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The Chromophore of asFP595: A Theoretical Study

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We investigate the electronic and structural properties of the chromophore of the asCP/asFP595, a newly discovered protein of the (green) fluorescent protein family. The use of theoretical methods with different degrees of accuracy and efficiency (DFT, TDDFT, CASSCF and perturbative corrections) allows us to compare the properties of a large number of hypothetic molecular models for the chromophore. The models are sorted on the basis of the relative stability and through a comparison with the experimental values of the excitation energy. Our study indicates that the most probable structure of the photoactive moiety in the protein and in water is the one resulting from the GFP-like rather than the "alternative" cyclization scheme.

1. Introduction

The green fluorescent protein from the *Aequorea victoria* (avGFP) jellyfish[1,2] has been widely used in the past decades in molecular biology as a marker for gene expression and protein localization in living cells.[3] The strong efforts of genetical engineering of the last years have produced a large variety of mutants of avGFP, covering the visible spectrum from blue to yellow. The red shift that can be achieved by mutation is limited;[4−7] however, several tens of new GFP homologues have been recently cloned[7,8] with absorption and emission wavelengths in the red region of the spectrum.[9,10] All of these seem to have evolved from the same common ancestor as avGFP[8] and share the same tertiary structure although the quaternary structure can vary from monomeric to tetrameric.

A very peculiar member of the family is the recently discovered asCP562/asFP595 from *Anemonia sulcata*, which is among the most red-shifted proteins in the family.[11,12] Its double name is due to the fact that two forms with different spectra can be extracted from *Anemonia*: the nonfluorescent purple protein asCP562 and the (weakly) fluorescent asFP595. These were shown to have the same molecular weight and sequence and can be interconverted upon denaturation−renaturation.[13] Furthermore, it is possible to pass from the chromo (nonfluorescent) to the fluoro form by illuminating with green and blue light, respectively,[11,12] and the chromo and fluoro forms can be stabilized by specific mutations in the chromophore environment.[12,14,15] The fluoro/chromo transition was hypothetically attributed to a cis−trans isomerization of the chromophore.[16]

The chromophore forms autocatalytically as in GFP, through a cyclization of the three amino acids in position Met65-Tyr66-Gly67 (residue numbering with respect to GFP sequence alignment), but at variance with GFP the formation is followed by a fragmentation of the protein chain in two parts.[12] On the basis of a structural chemical study revealing that the cleavage occurs through hydrolysis of the Cys64-Met65 peptide bond,[13] a cyclization mechanism "alternative" with respect to the GFP cyclization was initially proposed by Martynov et al.[13] and is represented in Figure 1a. According to this scheme, the cyclization would occur through dehydration of Tyr66 carbonyl oxygen and hydrogen of Met65 nitrogen, leading to a six-atom heterocycle in the chromophore. A subsequent maturation would include hydrolysis of the Cys-Met bond and tautomerization. Conversely, Zagranichny et al.[17] proposed a "traditional" (GFP-like) cyclization scheme (see Figure 1b) based on chemical analysis and NMR spectroscopy. In this scheme the cyclization leads to a GFP-like chromophore but is followed by a cleavage of the Cys64-Met65 peptide bond. Two forms of cleaved peptide deriving from the denatured protein are observed in water, namely, asFP595II and asFP595III in Figure 1b. This cyclization scheme was further supported by the X-ray structures of the wild-type asFP595 by Andresen and co-workers[18] and of the A148G mutant of asFP595 (named KFP), released by Wilmann et al.[19] and by Quillin et al.[20] asFP595III seems to be rather unstable in water and rapidly converts to asFP595II. Although asFP595II is the structure of the chromophore in the protein hypothesized by Quillin,[20] the actual attribution is still being discussed, since Wilmann and Andresen modeled the chromophore as asFP595III.

