Interplay between Locally Excited and Charge Transfer States Governs the Photoswitching Mechanism in the Fluorescent Protein Dreiklang: Supplemental Information

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1. DEFINITIONS OF PROTONATION STATES

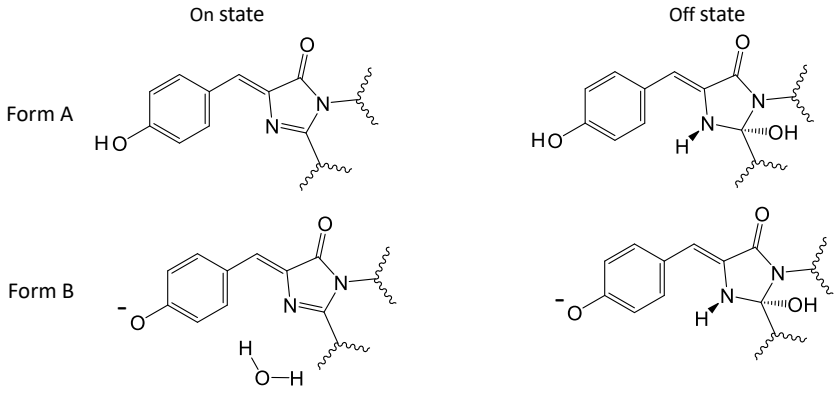


FIG. S1: Definition of chromophore states.

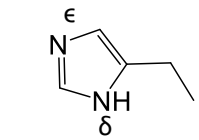
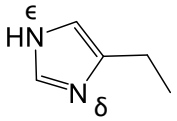
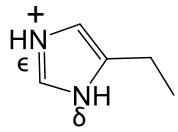
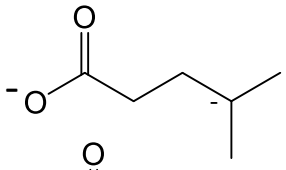
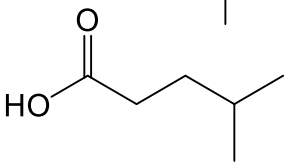
	Charge	
HSD/HID	0	
HSE/HIE	0	
HSP/HIP	+1	
GLU	-1	
GLUP	0	

FIG. S2: Definition of protonation states of Glu222 and His145 in Dreiklang. GLUP can exist in two conformations: As shown or protonated on the other oxygen (GLUP2).

2. COMPUTATIONAL DETAILS

In addition to the structures obtained by the computational protocol described in the main manuscript, we also consider the structures from our previous study[1] in which we started with 3ST2 structure and used QM/MM optimization with electrostatic embedding, as implemented in *NWChem*. The QM part was described by M06-L/cc-pVDZ and the MM part was described by the *AMBER* forcefield[2]. In these calculations[1], QM included the chromophore, side chains of Gln94, Arg96, His145, Tyr203, Ser205, and Glu222, and seven water molecules. This definition is similar to our extended QM. We note that these model structures also included additional water molecule, which is present in 3ST3 structure (OFF-state) but not seen in 3ST2 and 3ST4. The comparisons between the two protocols quantify the effect of the level of theoretical treatment.

The key structural parameters two sets of structures are compared in Tables S7-S12 below and graphically in Figs. S4 and S5.

3. FORCEFIELD PARAMETERS FOR THE NEUTRAL HYDRATED CHROMOPHORE

To derive missing forcefield parameters (for the OFF-form of the chromophore) we followed a protocol described in our previous work[3,4]. The key equations and the values of the forcefield parameters are given below.

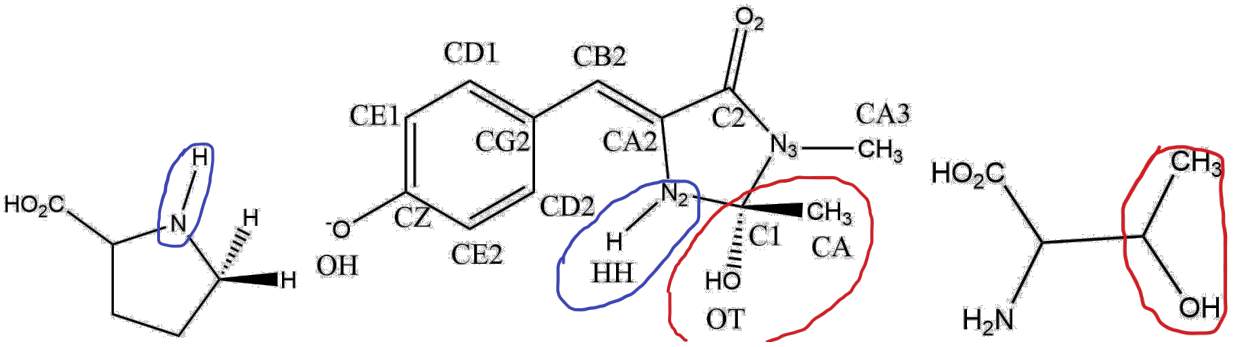


FIG. S3: From left to right: proline, chromophore in off-state, threonine.

$$\Delta q_{(on,charmm-qm)} = q_{on,charmm} - q_{NBO(on,qm)} \quad (1)$$

$$q_{(off,charmm)} = q_{NBO(off,qm)} + \Delta q_{(on,charmm-qm)} \tag{2}$$

$$E = k(b - b_0)^2 \tag{3}$$

$$k_{off,param} = \frac{k_{off,theory}}{k_{on,theory}} \times k_{on-charmm} \tag{4}$$

b_0 is the equilibrium bond length.

$$E = k(A - A_0)^2 \tag{5}$$

$$k_{off,param} = \frac{k_{off,theory}}{k_{on,theory}} \times k_{on-charmm} \tag{6}$$

A_0 is the equilibrium bond angle.

$$E = k[1 + cos(n\phi - \delta)] \tag{7}$$

where n is the phase, δ is the optimized dihedral angle.

$$k_{off,param} = \frac{k_{off,theory}}{k_{on,theory}} \times k_{on-charmm} \tag{8}$$

TABLE S1: Partial charges in the OFF-state.

Atom, off	on, charmm	on, qm	$\Delta q_{(on,charmm-qm)}$	$\Delta q_{adjusted}$	off, qm	off, charmm
C1(threonine)	0.10	0.15	-0.05	-0.02	0.67	0.69
N2 (proline)	-0.74	-0.28	-0.46	-0.43	-0.68	-0.25
N3	-0.64	-0.52	-0.12	-0.09	-0.56	-0.47
C2	0.8	0.74	0.06	0.09	0.74	0.65
O2	-0.61	-0.60	-0.01	0.02	-0.63	-0.65
CA2	0.24	0.05	0.19	0.22	0.12	-0.10
CB2	-0.10	-0.09	-0.01	0.02	-0.24	-0.26
HB2	0.1	0.28	-0.18	-0.15	0.27	0.42
CG2	0.00	-0.11	0.11	0.14	-0.09	-0.23
CD1	-0.115	-0.14	0.025	0.06	-0.185	-0.245
HD11	0.115	0.27	-0.155	-0.12	0.25	0.37
CD2	-0.115	-0.14	0.025	0.06	-0.185	-0.245
HD21	0.115	0.27	-0.155	-0.12	0.25	0.37
CE1	-0.115	-0.27	0.155	0.19	-0.275	-0.465
HE11	0.115	0.27	-0.155	-0.12	0.25	0.37
CE2	-0.115	-0.27	0.155	0.19	-0.275	-0.465
HE21	0.115	0.27	-0.155	-0.12	0.25	0.37
CZ	0.11	0.38	-0.27	-0.24	0.34	0.58
OH	-0.54	-0.68	0.14	0.17	-0.70	-0.87
OHH	0.43	0.52	-0.09	-0.06	0.50	0.56
OT (threonine)	-0.78	-0.65	-0.13	-0.10	-0.75	-0.65
HT(threonine)	0.50	0.44	0.06	0.09	0.50	0.41
HH (proline)	0.41	0.11	0.30	0.33	0.44	0.11
CA3		-0.18				-0.18
HA31		0.09				0.09
HA32		0.09				0.09
C		0.51	–	–	–	0.51
O		-0.51	–	–	–	-0.51
N		-0.47	–	–	–	-0.47
HN		0.31	–	–	–	0.31
CA		-0.02				-0.02
HA1		0.09				0.09
HA2		0.09				0.09

TABLE S2: Optimized bond lengths (in Å) involving key atoms.

Bonds	$B_{on,charmm}$	$B_{on,opt}$	$B_{off,opt}$
C1-N2 (proline)	1.434	1.46	1.45
N2-CA2	1.40	1.41	1.39
N2-HH (proline)	0.997	1.02	1.01
C1-OT (threonine)	1.42	1.40	1.41
C1-CA	1.49	1.49	1.53
C1-N3	1.39	1.38	1.45

TABLE S3: Parameterization of the force constant k for bond lengths in kcal/mol/Å^2.

