A computational study of the absorption spectra of the photoconvertible fluorescent protein EosFP in different protonation states–Supplementary Material

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Table 1: Absolute Energies (in E_H) from CASSCF(12,12) and CASMP2(12,12) calculations. The state used for geometry optimisation is given in parentheses.

	neutral	anion	zwitter
CASSCF S_0 (S_0)	-642.135777441	-641.547939820	-642.061028029
CASMP2 S_0 (S_0)	-643.993111175	-643.426493464	-643.935944538
CASSCF S_1 (S_0)	-641.929346219	-641.422975651	-641.928761865
CASMP2 S_1 (S_0)	-643.817952147	-643.333890853	-643.818692529
CASSCF S_1 (S_1)	-641.973944138	-641.428747662	-641.965202314
CASMP2 S_1 (S_1)	-643.849614107	-643.341081411	-643.847489665

Table 2: Occupation numbers of the active orbitals from CASMP2(12,12) calculations

neutral		anion		zwitter		
S_0	S_1	S_0	S_1	S_0	S_1	$S_1(S_0)$
1.96	1.95	1.97	1.96	1.98	1.98	1.98
1.95	1.94	1.96	1.95	1.96	1.95	1.95
1.94	1.91	1.95	1.94	1.95	1.89	1.90
1.91	1.84	1.94	1.91	1.92	1.87	1.87
1.90	1.46	1.92	1.89	1.91	1.55	1.77
1.87	1.34	1.91	1.07	1.89	1.19	1.34
0.13	0.65	0.14	0.95	0.13	0.85	0.66
0.10	0.59	0.08	0.12	0.09	0.43	0.26
0.08	0.13	0.07	0.08	0.08	0.13	0.13
0.06	0.08	0.05	0.07	0.05	0.10	0.09
0.05	0.06	0.04	0.05	0.04	0.05	0.05
0.04	0.05	0.02	0.03	0.01	0.02	0.02

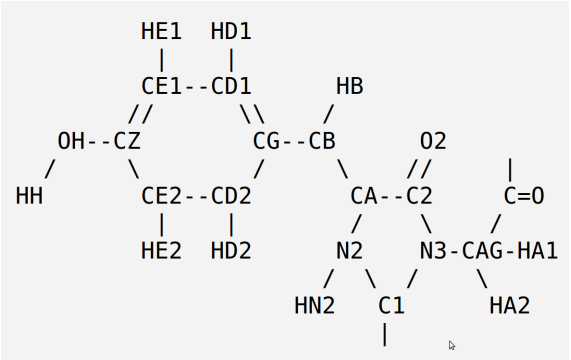

Figure 1: Atom names of the zwitter ionic chromophore

Table 3: Charge paramters for the zwitter ionic chromophore. For atom names see Fig. 1

Atom	Atom type	charge
C	C	0.51
O	O	-0.51
N3	N3	-0.59
CAG	CT2	-0.18
HA1	HB	0.09
HA2	HB	0.09
C1	C1	0.79
N2	N2	-0.58
HN2	H	0.35
CA	CPH1	0.11
CB	CE1	-0.04
HB	HA1	0.25
C2	C2	0.80
O2	O2	-0.68
CG	CA	-0.00
CD1	CA	-0.08
HD1	HP	0.14
CE1	CA	-0.28
HE1	HP	0.10
CZ	CA	0.45
OH	OH1	-0.62
CD2	CA	-0.08
HD2	HP	0.14
CE2	CA	-0.28
HE2	HP	0.10

Table 4: Bond Parameters for the zwitter ionic chromophore. For atom names see Fig. 1

BONDS	$V(b) = K_b (b - b_0)^2$	
atom types	K_b: kcal/mol/Å^2	b_0/Å
C1 CT1	320.0	1.490
N3 CT2	352.0	1.450
N3 C1	400.0	1.390
N3 C2	400.0	1.410
C2 O2	807.0	1.220
C1 N2	400.0	1.300
C2 CPH1	410.0	1.490
N2 CPH1	400.0	1.400
CPH1 CE1	560.0	1.360
CE1 CA	370.0	1.450
CE1 HA1	360.5	1.100
C1 HB	330.0	1.080

Table 5: Angle Parameters for the zwitter ionic chromophore. For atom names see Fig. 1

