom (Figure 5). According to these observations, it can be expected that the zwitterionic chromophore with the entire conjugated system being involved in the electron density transfer upon excitation should be the most sensitive system to the reduction of the active space.

The data of Table 2 demonstrate that all protonation states of the chromophore in both cis and trans conformations possess high oscillator strength values for the S0−S1 transitions, and therefore these bands should be easily spectrally detectable. It is worth noting that despite a somewhat higher amount of charge transfer in the neutral and zwitterionic chromophores, these forms are characterized by smaller values of the transition dipole moment in comparison to the anionic protonation state. This may be explained by the decreased overlap of the ground- and excited-state wave functions for the zwitterionic and neutral forms of the chromophore.

TABLE 2: Transition Dipole Moments (μ_T) and Oscillator Strengths (f) According to the aug-MCQDPT2/CASSCF(16/14)/(aug)-cc-pVDZ Calculations

		μ_T, D	f
anion	cis	4.02	0.918
	trans	5.05	1.482
zwitterions	cis	3.15	0.634
	trans	2.47	0.395
neutral	cis	3.38	0.780
	trans	3.72	0.989

Conclusions

The choice of an adequate quantum chemistry method for simulations of the chromophore properties in the gas phase should precede simulations in the condensed phases. In this paper we present the high level quantum chemistry estimates of the vertical excitation energies for all relevant protonation states of the isolated chromophore of the asFP595 photoswitchable protein. On the basis of the presented results, we conclude that the aug-MCQDPT2/CASSCF(16/14)//PBE0/(aug)-cc-pVDZ methodology allows us to provide accurate estimates of absorption band maxima of the asFP595 chromophore. Therefore, this

level of a theoretical treatment should be suitable for the QM/MM studies of the environmental effects on the chromophore photoabsorption properties which are presently under consideration. The slightly simplified aug-MCQDPT2/CASSCF(14/12)//PBE0/(aug)-cc-pVDZ approach permits a qualitative prediction of relative ordering of absorption maxima for the different forms of the chromophore: anionic, zwitterionic, and neutral. The zwitterionic form of the asFP595 chromophore is shown to be the most sensitive to reduction of the number of active orbitals and their particular choice because of the charge-transfer character of S0−S1 excitation that involves the entire conjugated system of the chromophore. The longest absorption wavelength is assigned to the anionic protonation state while the zwitterionic and neutral forms are blue-shifted. This contradicts the relative ordering of the absorption bands of the zwitterionic and anionic chromophore obtained at the TDDFT level of theory. The latter predicts the zwitterionic form as the most red-shifted absorbing species.[13,18] In addition, the TDDFT approach results in the same order of the absorption band for the gas-phase chromophore and the protein.[13] Therefore, modeling photoabsorption properties of the asFP595 chromophore with the TDDFT approach both in the gas phase and in the condensed phases can cause misleading results. The vertical excitation energies of the chromophore obtained at the aug-MCQDPT2/SA-CASSCF(16/14) level of the theory are 543 (535), 470 (476), and 415 (417) nm for the anionic, zwitterionic, and neutral forms of the trans and cis (in parentheses) isomers. These data can be used as a reference for future studies of the role of the protein environment in the modulation of chromophore optical properties.

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Supporting Information Available: The optimized geometries of anionic, zwitterionic, and neutral protonation states of the asFP595 chromophore in cis and trans conformations. Active space natural orbitals according to CASSCF(16/14)/(aug)-cc-pVDZ and CASSCF(14/12)/(aug)-cc-pVDZ computations for all considered model systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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