Bonds	$k_{on,charmm}$	$k_{on,theory}$	$k_{off,theory}$	$k_{off,theory}/k_{on,theory}$	$k_{off,param}$
C1-N2 (proline)	320	1156.50	1226.15	1.06	339.27
N2-CA2	400	940.00	1257.53	1.34	535.12
N2-HH (proline)	440	976.40	1044.80	1.07	470.82
C1-OT (threonine)	428	847.14	744.22	0.88	376.00
C1-CA	354	562.25	542.79	0.965	341.75
C1-N3	320	1156.50	1226.15	1.06	339.27

TABLE S4: Optimized bond angles (in degrees) involving key atoms.

Angles	$A_{on,charmm}$	$A_{on,opt}$	$A_{off,opt}$
N2-C1-N3	114.0	113.99	102.31
C1-N2-CA2	106.0	106.18	111.21
HH-N2-C1 (proline)	117.0	111.44	115.90
HH-N2-CA2	117.0	111.44	117.8
OT-C1-CA (threonine)	110.1	112.6	110.31
OT-C1-N2 (threonine)	110.1	112.6	107.90
OT-C1-N3 (threonine)	110.1	112.6	111.39
N2-CA2-CB2	129.5	129.58	130.17
N2-CA2-C2	108.3	108.73	106.54
C1-N3-C2	107.9	113.47	108.26
CA-C1-N3 (threonine)	113.5	111.6	112.01

TABLE S5: Parameterization of the force constant k for bond angles in kcal/mol/rad$^{\circ 2}$.

Angles	$k_{on,charmm}$	$k_{on,theory}$	$k_{off,theory}$	$k_{off,theory}/k_{on,theory}$	$k_{off,param}$
N2-C1-N3	130.0	444.28	347.33	0.78	101.6
C1-N2-CA2	130.0	438.63	259.98	0.59	77.05
HH-N2-C1 (proline)	35.0	89.73	79.69	0.89	31.08
HH-N2-CA2	35.0	89.73	79.69	0.89	31.08
OT-C1-CA (threonine)	75.7	232.18	259.37	1.12	84.56
OT-C1-N2(N3) (threonine)	75.7	232.18	259.37	112	84.56
N2-CA2-CB2	45.8	151.23	169.42	1.12	51.3
N2-CA2-C2	130.0	472.5	376.50	0.797	103.6
C1-N3-C2	130.0	498.24	305.6	0.61	79.7
CA-C1-N3 (threonine)	70.0	180.72	179.47	0.99	69.5

TABLE S6: Parameterization of the force constant k for dihedral angles; δ in degrees, k in kcal/mol.

Angles	$k_{on,charmm}$	n	δ	$k_{off,theory}/k_{on,theory}$	$k_{off,param}$
OT-C1-N2-HH	0.16	3	180	0.263	0.053
OT-C1-N2-CA2	0.20	3	0	13.83	2.213
CA-C1-N2-HH	0.16	3	180	0.263	0.053
HH-N2-CA2-CB2	0.16	3	0	0.263	0.053
HH-N2-CA2-C2	0.20	3	180	13.83	2.213
HH-N2-C1-N3	0.20	3	180	13.83	2.213
CA-C1-N3-CA3	0.16	3	0	0.263	0.053
OT-C1-N3-CA3	0.16	3	0	0.263	0.053
CA-C1-N3-C2	0.20	3	180	13.83	2.213

4. STRUCTURES OF MODEL SYSTEMS

TABLE S7: Comparison of the distances (in Å) from MD and QM/MM simulations with crystal structure 3ST4 (ON-state). The chromophore is neutral (A-form). 'md' denotes structures averaged over equilibrium MD trajectories. 'opt' and 'opt2' denote the QM/MM optimized structures obtained with present protocol and with the protocol from Ref. 1, respectively.

D		3ST4	HSE-GLU	Δ	HSE-GLUP	Δ	HSD-GLU	Δ	HSD-GLUP	Δ	HSP-GLU	Δ
d1	md	3.52	3.42(0.3)	0.10	3.96(0.8)	0.44	3.74(0.4)	0.22	3.63(0.4)	0.11	4.68(1.0)	1.16
	opt	–	3.31	0.21	3.92	0.4	3.53	0.01	4.2	0.68	3.78	0.26
	opt2	–	3.45	0.07	3.50	0.02	3.49	0.03			3.46	0.06
d2	md	2.97	3.94(0.3)	0.97	3.44(0.3)	0.47	3.92(0.3)	0.95	3.54(0.4)	0.57	4.00(0.3)	1.03
	opt	–	3.99	1.02	2.78	0.19	4.06	1.09	2.86	0.11	4.00	1.03
	opt2	–	3.46	0.49	3.09	0.12	3.44	0.47			3.49	0.52
d3	md	2.73	3.42(0.3)	0.69	2.77(0.1)	0.04	2.74(0.1)	0.01	2.77(0.1)	0.04	2.74(0.1)	0.01
	opt	–	2.58	0.15	2.62	0.11	2.6	0.13	2.67	0.06	2.61	0.12
	opt2	–	2.77	0.04	2.85	0.12	2.80	0.07			2.81	0.08
d4	md	3.81	3.60(0.2)	0.21	4.82(0.8)	1.01	3.62(0.2)	0.19	3.91(0.3)	0.10	4.02(0.7)	0.21
	opt	–	3.53	0.28	3.67	0.14	3.52	0.29	3.77	0.04	3.38	0.43
	opt2	–	3.96	0.15	3.92	0.11	3.96	0.36			4.16	0.35
d5	md	4.04	4.38(0.4)	0.34	4.08(0.3)	0.04	4.05(0.4)	0.01	3.86(0.3)	0.18	4.67(0.4)	0.63
	opt	–	—	–	—	—	—	—-	—	–	—-	–
	opt2	–	—	–	—	—	—	—	—	—	—-	—-
d6	md	3.64	3.85(0.2)	0.21	3.82(0.2)	0.18	3.88(0.2)	0.24	3.87(0.2)	0.23	3.82(0.2)	0.18
	opt	–	3.69	0.05	3.52	0.12	3.63	0.01	3.6	0.04	3.59	0.05
	opt2	–	3.85	0.21	3.63	0.01	3.68	0.04			3.67	0.03
d7	md	2.63	V.L	–	2.69(0.1)	0.06	2.71(0.1)	0.08	2.74(0.1)	0.11	3.19(0.8)	0.56
	opt	–	2.97	0.34	2.59	0.04	2.63	0	2.63	0	3.59	0.18
	opt2	–	2.59	0.04	2.66	0.03	2.66	0.03			2.67	0.04
d8	md	3.41	3.18(0.2)	0.23	3.43(0.2)	0.02	3.49(0.3)	0.08	3.43(0.2)	0.02	3.6(0.3)	0.19
	opt	–	3.25	0.16	3.22	0.19	3.71	0.3	3.27	0.14	3.59	0.18
	opt2	–	3.05	0.36	3.26	0.15	3.02	0.39			3.01	0.40
d9	md	2.89	2.92(0.2)	0.03	2.99(0.3)	0.10	2.88(0.2)	0.01	2.99(0.3)	0.10	2.96(0.2)	0.07
	opt	–	2.64	0.25	2.68	0.21	2.78	0.11	2.67	0.22	2.79	0.10
	opt2	–	2.82	0.07	2.81	0.08	2.79	0.10			2.81	0.08
d10	md	2.69	2.63(0.1)	0.06	2.81(0.2)	0.12	2.63(0.1)	0.06	2.78(0.2)	0.09	2.66(0.1)	0.03
	opt	–	2.58	0.11	2.7	0.01	2.8	0.11	2.72	0.03	2.73	0.04
	opt2	–	2.67	0.02	2.75	0.06	2.67	0.02			2.67	0.02

TABLE S8: Comparison of the distances (in Å) from MD and QM/MM simulations with crystal structure 3ST4 (ON-state). The chromophore is neutral (A-form). 'md' denotes structures averaged over equilibrium MD trajectories. 'opt' and 'opt2' denote the QM/MM optimized structures obtained with present protocol and with the protocol from Ref. 1, respectively.