ANGLES	$V(\theta) = K_\theta(\theta - \theta_0)^2$	
atom types	K_θ: kcal/mol/rad^2	θ_0/degrees
N CT1 C1	50.0	107.0
CT2 CT1 C1	52.0	108.0
N2 C1 CT1	40.0	125.0
N2 C1 N3	130.0	114.0
C1 N2 CPH1	130.0	106.0
N3 C1 CT1	35.0	121.4
C1 N3 C2	130.0	107.9
C1 N3 CT2	36.0	129.0
N2 CPH1 C2	130.0	108.3
N2 CPH1 CE1	45.8	129.5
C2 N3 CT2	32.0	123.4
N3 C2 O2	42.0	126.0
N3 C2 CPH1	130.0	103.0
N3 CT2 CA	50.0	107.0
O2 C2 CPH1	38.0	132.0
C2 CPH1 CE1	45.8	122.0
CPH1 CE1 CA	130.0	130.0
CPH1 CE1 HA1	42.0	114.0
NH1 CT1 C1	50.0	107.0
HB CT1 C1	50.0	109.5
N3 CT2 HB	48.0	108.0
HA1 CE1 CA	42.0	116.0
N3 CT2 C	50.0	107.0
HB CT1 HB	36.0	115.0
HB C1 N2	48.0	125.0
HB C1 N3	48.0	125.0

Table 6: Dihedral Parameter for the zwitter ionic chromophore. For atom names see Fig. 1

DIHEDRALS	$V(\chi) = K_\chi(1 + cos(n(\chi) - \delta))$		
atom type	K_χ/kcal/mol	n	δ/degree
C2 CPH1 CE1 CA	6.840	2	180.00
C2 CPH1 CE1 HA1	6.840	2	180.00
N2 CPH1 CE1 CA	6.840	2	180.00
N2 CPH1 CE1 HA1	6.840	2	180.00
CPH1 CE1 CA CA	1.400	2	180.00
HA1 CE1 CA CA	1.400	2	180.00
C1 N3 CT2 HAG	0.067	3	0.00
C1 N3 CT2 C	0.067	3	0.00
C2 N3 CT2 HAG	0.067	3	180.00
C2 N3 CT2 C	0.067	3	180.00
N3 C1 CT1 NH1	0.100	3	0.00
N3 C1 CT1 CT1	0.100	3	0.00
N3 C1 CT1 HB	0.100	3	0.00
N2 C1 CT1 NH1	0.100	3	180.00
N2 C1 CT1 HB	0.100	3	180.00
N2 C1 CT1 CT1	0.100	3	180.00
CT2 CT1 C1 N3	0.100	3	180.00
CT2 CT1 C1 N2	0.100	3	0.00
C NH1 CT1 C1	0.200	1	180.00
H NH1 CT1 C1	0.000	1	0.00
N3 C1 N2 CPH1	14.000	2	180.00
N3 C2 CPH1 N2	14.000	2	180.00
C1 N3 C2 CPH1	14.000	2	180.00
N2 C1 N3 C2	14.000	2	180.00
CE1 CA CA CA	3.100	2	180.00
CE1 CA CA HP	4.200	2	180.00
NH1 C CT2 N3	0.6000	1	0.00
CT1 C1 N3 CT1	1.600	1	0.00
CT1 C1 N3 CT1	2.500	2	180.00
CT1 C1 N3 CT2	1.600	1	0.00
CT1 C1 N3 CT2	2.500	2	180.00
CT1 C1 N2 CT1	1.600	1	0.00
CT1 C1 N2 CT1	2.500	2	180.00
CT1 C1 N3 C2	1.600	1	0.00
CT1 C1 N3 C2	2.500	2	180.00
CT1 C1 N3 CPH1	1.600	1	0.00
CT1 C1 N3 CPH1	2.500	2	180.00
N3 C2 CPH1 CE1	0.000	1	0.00
CPH1 C2 N3 CT2	1.600	1	0.00
CPH1 C2 N3 CT2	2.500	2	180.00
CT2 N3 C2 O2	2.500	2	180.00
HB CT2 N3 C1	0.000	1	0.00
HB CT2 N3 C2	0.000	1	0.00
C1 N3 C2 O2	2.500	2	180.00
N2 CPH1 C2 O2	0.000	1	0.00
CE1 CPH1 C2 O2	1.400	1	0.00
CT1 C1 N2 CPH1	1.600	1	0.00
CT1 C1 N2 CPH1	2.500	2	180.00
CE1 CPH1 N2 C1	1.800	1	0.00
N2 C1 N3 CT2	14.000	2	180.00
HB C1 N2 CPH1	0.000	1	0.00
CT2 N3 C1 HB	0.000	1	0.00

Table 7: Improper Angle Parameters for the zwitter ionic chromophore. For atom names see Fig. 1

IMPROPER		
atom types	K_ψ: kcal/mol/rad^2	ψ_0/rad
C2 CPH1 N3 O2	0.500	0.0
C1 N3 N2 CT1	0.500	0.0
N3 C2 C1 CT2	0.500	0.0
CPH1 C2 CE1 N2	220.00	0.0
CE1 CA CPH1 HA1	30.00	0.0
CPH1 CPH1 NR3 HR1	0.500	0.0
CPH2 NR3 NR3 HR2	0.500	0.0
CPH1 NR3 CPH1 HR1	0.500	0.0

Figures 2 and 3 show a comparison of absorption spectra with differently-sized quantum regions, together with spectra computed from the same MD ensemble but without inclusion of the protein matrix in the calculation of the excitation energies.