We observe (see Figure 1) that the forms from Zagranichny do not have the same stoichiometry as the Martynov form, since in the Zagranichny scheme the maturation includes an additional oxidation of the C_α carbon of Met65, with the elimination of two hydrogen atoms.

The chromophore containing pentapeptide absorbs at 430 nm and at 535 nm in acid and basic conditions, respectively, with $pK_a = 6.8$.[13] These peaks were attributed to cationic−neutral or to neutral−anionic forms by Martynov and Zagranichny, respectively. In both cases the transition was attributed to the deprotonation of the phenolic oxygen. Zagranichny et al. also observed the absorption and emission of the neutral and anionic forms of asFP595III, 420 and 520 nm, respectively. Within the scheme proposed by Martynov, the large red shift in the absorption with respect to GFP chromophore would be due to

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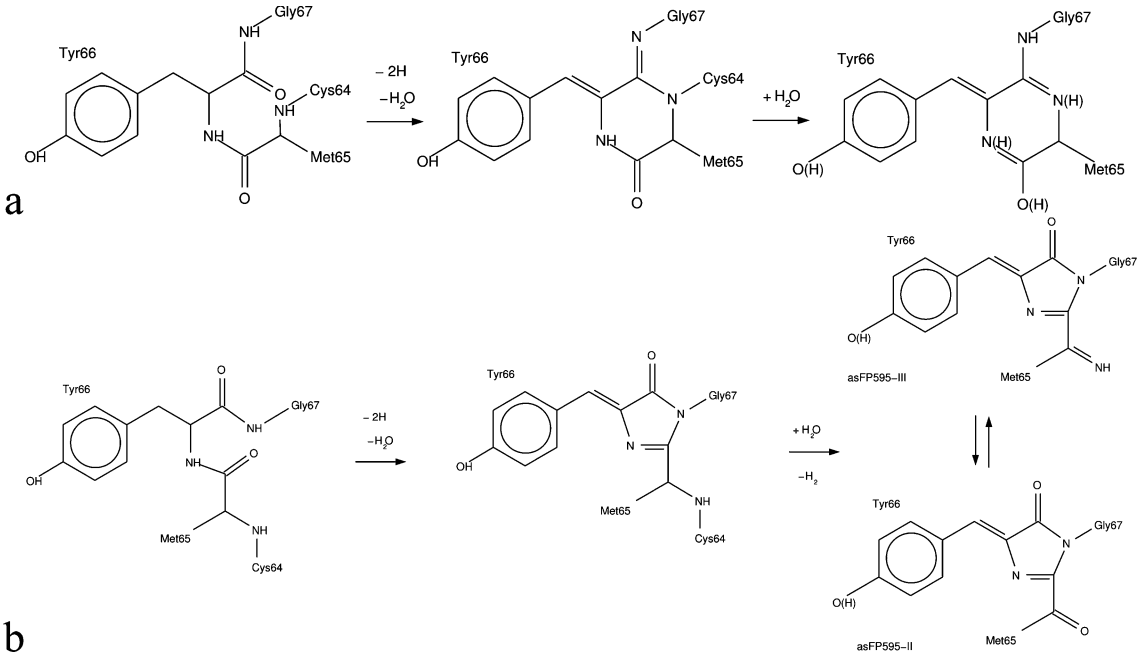

Figure 1. Chromophore formation: (a) according to Martynov[13] and (b) from Zagranichny's data.[17,21]