D		3ST4	HSE-GLU	Δ	HSE-GLUP	Δ	HSD-GLU	Δ	HSD-GLUP	Δ	HSP-GLU	Δ
d11	md	2.73	V.L	–	3.76(0.8)	1.03	2.9(0.2)	0.17	3.02(0.3)	0.29	2.93(0.3)	0.20
	opt	–	3.01	0.28	2.7	0.03	2.52	0.21	2.59	0.14	2.89	0.16
	opt2	–	2.72	0.01	2.69	0.04	2.67	0.06			2.63	0.10
d12	md	3.05	V.L	–	3.06(0.3)	0.01	3.53(0.4)	0.48	3.42(0.4)	0.37	3.89(0.4)	0.84
	opt	–	3.33	0.28	2.58	0.47	2.83	0.22	2.95	0.1	3.23	0.18
	opt2	–	3.53	0.48	2.70	0.35	3.10	0.05			2.74	0.31
d13	md	2.89	V.L	–	2.84(0.3)	0.05	3.4(0.4)	0.51	3.41(0.6)	0.52	3.87(0.4)	0.98
	opt	–	–	–	–	–	–	–	–	–	–	–
	opt2	–	—	–	—	—	—	—	—	—	—-	—-
d14	md	4.18	2.67(0.1)	1.51	4.28(0.5)	0.10	2.66(0.1)	1.52	3.48(0.4)	0.70	2.68(0.1)	1.5
	opt	–	2.7	1.48	4.72	0.54	2.56	1.62	4.32	0.14	2.5	1.68
	opt2	–	5.06	0.88	4.38	0.30	5.02	0.84			5.03	0.85

TABLE S9: Comparison of the distances (in Å) from MD and QM/MM simulations with crystal structure 3ST4 (ON-state). Chromophore is anionic (B-form). 'md' denotes structures averaged over equilibrium MD trajectories. 'opt' and 'opt2' denote the QM/MM optimized structures obtained with present protocol and with the protocol from Ref. 1, respectively.

D		3ST4	HSE-GLU	Δ	HSE-GLUP	Δ	HSD-GLU	Δ	HSD-GLUP	Δ	HSP-GLUP	Δ
d1	md	3.52	3.90(0.3)	0.38	3.19(0.2)	0.33	3.57(0.4)	0.05	3.32(0.3)	0.20	3.05(0.3)	0.47
	opt	–	3.66	0.14	3.39	0.13	3.39	0.13	3.47	0.05	2.88	0.64
	opt2	–			3.30	0.22			3.24	0.28	3.10	0.42
d2	md	2.97	3.98(0.3)	1.01	3.17(0.3)	0.20	4.62(0.4)	1.65	3.13(0.2)	0.16	3.07(0.2)	0.10
	opt	–	4.02	1.05	2.74	0.23	4.11	1.14	2.9	0.07	2.79	0.18
	opt2	–			3.47	0.50			3.49	0.52	3.08	0.11
d3	md	2.73	2.72(0.1)	0.01	2.75(0.1)	0.02	2.74(0.1)	0.01	2.72(0.1)	0.04	2.73(0.1)	0.00
	opt	–	2.52	0.21	2.57	0.16	2.51	0.22	2.57	0.16	2.64	0.09
	opt2	–			2.74	0.01			2.76	0.03	2.81	0.08
d4	md	3.81	3.78(0.3)	0.03	4.83(0.8)	1.02	3.73(0.3)	0.08	3.97(0.3)	0.16	4.42(0.4)	0.61
	opt	–	3.93	0.12	3.84	0.03	3.33	0.48	4.06	0.25	4.51	0.7
	opt2	–			4.01	0.20			4.01	0.20	3.99	0.18
d5	md	4.04	4.22(0.2)	0.18	4.74(0.3)	0.70	4.15(0.3)	0.11	3.82(0.2)	0.22	4.14(0.3)	0.10
	opt	–	—	–	—	—	—	—-	—	–	—-	–
	opt2	–	–	–	–	–	—	—	—	—	–	—
d6	md	3.64	3.96(0.2)	0.32	3.78(0.2)	0.14	4.00(0.3)	0.36	3.84(0.2)	0.20	3.94(0.2)	0.30
	opt	–	3.84	0.20	3.64	0.0	3.97	0.33	3.67	0.03	3.64	0.0
	opt2	–			3.68	0.04			3.83	0.19	3.68	0.04
d7	md	2.63	2.79(0.2)	0.16	5.24(1.0)	2.61	2.8(0.2)	0.17	2.75(0.2)	0.12	2.79(0.2)	0.16
	opt	–	2.86	0.23	5.14	2.51	2.87	0.24	2.59	0.04	2.54	0.09
	opt2	–			2.74	0.11			2.68	0.05	2.63	0.0
d8	md	3.41	3.00(0.1)	0.41	3.86(0.5)	0.45	3.01(0.1)	0.40	3.72(0.4)	0.31	3.88(0.4)	0.47
	opt	–	3.19	0.22	3.29	0.12	3.47	0.06	3.23	0.18	3.51	0.10
	opt2	–			3.34	0.07			3.02	0.39	3.27	0.14
d9	md	2.89	2.83(0.1)	0.06	2.86(0.2)	0.03	2.84(0.1)	0.05	2.84(0.1)	0.05	2.85(0.2)	0.04
	opt	–	2.71	0.18	2.88	0.01	2.58	0.41	2.77	0.12	2.77	0.12
	opt2	–			2.87	0.02			2.86	0.03	2.80	0.09
d10	md	2.69	2.66(0.1)	0.03	2.8(0.2)	0.11	2.64(0.1)	0.05	2.78(0.2)	0.09	2.78(0.2)	0.09
	opt	–	2.46	0.23	2.64	0.05	2.89	0.20	2.77	0.08	2.81	0.12
	opt2	–			2.91	0.22			2.89	0.20	2.75	0.06

TABLE S10: Comparison of the distances (in Å) from MD and QM/MM simulations with crystal structure 3ST4 (ON-state). Chromophore is anionic (B-form). 'md' denotes structures averaged over equilibrium MD trajectories. 'opt' and 'opt2' denote the QM/MM optimized structures obtained with present protocol and with the protocol from Ref. 1, respectively.

D		3ST4	HSE-GLU	Δ	HSE-GLUP	Δ	HSD-GLU	Δ	HSD-GLUP	Δ	HSP-GLUP	Δ
d11	md	2.73	3.67(0.7)	0.94	4.54(0.8)	1.81	2.9(0.2)	0.17	2.9(0.2)	0.17	2.97(0.4)	0.24
	opt	–	3.95	1.22	4.11	1.38	2.66	0.07	2.74	0.01	2.76	0.03
	opt2	–			2.62	0.11			2.63	0.10	2.70	0.03
d12	md	3.05	5.41(0.3)	2.36	3.07(0.4)	0.02	3.43(0.4)	0.38	3.25(0.3)	0.20	3.33(0.3)	0.28
	opt	–	3.31	0.26	2.89	0.16	3.76	0.71	2.85	0.2	2.65	0.40
	opt2	–			3.37	0.32			3.31	0.26	2.76	0.29
d13	md	2.89	3.38(0.6)	0.49	4.73(0.5)	1.84	3.25(0.3)	0.36	2.81(0.2)	0.08	3.24(0.5)	0.35
	opt	–	–	–	–	–	–	–	–	–	–	–
	opt2	–	—	—	–	–	–	–	–	—	—	—
d14	md	4.18	2.68(0.1)	1.50	4.33(0.5)	0.15	2.65(0.1)	1.53	4.1(0.4)	0.08	5.24(0.4)	1.06
	opt	–	2.65	1.53	4.27	0.09	2.55	1.63	4.59	0.41	5.79	1.61
	opt2	–			4.90	0.72			4.99	0.81	4.53	0.35

TABLE S11: Comparison of the distances (in Å) from MD and QM/MM simulations with crystal structure 3ST3 (OFF-state). Chromophore is neutral. 'md' denotes structures averaged over equilibrium MD trajectories. 'opt' and 'opt2' denote the QM/MM optimized structures obtained with present protocol and with the protocol from Ref. 1, respectively.