The absorption maxima calculated with additional inclusion of His62 in the QM region (medium size) result in a blue shift compared to the spectra obtained with less atoms treated quantum mechanically (small QM region), see Figure 2b). For the anionic and zwitter ionic forms the blue shift amounts to about 50nm (maxima at 510nm and 623nm, respectively) and is even larger when His62 is protonated, resulting in absorption maxima at 500 nm and 587 nm, for the anionic and zwitter ionic forms, respectively (Figure 2a and b)). The absorption of the neutral form is at almost the same wavelength, 358 nm, for both, small and medium-sized QM region (Figure 2a and b)).

Absorption spectra computed with the large QM region again show no change in the electronic excitation energies of the neutral form (Figure 2c)). The spectrum computed for the anionic form shows a maximum at 494 nm, independent of the protonation states of His62 and His194/Glu212. The absorption spectrum of the zwitter ionic form is also invariant to the protonation state of His62 and His194/Glu212. Its maximum is observed at 580/385 nm.

Comparison of the spectra computed from the molecular dynamics ensemble of the protein simulation with only taking the quantum mechanically treated atoms into account for the calculation of the excitation energies reveals the impact of the protein environment on the excitation energies and in particular of the residues in close proximity of the chromophore. Absorption spectra computed for the quantum mechanical region only exhibit a large difference in case of the zwitter ionic chromophore compared to the spectra computed in the full protein. The excitation energies computed for the conformations from the molecular dynamics ensemble are significantly red-shifted without inclusion of the environment and the absorption maximum is broadened. Extension of the QM region to His194/Glu212 for the computation of absorption spectra diminishes this effect significantly. The absorption maximum is shifted by only 50 nm (compared to ca. 200 nm) and its broadening is significantly reduced. The anionic and neutral forms exhibit only small shifts of ca. 12 nm for the large QM region compared to the full protein.