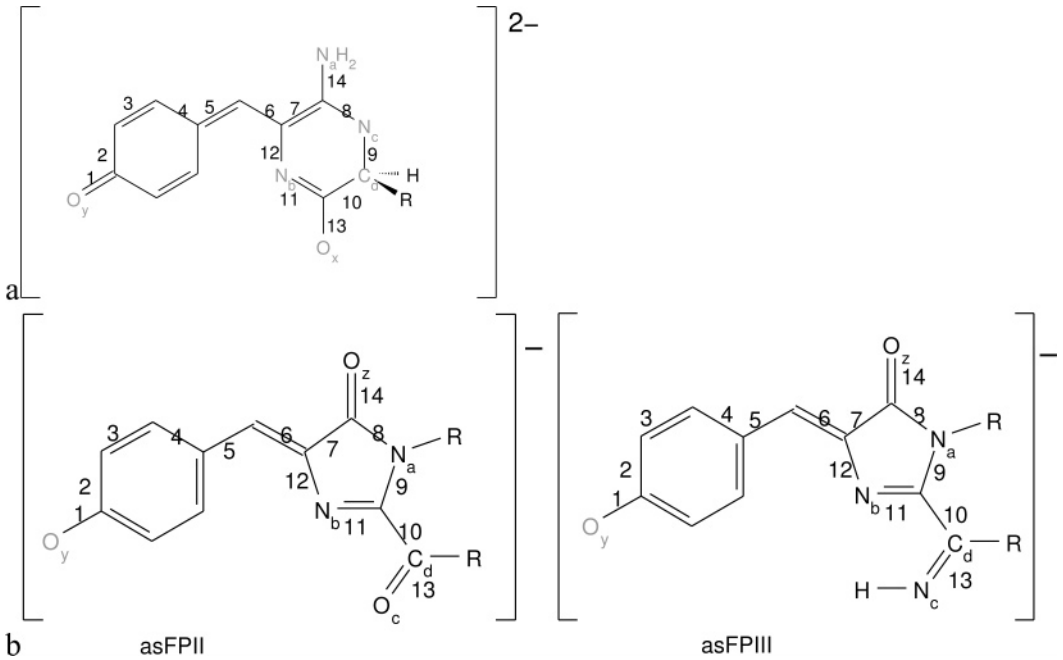

Figure 2. Model systems derived according to (a) Martynov's scheme and (b) Zagranichny's scheme. The atoms whose protonation is varied are represented in gray. R = CH_3 or H in the first or second set of models, respectively.

the different chemical structure of the chromophore, while for Zagranichny an acylimine bond would form on a GFP-like chromophore after cyclization, similarly to DsRed,[22] thus extending the π-bond conjugation.

In this paper we report a theoretical study based on density functional theory (DFT) and more accurate ab initio methods, aimed at clarifying some geometrical and electronic properties of the chromophore of asFP595. For the sake of completeness and in order to set up an instructive comparison, our study includes the neutral and anionic forms of the two structures proposed by Zagranichny and several different neutral, cationic, and anionic protonation forms of the structure formerly hypothesized by Martynov.

2. Model Systems

According to the indications from absorption data given in refs 13 and 17, we considered cationic, neutral, zwitterionic, and anionic forms from Martynov's model, and neutral and anionic for the two Zagranichny forms. A bianionic structure, corresponding to the "f " form of ref 13 was also considered. Figure 2 represents in panels a and b our model systems for the structures hypothesized by Martynov and Zagranichny, respectively. For each structure, two models were employed, thus generating two sets. The model systems were obtained by substituting, in the Martynov's structures, C_α of Gly67 and the atoms connected to it with a hydrogen, while the side chain of Met65 was substituted with a methyl group or a hydrogen (in

TABLE 1: Protonation of the Different Structures Analyzed[a]