D		3ST3	HSE GLU P	Δ	HSD GLU P	Δ	HSE GLU P2	Δ	HSD GLU P2	Δ	HSE GLU	Δ	HSD GLU	Δ	HSP GLU	Δ
d1	md	3.35	3.37	0.0	3.75	0.4	3.71	0.3	3.68	0.3	3.63	0.3	3.53	0.2	2.97	0.4
			(0.3)		(0.5)		(0.6)		(0.2)		(0.5)		(0.4)		(0.2)	
	opt	–	4.1	0.7	2.96	0.4	3.17	0.2	4.2	0.8	4.29	0.9	3.48	0.1	3.38	0.0
	opt2	–	3.58	0.2	3.27	0.1					3.44	0.1	3.47	0.1	3.46	0.1
d2	md	2.46	3.25	0.8	3.28	0.8	4.21	1.7	4.53	2.1	3.20	0.7	3.23	0.8	3.13	0.7
			(0.2)		(0.2)		(0.4)		(0.4)		(0.2)		(0.2)		(0.1)	
	opt	–	2.8	0.3	2.85	0.4	2.76	0.3	2.67	0.2	2.66	0.2	2.67	0.2	2.78	0.3
	opt2	–	2.82	0.4	2.86	0.4					2.62	0.2	2.63	0.2	2.63	0.2
d3	md	2.85	4.23	1.4	4.32	1.5	3.56	0.7	3.49	0.6	3.91	1.1	3.67	0.8	3.51	0.7
			(0.6)		(0.4)		(0.3)		(0.3)		(0.4)		(0.5)		(0.3)	
	opt	–	3.5	0.6	3.45	0.6	3.13	0.3	3.09	0.2	2.96	0.1	3.03	0.2	3.0	0.1
	opt2	–	3.17	0.3	3.17	0.3					2.96	0.1	2.96	0.1	2.98	0.1
d4	md	3.01	2.69	0.3	2.7	0.3	2.71	0.3	2.67	0.3	2.68	0.3	2.68	0.3	2.67	0.3
			(0.1)		(0.1)		(0.1)		(0.1)		(0.1)		(0.1)		(0.1)	
	opt	–	2.66	0.3	2.68	0.3	2.66	0.3	2.67	0.3	2.6	0.4	2.62	0.4	2.61	0.4
	opt2	–	2.82	0.2	2.79	0.2					2.80	0.2	2.82	0.2	2.82	0.2
d5	md	3.82	4.13	0.3	4.93	1.1	4.84	1.0	5.51	1.7	5.66	1.8	3.81	0.0	4.69	0.9
			(0.3)		(0.4)		(0.6)		(0.7)		(0.5)		(0.3)		(0.4)	
	opt	–	3.6	0.2	4.12	0.3	3.96	0.1	4.05	0.2	3.85	0.0	3.79	0.0	3.78	0.0
	opt2	–	4.16	0.3	3.95	0.1					4.02	0.2	4.04	0.2	4.15	0.3
d6	md	3.98	3.91	0.1	4.96	1.0	4.62	0.6	4.14	0.2	4.50	0.5	3.98	0.0	4.44	0.5
			(0.3)		(0.4)		(0.4)		(0.3)		(0.4)		(0.3)		(0.4)	
	opt	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	opt2	–	–	–	–	–	—	–	–	–	–	–	–	–	–	–
d7	md	3.91	3.98	0.1	3.78	0.1	3.80	0.1	3.94	0.0	3.99	0.1	4.05	0.1	4.21	0.3
			(0.3)		(0.2)		(0.2)		(0.3)		(0.3)		(0.3)		(0.3)	
	opt	–	3.68	0.2	3.67	0.2	3.66	0.2	3.74	0.2	3.95	0.0	3.7	0.2	3.67	0.2
	opt2	–	3.70	0.2	3.73	0.2					3.72	0.2	3.69	0.2	3.67	0.2

TABLE S12: Comparison of the distances (in Å) from MD and QM/MM simulations with crystal structure 3ST3 (OFF-state). Chromophore is neutral. 'md' denotes structures averaged over equilibrium MD trajectories. 'opt' and 'opt2' denote the QM/MM optimized structures obtained with present protocol and with the protocol from Ref. 1, respectively.

D		3ST3	HSE GLU P	Δ	HSD GLU P	Δ	HSE GLU P2	Δ	HSD GLU P2	Δ	HSE GLU	Δ	HSD GLU	Δ	HSP GLU	Δ
d8	md	2.59	L.V	–	3.86	1.3	10.4	7.8	2.87	0.3	L.V	–	2.64	0.0	L.V	–
					(1.1)		(7.9)		(0.4)				(0.1)			
	opt	–	2.7	0.1	2.71	0.1	2.96	0.4	2.61	0.0	2.71	0.1	2.83	0.2	2.67	0.1
	opt2	–	2.7	0.1	2.67	0.1					2.63	0.0	2.66	0.1	2.68	0.1
d9	md	2.64	L.V	–	7.28	4.6	7.38	4.7	3.97	1.3	L.V	–	2.80	0.2	L.V	–
					(0.7)		(8.2)		(0.6)				(0.1)			
	opt	–	2.71	0.1	4.36	1.7	2.62	0.0	2.53	0.1	2.6	0.0	2.62	0.0	2.51	0.1
	opt2	–	2.65	0.0	2.62	0.0					2.75	0.1	2.67	0.0	2.63	0.0
d10	md	3.17	L.V	–	4.79	1.6	9.60	6.4	5.40	2.2	L.V	–	3.37	0.2	L.V	–
					(0.7)		(8.3)		(0.3)				(0.4)			
	opt	–	4.5	1.3	3.8	0.6	2.62	0.5	2.83	0.3	4.03	0.9	2.87	0.3	2.54	0.6
	opt2	–	2.68	0.5	3.55	0.4					3.44	0.3	3.13	0.0	2.75	0.4
d11	md	2.80	L.V	–	3.95	1.1	7.37	4.6	2.82	0.0	L.V	–	3.21	0.4	L.V	–
					(1.4)		(8.6)		(0.2)				(0.4)			
	opt	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	opt2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
d12	md	2.67	4.29	1.6	4.38	1.7	4.72	2.0	4.64	2.0	2.79	0.1	3.04	0.4	2.62	0.0
			(0.9)		(0.8)		(0.1)		(0.5)		(0.3)		(0.6)		(0.1)	
	opt	–	3.53	0.9	3.6	0.9	2.73	0.1	2.71	0.0	2.68	0.0	2.58	0.1	2.53	0.1
	opt2	–	2.55	0.2	2.54	0.1					2.68	0.0	2.65	0.0	2.65	0.0
d13	md	2.47	L.V	–	3.03	0.6	2.78	0.3	2.89	0.4	4.70	2.2	4.22	1.7	2.81	0.3
					(0.4)		(0.1)		(0.2)		(1.8)		(1.7)		(0.1)	
	opt	–	2.64	0.2	2.69	0.2	2.6	0.1	2.56	0.1	3.16	0.7	2.56	0.1	2.5	0.0
	opt2	–	2.65	0.2	2.66	0.2					2.60	0.2	2.58	0.1	2.56	0.1
d14	md	4.88	3.74	1.1	5.18	0.3	6.56	1.7	6.36	1.5	4.52	0.4	3.69	1.2	4.59	0.3
			(1.2)		(0.9)		(0.4)		(0.5)		(0.6)		(1.0)		(0.4)	
	opt	–	6.02	1.1	5.95	1.1	4.91	0.0	5.04	0.2	5.27	0.4	4.76	0.1	4.82	0.1
	opt2	–	4.79	0.1	4.91	0.0					5.01	0.1	4.95	0.1	4.93	0.0