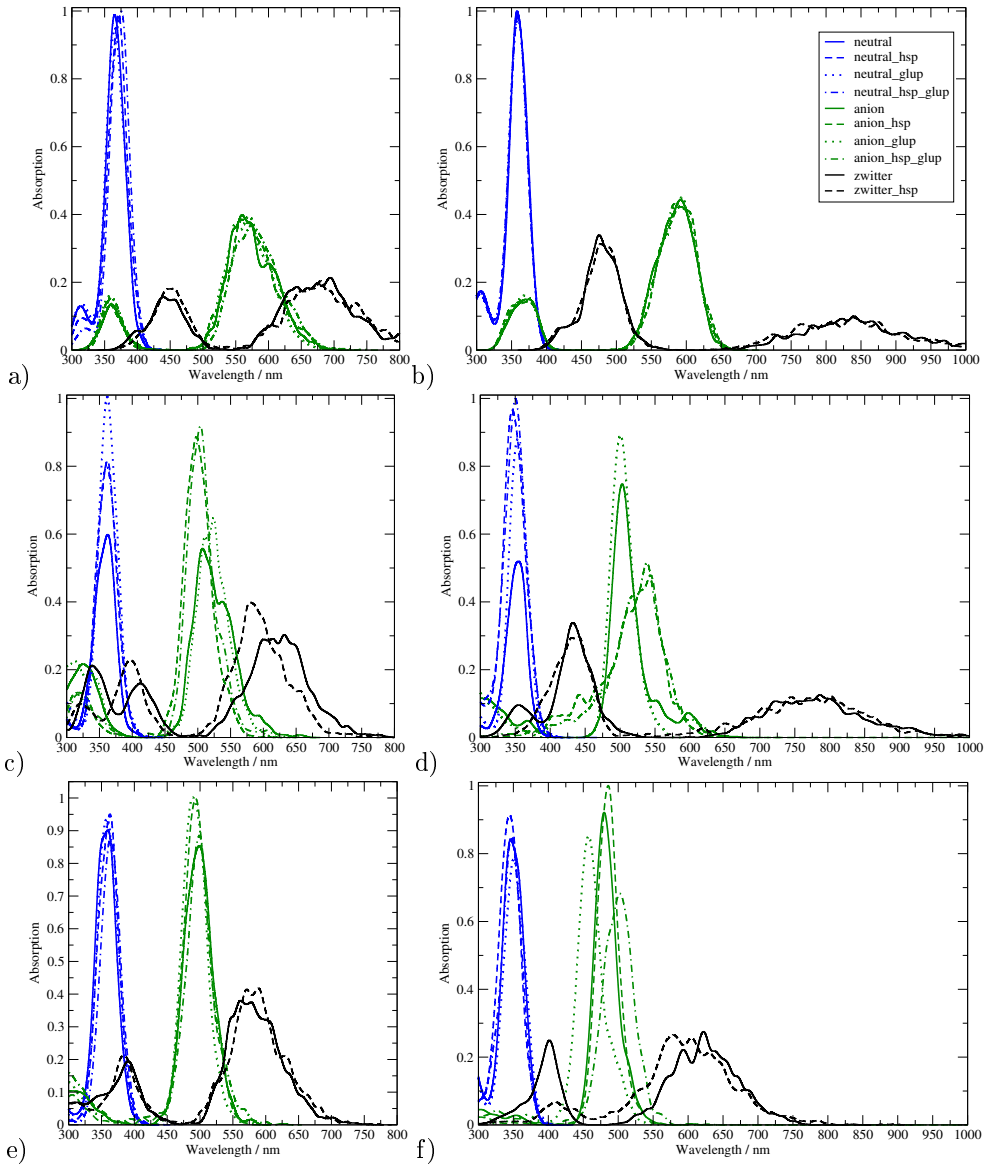

Figure 2: Left: AM1/MRCI/MM-simulated absorption spectra of EosFP with the chromophore in neutral, anionic and zwitter ionic state with variation of the QM region. Right: Absorption spectra of the EosFP chromophore calculated from the configurations of the protein molecular dynamics ensemble. a) and b) small QM region: chromophore only, c) and d) medium-sized QM region: chromophore + His62, and e) f) large QM region: chromophore + His62 + Glu212+ His192. Solid lines represent spectra from simulations with neutral His62, whereas dashed lines correspond to protonated His62 (hsp). Spectra from simulations in which Glu212 is protonated (i.e. neutral) are shown with dotted lines (glup) and those from simulations with an additional proton at His62 and at Glu212 (hsp_glup) are shown as dot-dashed curves.

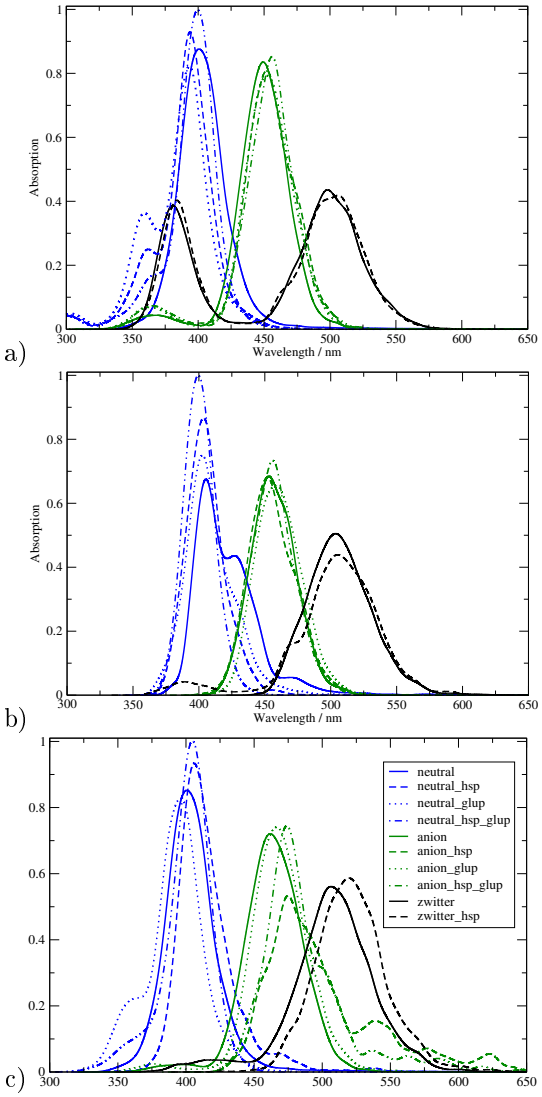

Figure 3: TD-BP86/MM-simulated spectra of EosFP with the chromophore in neutral, anionic and zwitter ionic state with variation of the QM region. a) small QM region: chromophore only, b) medium-sized QM region: chromophore + His62, and c) large QM region: chromophore + His62 + Glu212+ His192. Solid lines represent spectra from simulations with neutral His62, whereas dashed lines correspond to protonated His62 (hsp). Spectra from simulations in which Glu212 is protonated (i.e. neutral) are shown with dotted lines (glup) and those from simulations with an additional proton at His62 and at Glu212 (hsp_glup) are shown as dot-dashed curves.