cation1	N_aH_2	O_yH	N_b	N_cH	O_xH	C_dHR
cation2	N_aH_2	O_yH	N_bH	N_cH	O_x	C_dHR
neutral1	N_aH_2	O_y	N_b	N_cH	O_xH	C_dHR
neutral2	N_aH_2	O_yH	N_b	N_c	O_xH	C_dHR
neutral3	N_aH_2	O_yH	N_b	N_cH	O_x	C_dHR
neutral4	N_aH_2	O_yH	N_bH	N_c	O_x	C_dHR
neutral5	N_aH_2	O_y	N_bH	N_cH	O_x	C_dHR
neutral6	N_aH_2	O_yH	N_b	N_cH	O_xH	C_dHR
neutral7	N_aH_2	O_yH	N_bH	N_cH	O_x	C_dHR
neutral8	$N_aH_3^+$	O_yH	N_b	N_c	O_x^-	C_dHR
neutral9	$N_aH_3^+$	O_y	N_b	N_cH	O_x^-	C_dHR
neutral10	$N_aH_3^+$	O_y^-	N_bH	N_c	O_x	C_dHR
neutral11	$N_aH_3^+$	O_y^-	N_b	N_c	O_xH	C_dHR
neutral12	N_aH_2	O_y	N_bH	N_cH	O_xH	C_dR
anion1	N_aH_2	O_y^-	N_bH	N_c	O_x	C_dHR
anion2	N_aH_2	O_yH	N_b	N_c	O_x^-	C_dHR
anion3	N_aH_2	O_y^-	N_b	N_c	O_xH	C_dHR
anion4	N_aH_2	O_y	N_b	N_cH	O_x^-	C_dHR
bianion	N_aH_2	O_y^-	N_b	N_c	O_x^-	C_dHR
asFPII	N_aR	O_yH	N_b	O_c	O_z	C_dR
asFPIII	N_aR	O_yH	N_b	N_cH	O_z	C_dR
asFPIIanion	N_aR	O_y^-	N_b	O_c	O_z	C_dR
asFPIIIanion	N_aR	O_y^-	N_b	N_cH	O_z	C_dR

[a] R = CH_3 or H in the first or second set of models, respectively. Labels of the heteroatoms of the chromophore are referred to Figure 2. For the sake of clarity, the protonation state of some heteroatoms is indicated in the table even if it is not variable.

the first or second set of models, respectively); from the list of all possible protonation forms for Martynov's hypothesis, those protonated both on N_b and O_x where excluded. The models for Zagranichny's structures were obtained substituting both C_α of Gly67 and the side chain of Met65 either with a methyl group or with a hydrogen each (in the first or second set of models, respectively); only deprotonation on the phenolic oxygen was considered for these structures.

The need for a second, simpler set of models was due to limitations in the implementation of the program code performing the perturbation on the energy.

All the trans isomers with respect to bond 6 (see Figure 2) were also considered. The complete set of models considered is reported in Table 1.

3. Computational Methods

All geometries were optimized within the DFT framework; plane waves (PW) and localized basis (LB) approaches were used for the expansion of the wave functions. In the PW approach we optimized the geometries of the first set of models using the CPMD code[23,24] with the Becke−Lee−Yang−Parr (BLYP) exchange and correlation functional,[25,26] Troullier−Martins pseudopotentials[27,28] and orthorhombic simulation boxes with cell sides of 11−12 Å, to leave at least 5 Å of empty space between periodic images. Fictitious interaction between periodic images was removed.[29] In the LB approach, geometry optimizations were run on the second set of models with the Gaussian program[30] with the B3LYP functional and the 6-31G* basis set.

Ground-state energies were evaluated for the first set of models with DFT methods using the CPMD[24] and ADF 2002.3[31] packages. With ADF we used the BLYP functional and the TZP basis set. Ground-state energies for the second set of models were calculated using the Dalton 1.2[32] and the Gamess[33] packages. With Dalton we computed a CAS(8/7) wave function using the 6-31G* basis set, and we corrected the energies by second-order partially contracted *N*-electron Valence state Perturbation Theory (NEVPT2).[34,35] With Gamess we used the same basis set and CAS as with Dalton, and we performed a calculation with the polarizable continuum model (PCM) to determine the solvent effect on the relative stabilities of the different structures.