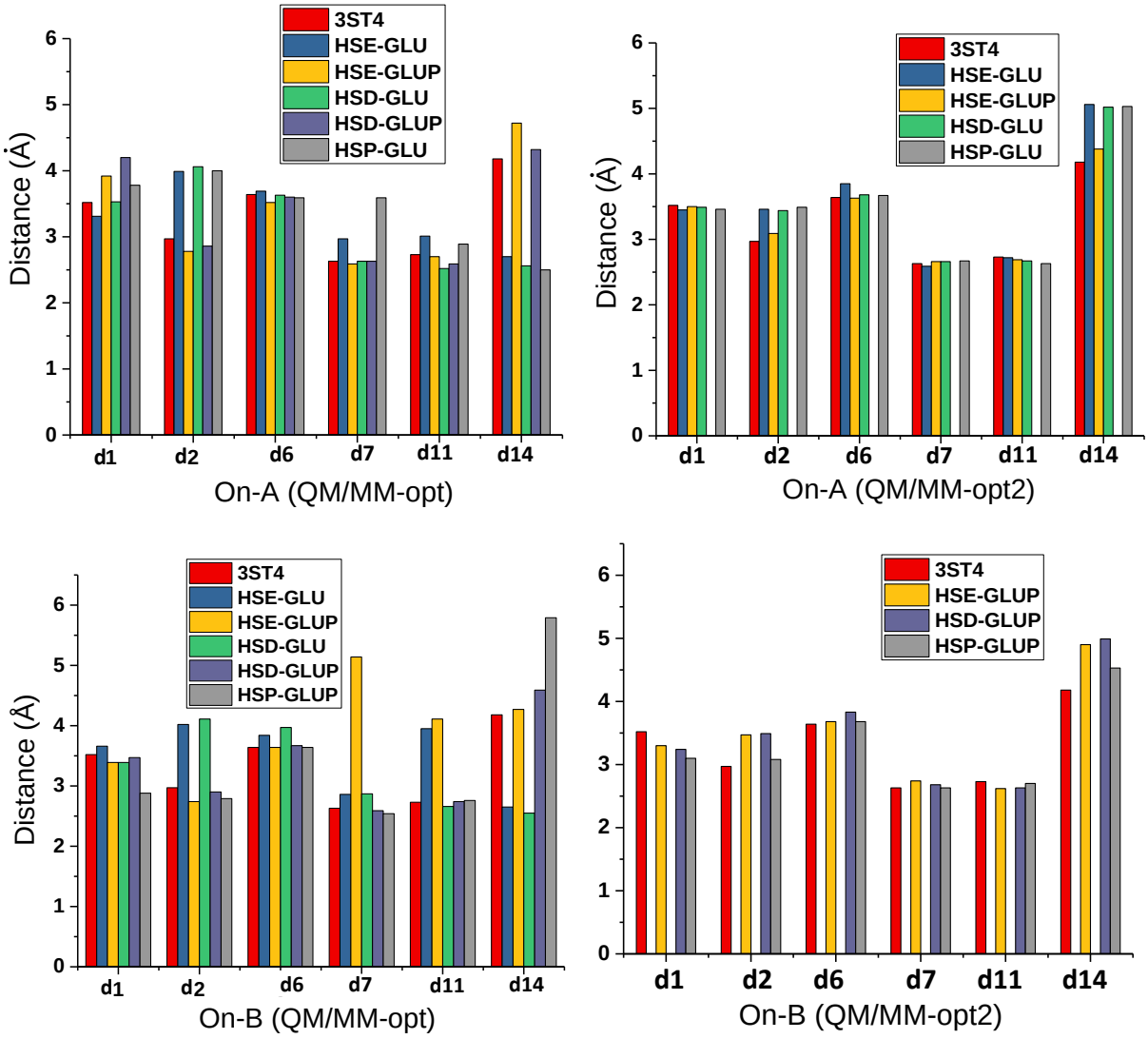


FIG. S4: Key distances for ON-states: Comparison between crystal structure and QM/MM optimization. OPT1 and OPT2 denote two different protocols (see text). See Fig. 5 in the main text for definitions.

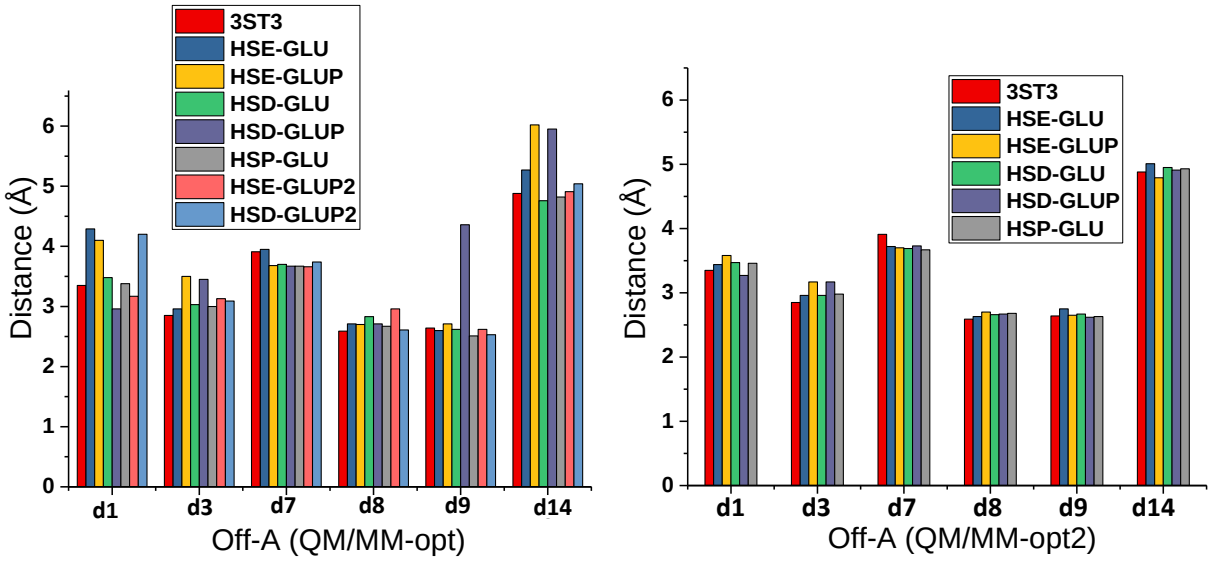


FIG. S5: Key distances for OFF-states: Comparison between crystal structure, average MD values, and QM/MM optimizations. See Fig. 6 of the main text for definitions.

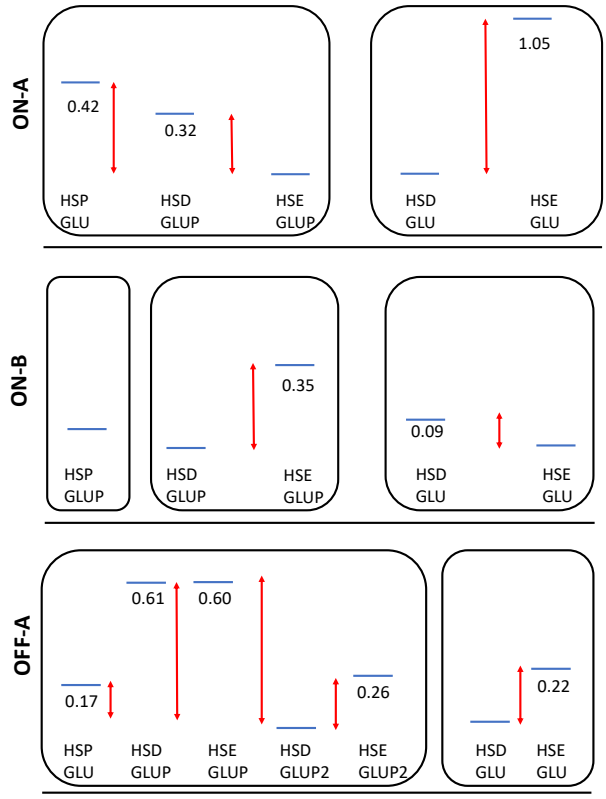


FIG. S6: Energy ordering (eV) of QM/MM (ONIOM) optimized structures (boxes mark the structures with the same number of atoms in QM).

5. ANALYSIS OF EXCITED STATES

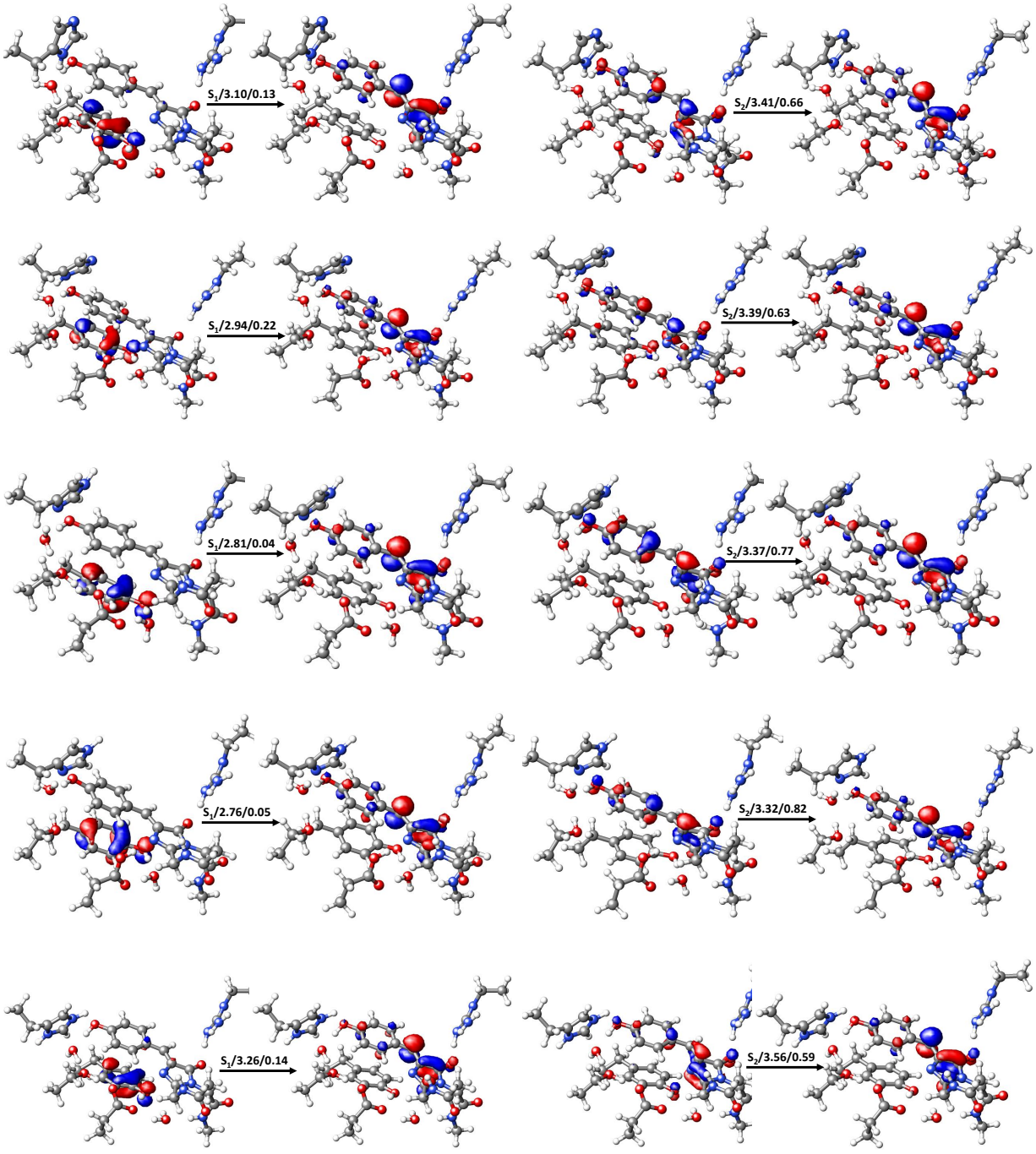


FIG. S7: NTOs of the lowest excited states of the neutral form and different protonation states of His145 and Glu222; TD-DFT, extended QM. Left: CT state; right: LE state; top-to-bottom: HSD-GLU, HSD-GLUP, HSE-GLU, HSE-GLUP, HSP-GLU.