Excitation energies were computed, for the first set of models, with time-dependent DFT (TDDFT) and the BLYP functional. The PW calculations were performed within linear response and Tamm−Dancoff approximation[36] as implemented in the CPMD code,[23,24] while in the LB approach excitation energies were calculated with ADF code,[31] within linear response TDDFT[37] for singlet−singlet transitions and TZP basis set. For the second set of structures, excitation energies were evaluated as differences between the excited and the ground-state energies, computing the CASSCF wave function with the same basis as for ground states, estimating the NEVPT2 correction to the energies (with Dalton) or the PCM solvent effect (with Gamess).

4. Geometries and Stability in Vacuum

The neutral optimized structures can roughly be divided into two groups, depending on the computed bond length (alternate single/double bond) pattern they present (see Supporting Information, Tables 1S and 2S). These two subsets correspond approximately to the phenol/quinonoid and phenone/benzenoid forms of the two rings of the chromophore. Both cis and trans anionic forms of asFP-II and asFP-III show an overall good agreement with the X-ray structures of wild type asFP595[18] and KFP[20] (see Supporting Information). The close similarity between our asFP-III model and Quillin's chromophore structure (although modeled as asFP-II) indicates that the chromophore in the native protein has a phenolate and a heterocyclic ring like those of asFP-III, as suggested by Zagranichny.[17]

In Table 2 the (relative) ground state energies of the optimized structures are reported. Comparison of the energies of differently charged structures is not reliable. Within groups with the same charge state, however, a reference structure is chosen and the relative energy of models with the same charge state and different stoichiometry is evaluated using the thermodynamic cycle represented in Figure 3.[38] Thus formation energies of Martynov's and Zagranichny's models can be compared. Table 2 shows that Zagranichny's neutral structures are more stable in vacuo than Martynov's. The same is true for anionic structures as well. asFP-III higher energy, however, is in accordance with Zagranichny's observation that this structure may easily be hydrolyzed.

The energies evaluated within the PW (with CPMD) and the LB set of Slater-type orbitals (with ADF) and Gaussians (with Gaussian) display an average difference of about 1.4 kcal/mol for the same cis structures. Conversely, for the trans structures Gaussian LB results are systematically smaller, of about 4 kcal/mol. This difference can be attributed to the use of two different models rather than to a basis set bias. In fact, the sterical hindrance of the methyl group influences the cis and trans isomers in a different way. A direct comparison between cis and trans energies indicates that cis configurations are on average 7−8 kcal/mol more stable in the complete model (PW), while they are on average only 3−4 kcal/mol more stable in the simplified models (Gaussian LB).

The Gaussian LB calculations confirm that asFP-II and asFP-II anion are the most stable structures among neutral and anionic structures, respectively. Despite relevant numerical differences in the relative ground-state energies with different methods, this picture is further confirmed by all the ab initio CASSCF calculations.

The simulation of the solvation effect within the PCM (Table 2) shows that the relative ground state stabilities are generally

TABLE 2: Ground State Relative Energies[a]