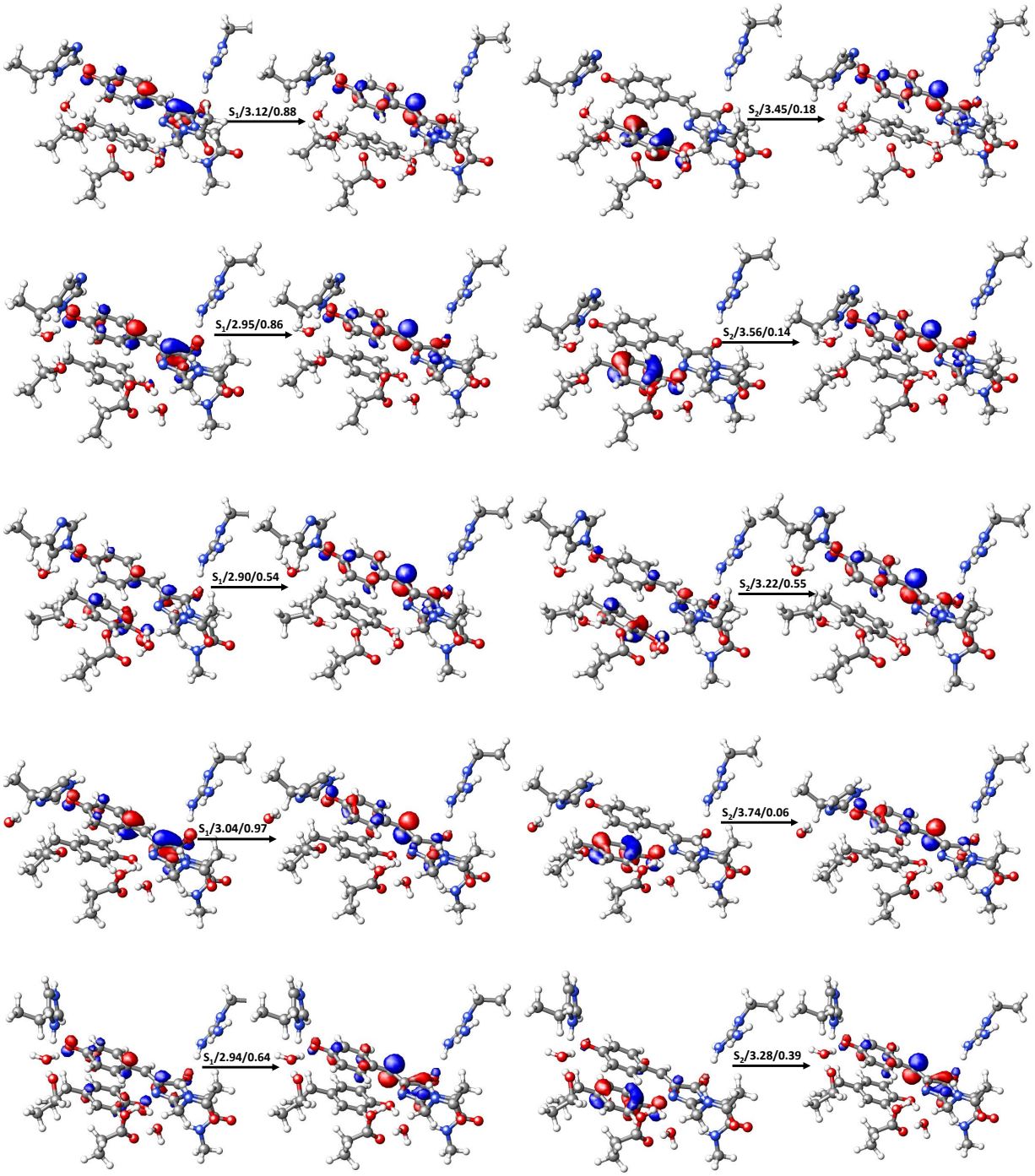


FIG. S8: NTOs of the lowest excited states of the anionic form and different protonation states of His145 and Glu222; TD-DFT, extended QM. Left: LE state; right: CT state; top-to-bottom: HSD-GLU, HSD-GLUP, HSE-GLU, HSE-GLUP, HSP-GLUP. CT state is pushed to much higher energies and disappears in QM/MM calculations.

TABLE S13: Effect of the protein environment beyond extended QM estimated from the 21 MD snapshots for the neutral chromophore in the ON-state. All energies are in eV; large QM.

System	State	TD-DFT aug-cc-pVDZ QM/MM (MD)	TD-DFT aug-cc-pVDZ QM only (MD)	TD-DFT aug-cc-pVDZ Δ^a	TD-DFT aug-cc-pVDZ QM only (opt)[b]	TD-DFT aug-cc-pVDZ QM/MM-corr[c]
HSD-GLUP	CT	4.01 (0.05)	3.76 (0.17)	+0.25	3.07 (0.26)	3.32 (0.26)
	LE	3.49 (0.56)	3.42 (0.52)	+0.07	3.49 (0.54)	3.56 (0.54)
HSE-GLUP	CT	3.96 (0.10)	3.70 (0.38)	+0.26	2.88(0.06)	3.14 (0.06)
	LE	3.58 (0.52)	3.44 (0.30)	+0.14	3.49 (0.73)	3.63 (0.73)
HSD-GLU	CT	3.84 (0.10)	3.58 (0.19)	+0.26	3.10 (0.13)	3.36 (0.13)
	LE	3.39 (0.49)	3.30 (0.51)	+0.09	3.41 (0.66)	3.50 (0.66)
HSE-GLU	CT	3.93 (0.08)	3.62 (0.11)	+0.31	2.81 (0.04)	3.12 (0.04)
	LE	3.45 (0.56)	3.37 (0.60)	+0.08	3.37 (0.77)	3.45 (0.77)
HSP-GLU	CT	3.88 (0.16)	3.71 (0.12)	+0.17	3.26 (0.14)	3.43 (0.14)
	LE	3.56 (0.63)	3.53 (0.69)	+0.03	3.56 (0.59)	3.59 (0.59)

[a] Δ is the difference in excitation energies in QM/MM and QM only calculation evaluated using structures from 21 MD snapshots.

[b] QM only excitation energies computed using ONIOM optimized structures.

[c] Extrapolated values: QM only excitation energies computed using ONIOM optimized structures plus Δ correction.

TABLE S14: Effect of the protein environment beyond extended QM estimated from the 21 MD snapshots for the anionic chromophore in the ON-state. All energies are in eV; large QM.

System	State	TD-DFT aug-cc-pVDZ QM/MM (MD)	TD-DFT aug-cc-pVDZ QM only (MD)	TD-DFT aug-cc-pVDZ Δ^a	TD-DFT aug-cc-pVDZ QM only (opt)[b]	TD-DFT aug-cc-pVDZ QM/MM-corr[c]
HSD-GLUP	LE	2.89 (0.91)	2.96 (0.86)	-0.07	3.01 (0.88)	2.94 (0.88)
HSE-GLUP	LE	2.99 (1.02)	3.02 (0.99)	-0.03	3.09 (0.95)	3.06 (0.95)
HSD-GLU	LE	3.07 (0.96)	3.02 (0.90)	+0.05	3.14 (0.98)	3.19 (0.98)
HSE-GLU	LE	3.03 (0.88)	2.92 (0.81)	+0.11	3.04 (0.94)	3.15 (0.94)
HSP-GLUP	LE	3.14 (0.94)	3.22 (0.88)	-0.08	3.05 (0.84)	2.97 (0.84)

[a] Δ is the difference in excitation energies in QM/MM and QM only calculation evaluated using structures from 21 MD snapshots.

[b] QM only excitation energies computed using ONIOM optimized structures.