	ΔE_{cis} (kcal/mol)						$\Delta E_{cis/trans}$ (kcal/mol)				
method code set of models	DFT CPMD first	DFT ADF first	DFT Gaussian second	CASSCF Dalton second	PC-NEVPT2 Dalton second	CASSCF/PCM Gamess second	DFT CPMD first	DFT Gaussian second	CASSCF Dalton second	PC-NEVPT2 Dalton second	CASSCF/PCM Gamess second
cation1	0.00	0.00	0.00	0.00	0.00	0.00	9.10	5.12	2.54	3.74	4.00
cation2	−0.30	−3.14	−3.96	−5.12	5.12	−10.20	4.89	3.86	2.00	2.47	2.41
neutral1	0.00	0.00	0.00	0.00	0.00	0.00	1.58	2.05	1.98	1.97	3.49
neutral2	−11.21	−14.11	−16.11	−21.37	−25.78	−17.36	7.60	5.05	4.39	4.00	5.06
neutral3	−1.53	−3.50	−2.09	0.32	−7.62	−15.99	10.47	8.22	7.67	7.08	5.83
neutral4	−18.57	−21.49	−25.50	−32.36	−35.66	−30.92	6.22	4.25	4.47	4.11	4.72
neutral5	−6.99	−7.84	−7.93	−7.32	−6.04	−9.97	5.70	0.46	−0.60	−0.12	0.64
neutral6			−9.66	−14.98	−18.86	−12.75		5.79	8.89	8.84	9.89
neutral7			−20.42	−27.42	−30.01	−26.49		4.74	8.34	7.86	8.27
neutral8			29.72	36.86	21.52	9.46		1.95	5.92	0.23	9.11
neutral9			49.13	48.27	54.41	21.48		2.93	4.91	−5.60	8.21
neutral10			26.20	34.45	19.25	8.51		−6.30	−4.24	−5.13	7.26
neutral11			34.69	40.27	29.63	19.47		−6.09	−1.67	−7.29	8.09
neutral12			20.56	22.81	23.18	22.42		3.66	1.57	3.78	2.76
anion1	0.00	0.00	0.00				8.46				
anion2	12.70	11.48	12.17				11.09				
anion3	9.50	12.84	13.84				10.52				
anion4	19.24		23.89				7.15				
asFP−II			−36.91				1.61	2.07	3.22	2.52	3.44
asFP−III			−3.02				1.80	6.28	2.85	2.24	3.85
asFP−IIanion			−31.87				1.27	1.71	2.53	1.51	2.57
asFP−IIIanion			15.64				2.75	1.74	2.60	1.78	2.78

[a] All energies are in kcal/mol. The energies of the cis forms are referred to the first model of each charge group (cation1, neutral1, and anion1). The energies of the trans form are referred to the correspondent cis form. Absolute ground state energies are reported as Table 5 of the Supporting Information.

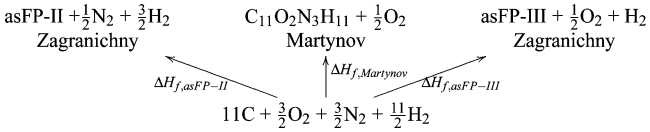

Figure 3. Thermodynamic cycle used to determine the relative stability of the neutral forms by Martynov and Zagranichny with different stoichiometry.

increased, with respect to the reference structures. We mention here that the structures where this behavior is not displayed (such as neutral 2, 6, and 12) have a lesser charge separation in the ground state and consequently are less affected by the PCM polarization.

Concerning the existence of cis and trans forms, at variance with Zagranichny's structures, the trans forms in Martynov's structures would be even more disfavored, both in a vacuum and in the solvent, due to the distortion produced by the sterical hindrance of the NH_2 substituent in the heterocyclic ring that mimics the protein chain.

We note here that some structures (namely, neutrals 8−12) have ground-state energies noticeably higher than the others. Since Martynov's neutrals differ from one another only by their protonation, it is clear that neutrals 8−12 would not be eligible to model the experimental structures.

5. Electronic Structure and Excitation Energies

The TDDFT excitation energies reported in Table 3 in most of the cases belong to HOMO−LUMO transitions (in percentages ranging from 50 to 80% depending on the model) while HOMO and LUMO have almost always π and π^* character, respectively (data not shown). The excitation energies, calculated within TDDFT with LB, are on average 5−10% smaller than those calculated with PW. This difference could be imputed to the Tamm−Dancoff approximation and to the use of a different basis set.

According to the TDDFT data, excitation energies of the trans states are on average 0.3−0.5 eV smaller than those of the cis states (corresponding to a red shift of about 40−50 nm), with the exception of the neutral2, whose trans form has a transition about 0.1 eV more energetic than the cis.