[c] Extrapolated values: QM only excitation energies computed using ONIOM optimized structures plus Δ correction.

TABLE S15: Effect of the protein environment beyond extended QM estimated from the 21 MD snapshots for the OFF-form (neutral chromophore). All energies are in eV; large QM.

System	State	TD-DFT aug-cc-pVDZ QM/MM (MD)	TD-DFT aug-cc-pVDZ QM only (MD)	TD-DFT aug-cc-pVDZ Δ^a	TD-DFT aug-cc-pVDZ QM only (opt)[b]	TD-DFT aug-cc-pVDZ QM/MM-corr[c]
HSD-GLUP	LE	4.10 (0.44)	3.97 (0.39)	+0.13	3.86 (0.41)	3.99 (0.41)
HSE-GLUP	LE	3.89 (0.51)	3.88 (0.48)	+0.01	3.92 (0.33)	3.93 (0.33)
HSD-GLU	LE	4.12 (0.56)	4.03 (0.36)	+0.09	3.69 (0.53)	3.78 (0.53)
HSE-GLU	LE	3.88 (0.40)	3.79 (0.41)	+0.09	3.52 (0.57)	3.61 (0.57)
HSP-GLU	LE	3.89 (0.63)	3.85 (0.57)	+0.04	3.85 (0.60)	3.89 (0.60)
HSD-GLUP2	LE	3.79 (0.66)	3.63 (0.59)	+0.16	3.52 (0.64)	3.68 (0.64)
HSE-GLUP2	LE	3.77 (0.40)	3.74 (0.32)	+0.03	3.87 (0.58)	3.90 (0.58)

[a] Δ is the difference in excitation energies in QM/MM and QM only calculation evaluated using structures from 21 MD snapshots.

[b] QM only excitation energies computed using ONIOM optimized structures.

[c] Extrapolated values: QM only excitation energies computed using ONIOM optimized structures plus Δ correction.

TABLE S16: TD-DFT excitation energies (eV) of the two lowest states of protein-bound neutral chromophore in the ON-state with different basis sets and different size of QM region; oscillator strength is shown in parentheses.

System	State	Extended QM cc-pVDZ QM only	Extended QM mixed basis[a] QM only	Extended QM aug-cc-pVDZ QM only	Large QM aug-cc-pVDZ QM only
HSD-GLUP	LE	3.43 (0.72)	3.40 (0.61)	3.39 (0.63)	3.49 (0.54)
	CT	2.91 (0.16)	2.96 (0.24)	2.94 (0.22)	3.07 (0.26)
HSE-GLUP	LE	3.38 (0.87)	3.34 (0.82)	3.32 (0.82)	3.39 (0.73)
	CT	2.71 (0.03)	2.80 (0.05)	2.76 (0.05)	2.88 (0.06)
HSD-GLU	LE	3.46 (0.73)	3.41 (0.65)	3.41 (0.66)	3.44 (0.60)
	CT	3.09 (0.08)	3.12 (0.14)	3.10 (0.13)	3.16 (0.11)
HSE-GLU	LE	3.42 (0.80)	3.38 (0.75)	3.37 (0.77)	3.48 (0.67)
	CT	2.74 (0.03)	2.85 (0.05)	2.81 (0.04)	2.88 (0.02)
HSP-GLU	LE	3.62 (0.65)	3.57 (0.58)	3.56 (0.59)	3.58 (0.52)
	CT	3.26 (0.09)	3.28 (0.14)	3.26 (0.14)	3.32 (0.13)

[a] mixed basis: aug-cc-pVDZ for the chromophore and tyrosine and cc-pVDZ for rest of QM.

TABLE S17: TD-DFT excitation energies (eV) of the two lowest states of protein-bound anionic chromophore in the ON-state; oscillator strength is shown in parentheses.

System	State	Extended QM cc-pVDZ QM only	Extended QM aug-cc-pVDZ QM only	Large QM aug-cc-pVDZ QM only
HSD-GLUP	LE	3.03 (0.86)	2.95 (0.86)	3.01 (0.88)
HSE-GLUP	LE	3.12 (0.98)	3.04 (0.97)	3.09 (0.95)
HSD-GLU	LE	3.10 (0.79)	3.12 (0.88)	3.14 (0.98)
HSE-GLU	LE	2.89 (0.36)	2.90 (0.54)	3.04 (0.94)
HSP-GLUP	LE	2.96 (0.49)	2.94 (0.64)	3.05 (0.84)

TABLE S18: TD-DFT excitation energies (eV) of the two lowest states of protein-bound neutral chromophore in the OFF-state; oscillator strength is shown in parentheses.

System	State	Extended QM cc-pVDZ QM only	Extended QM aug-cc-pVDZ QM only	Large QM aug-cc-pVDZ QM only
HSD-GLUP	LE	4.03 (0.19)	3.86 (0.41)	3.80 (0.58)
HSE-GLUP	LE	3.97 (0.63)	3.92 (0.33)	3.93 (0.56)
HSD-GLU	LE	4.07 (0.11)	3.69 (0.53)	3.70 (0.49)
HSE-GLU	LE	3.57 (0.56)	3.52 (0.57)	3.58 (0.54)
HSP-GLU	LE	3.89 (0.58)	3.85 (0.60)	3.83 (0.52)
HSD-GLUP2	LE	3.58 (0.64)	3.52 (0.64)	3.57 (0.60)
HSE-GLUP2	LE	3.95 (0.56)	3.87 (0.58)	3.83 (0.52)

TABLE S19: Excitation energies (eV) of the two lowest states of protein-bound neutral chromophore in the ON-state; oscillator strength is shown in parentheses. Extended QM.

System	State	TD-DFT aug-cc-pVDZ QM only	SOS-CIS(D) aug-cc-pVDZ QM only	XMCQDPT2 cc-pVDZ QM only	XMCQDPT2[a] cc-pVDZ QM only	XMCQDPT2 aug-cc-pVDZ /cc-pVDZ QM only
HSD-GLUP	LE	3.39 (0.63)	2.87 (1.04)	2.62 (0.50)		2.59 (0.59)
	CT	2.94 (0.22)	3.40 (0.08)	2.98 (0.12)		3.04 (0.17)
HSE-GLUP	LE	3.32 (0.82)	2.87 (0.99)	2.64 (0.57)	2.88 (0.31)	2.79 (0.41)
	CT	2.76 (0.05)	3.17 (0.13)	2.89 (0.23)	3.09 (0.05)	2.51 (0.28)
HSD-GLU	LE	3.41 (0.66)	3.10 (0.97)	2.89 (0.72)	2.85 (0.40)	2.83 (0.24)
	CT	3.10 (0.13)	3.68 (0.04)	3.01 (0.03)	3.12 (0.03)	2.76 (0.28)
HSE-GLU	LE	3.37 (0.77)	2.98 (0.94)	2.70 (0.30)	2.74 (0.56)	2.87 (0.45)
	CT	2.81 (0.04)	3.21 (0.10)	2.98 (0.29)	3.08 (0.01)	2.59 (0.18)
HSP-GLU	LE	3.56 (0.59)	3.42 (0.93)	3.02 (0.14)	2.90 (0.16)	2.96 (0.26)
	CT	3.26 (0.14)	4.05 (0.04)	3.10 (0.15)	2.93 (0.02)	2.94 (0.11)

[a] Using structures and QM definition from the old protocol[1].

TABLE S20: Excitation energies of the protein-bound anionic chromophore in the ON-state; oscillator strength is shown in parentheses. Extended QM.

System	state	TD-DFT aug-cc-pVDZ QM only	SOS-CIS(D) aug-cc-pVDZ QM only	XMCQDPT2 cc-pVDZ QM only	XMCQDPT2[a] cc-pVDZ QM-only	XMCQDPT2 aug-cc-pVDZ /cc-pVDZ QM-only
HSD-GLUP	LE	2.95 (0.86)	2.41 (1.29)	2.30 (0.94)	2.39 (0.89)	2.16 (0.87)
HSE-GLUP	LE	3.04 (0.97)	2.68 (1.41)	2.58 (0.96)	2.37 (0.93)	2.39 (0.98)
HSD-GLU	LE	3.12 (0.88)	2.64 (1.34)	2.50 (0.93)		2.37 (0.92)
HSE-GLU	LE	2.90 (0.54)	2.41 (1.29)	2.41 (0.90)		2.29 (0.92)
HSP-GLUP	LE	2.94 (0.64)	2.43 (1.23)	2.39 (0.84)	2.41 (0.85)	2.27 (0.81)

[a] Using structures and QM definition from the old protocol[1].

TABLE S21: Excitation energies of the protein-bound neutral chromophore in the OFF-state; oscillator strength is shown in parentheses. Extended QM.