In the plain 6-31G*/CASSCF(8/7) calculation, the excitation energy of the trans states is generally higher than the corresponding cis. The NEVPT2 contribution lowers the transition energy by as much as 1.50 eV. Remarkably, the correction for the excitation energies of the trans states is generally smaller than that for the cis, so that the excitation energy difference between the two states decreases or even inverts, confirming generally the trend predicted by the TDDFT.

The CASSCF calculations confirm the ($\pi \rightarrow \pi^*$) nature of the excitation (data not shown). Furthermore, the excitation of asFP-II (in both the neutral and anionic forms) in the CASSCF calculation provides evidence that the excitation is accompanied by an electronic charge transfer toward the heterocyclic ring, and the same holds for asFP-III.

The effect of the solvent, i.e., the polarization on the solute induced by the solvent polarity, was evaluated by calculations in the presence of the PCM implicit solvent (see Table 3). Table 4 schematically depicts the qualitative dipole changes (along the heterocycle → phenol direction) after excitation. As has already been observed for GFP chromophore,[39] the electronic excitation can be accompanied by an electronic charge transfer from the phenol to the heterocyle.

The simulation of the solvent effect with the PCM does not affect much the excitation energies. Data reported in Table 3 show that, in fact, excitation energies are significantly abated, with respect to in vacuo CASSCF results, only for some structures, namely, neutrals 1, 5, and 12. This effect is clearly related to the dipole increase through excitation (see Table 4).

6. Discussion and Conclusions

We now review and discuss the results reported in the preceding sections, and two main criteria are taken into account.

TABLE 3: Excitation Energies (eV) Resulting from Different Methods

	excitation energy (eV)								
	cis					trans			
method code set of models	TDDFT CPMD first	TDDFT ADF first	CASSCF Dalton second	PC-NEVPT2 Dalton second	CASSCF/PCM Gamess second	DFT CPMD first	CASSCF Dalton second	PC-NEVPT2 Dalton second	CASSCF/PCM Gamess second
cation1	3.20	2.96	4.18	3.36	4.95	2.87	4.40	3.41	4.95
cation2	3.12	2.87	4.30	3.37	5.04	2.76	4.38	3.33	4.96
neutral1	3.33	3.00	4.75	3.50	3.70	2.89	3.90	2.68	3.39
neutral2	3.57	3.44	5.34	4.78	5.35	3.75	5.48	4.89	5.49
neutral3	2.35	2.11	3.41	3.17		2.14	3.21	2.98	4.80
neutral4	3.66	3.53	5.45	4.85	5.48	3.44	5.54	4.95	5.55
neutral5	3.28	2.86	4.09	2.72	3.53	2.88	3.75	2.44	3.36
neutral6			5.33	4.78	5.35		5.47	4.64	5.48
neutral7			5.51	4.89	5.53		5.49	4.69	5.51
neutral8			5.09	4.36	5.17		3.03	2.97	4.24
neutral9			3.27	2.53	3.54		2.79	2.03	
neutral10			3.61	2.67	3.95		3.51	2.53	3.73
neutral11			3.75	2.75	4.06		3.71	2.65	3.73
neutral12			3.93	2.24	3.43		3.52	1.89	3.15
anion1	3.13	2.70				2.98			
anion2	2.85	2.52				2.24			
anion3	2.92	2.77				2.96			
anion4	2.31					1.85			
bianion	2.40	2.33							
asFP−II	2.76	2.53	4.52	3.33	4.34		4.59	3.39	4.40
asFP−III	2.97	2.77	4.78	3.66	4.64		4.81	3.58	4.69
asFP−IIanion	2.71	2.39	3.54	2.55	3.74		3.67	2.17	3.61
asFP−IIIanion	2.71	2.44	3.71	2.68	3.27		3.67	2.19	3.60

TABLE 4: $\Delta\mu_{exc}$ Compared for Neutral Structures from CASSCF (CAS(8/7)/6-31G*//B3LYP/6-31G*) without and with PCM Field.

		neutral 1 to 12												asFP	
		1	2	3	4	5	6	7	8	9	10	11	12	II	III
CASSCF	cis	↑	=	↓	=	↑	=	=	=	↑	↑	↑	↑	↑	↑
CASSCF	trans	↑	=	↓	=	↑	=	=	↓	↑	=	=	↑	↑	↑
PCM	cis	↑	=	↓	=	↑	=	=	=	↑	↓	↓	↑	↑	↑
PCM	trans	↑	=	↓	=	↑	=	=	↓	↑	↓	↓	↑	↑	↑

[a] "↑" represents a dipole increase after electronic excitation (i.e., a negative charge transfer from the phenol toward the heterocyclic ring); "↓" dipole decreases; "=" dipole changes by less than 0.5 D.

The first one requires that the absorption wavelength be shorter in the chromophore at acidic pH than at neutral/alkaline pH (2.88 eV vs 2.32, respectively), while the second one may help selecting some structures comparing their ground-state stabilities.

Regarding the absorption red shift associated to pH change, two cases must be considered, namely, those of deprotonation from cationic to neutral and from neutral to anionic structures. In the first of these cases, the expected absorption energy decrease may result from one of cations 1 or 2 deprotonating to one of neutrals 1, 3, 5, 9, 10, 11, or 12. Neutrals 1 and 8−12, however, are disfavored because of their ground-state energies. The most stable structures, similarly, show excitation energies not compatible with the experimental data. In the case of deprotonation from neutral to anion, the structures eligible for their calculated excitation energies are neutrals 1, 2, 4, or 5 and all anions of Martynov's hypothesis; combining these results with the respective relative ground-state energies, none of Martynov's structures is eligible for the comparison with experimental data. Instead, all of Zagranichny structures respect both requirements about excitation and ground-state energies. All the calculations we performed support these observations. Within the hypothesis of Martynov, the most eligible structures would be cation 2/neutral 5.

However, the anionic structures from Zagranichny's hypothesis have energies more compatible than Martynov's with the experimental absorption maximum at neutral/alkaline pH. Our results show that a deprotonation of the phenolic oxygen leading from the neutral asFP-II and asFP-III to their anionic counterparts produces a decrease in the excitation energy. In fact, refs 19 and 20 made clear that the chromophore's structure is more similar to that of GFP than previously hypothesized and that the most probable forms responsible for absorption are the neutral and the anionic ones.

Our calculations for the ground and first excited states of asFP-II and asFP-III are thus strongly consistent with experimental data on the following points: (i) the trend of the excitation energy following phenolic oxygen deprotonation is correct and consistently shown by all calculations; (ii) there is a conjugated extended π-bond network on a substantially planar chromophore, though sterical hindrance within the chromophore and/or protein environment may cause a distortion not present in vacuo; (iii) anionic asFP-II and especially anionic asFP-III show a close resemblance with some X-ray structures of the protein in the dark state; (iv) the lower stability of asFP-III may explain why asFP-II was more easily found after protein degradation in the early experimental studies on the chromophore;[13,17] (v) the CASSCF/PC-NEVPT2 excitation energies for neutral asFP-III of the cis and trans isomers are compatible with the experimental red shift when cis−trans isomerization occurs.[16]

In conclusion, our calculations on the chromophore point to asFP595III as the absorbing form in the protein and to asFP595II as the absorbing form in water solution and lead to exclude Martynov's structures as absorbing forms both in the protein and in water solution; consistently, even the bianion has a calculated excitation energy not matching the experimental absorption of the "f" colorless form of ref 13. Although the low-energy difference found in our calculations between the ground states of the cis and trans isomers does not forbid that the two conformations are present at equilibrium, further calculations of the chromophore within the protein environment would help the understanding of the kindling phenomenon.

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Supporting Information Available: Two tables with bond lengths of all the structures, two tables reporting RMSD between computed and X-ray structures, and a table with absolute ground-state energies of the structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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