System	TDDFT aug-cc-pVDZ QM only	SOS-CIS(D) aug-cc-pVDZ QM only	XMCQDPT2 cc-pVDZ QM only	XMCQDPT2[a] cc-pVDZ QM-only	XMCQDPT2 aug-cc-pVDZ /cc-pVDZ QM-only
HSD-GLUP	3.86 (0.41)	3.98 (0.83)	4.06 (0.52)	3.79 (0.21)	3.50 (0.63)
HSE-GLUP	3.92 (0.33)	4.17 (0.79)	4.11 (0.53)	4.00 (0.61)	3.67 (0.58)
HSD-GLU	3.69 (0.53)	3.93 (0.76)	3.51 (0.58)	3.99 (0.66)	3.35 (0.55)
HSE-GLU	3.52 (0.57)	3.62 (0.80)	3.30 (0.63)	3.97 (0.43)	3.05 (0.55)
HSP-GLU	3.85 (0.60)	4.11 (0.72)	3.60 (0.55)	3.94 (0.60)	3.46 (0.60)
HSD-GLUP2	3.52 (0.64)	3.53 (0.87)	3.34 (0.52)		3.14 (0.52)
HSE-GLUP2	3.87 (0.58)	4.06 (0.78)	3.92 (0.21)		3.59 (0.54)

[a] Using structures and QM definition from the old protocol[1].

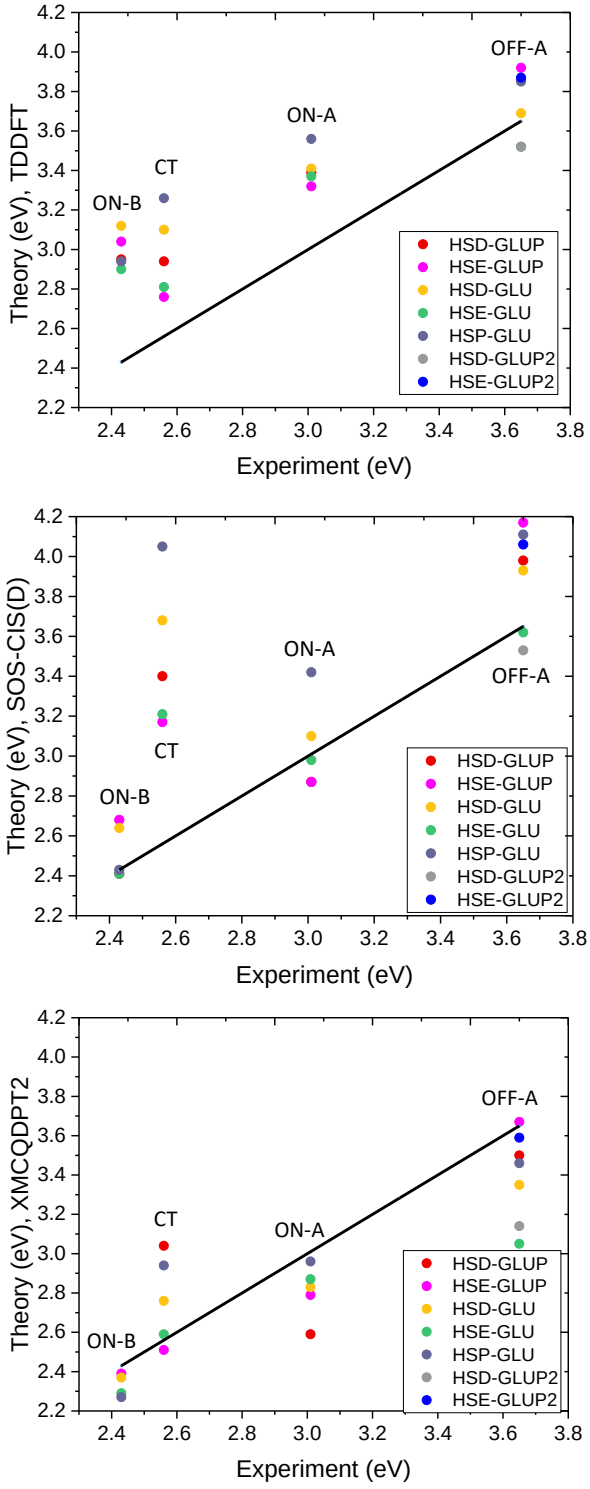


FIG. S9: Excitation energies for different model systems shown against the experimental values. Top: TD-DFT/aug-cc-pVDZ; middle: SOS-CIS(D)/aug-cc-pVDZ; bottom: XMCQDPT2/aug-cc-pVDZ/cc-pVDZ. Extended QM.

6. STRUCTURES OF POSSIBLE INTERMEDIATES

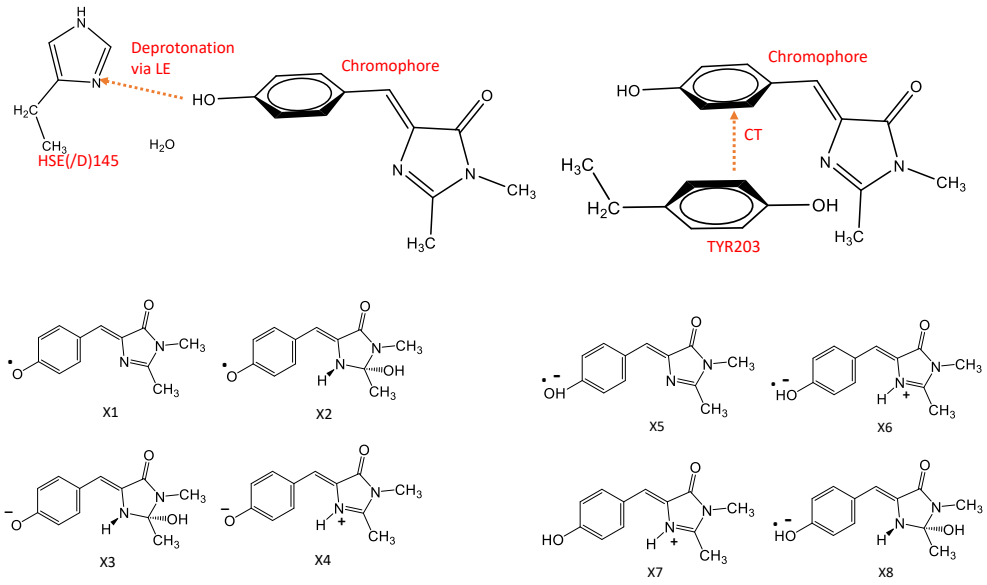


FIG. S10: Two possible initial steps for Dreiklang photoconversion. Ref. 5 proposed that the photoconversion begins by ESPT (left), forming anionic chromophore, which undergoes further transformation. Following this route, one can consider structures X1-X4 as possible candidates for reaction intermediate X. We propose an alternative mechanism via CT state (right). Following this route, one can consider structures X5-X8 as possible candidates for reaction intermediate X.

We considered several structures of the intermediates. Fig. S10 shows 2 possible scenarios for initiating photoconversion. Ref. 5 proposed that the photoconversion begins by ESPT, forming anionic chromophore, which undergoes further transformation. Following this route, one can consider structures X1-X4 as possible candidates for reaction intermediate X. As explained in the main text, there are several major objections to this mechanism. We propose an alternative mechanism via CT state. Following this route, one can consider structures X5-X8 as possible candidates for reaction intermediate X.

Intermediate X5 corresponds to the chromophore in the CT state ($\text{Chro}^{\cdot -}$). Intermediate X5 is the result of proton transfer to $\text{Chro}^{\cdot -}$, forming neutral radical. X7 is the result of the protonated chromophore after back-transfer of the electron. X8 is the result of the hydrated chromophore which still has the extra electron.

7. OPTIMIZATION AND AIMD SIMULATIONS: ADDITIONAL RESULTS

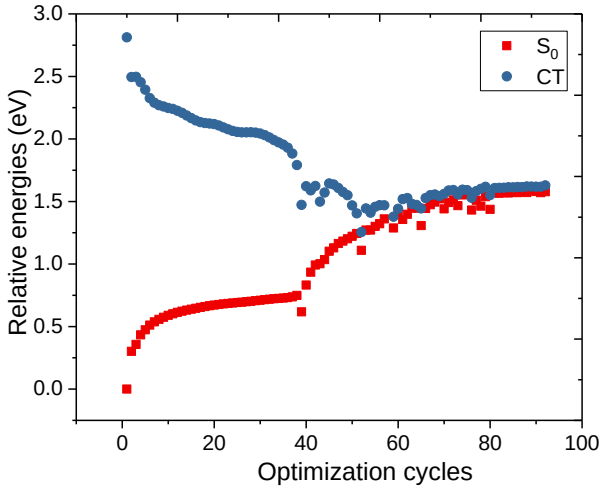


FIG. S11: Energies of the Kohn-Sham reference state (S_0) and CT state along optimization path (on-A-HSE-GLUP structure).

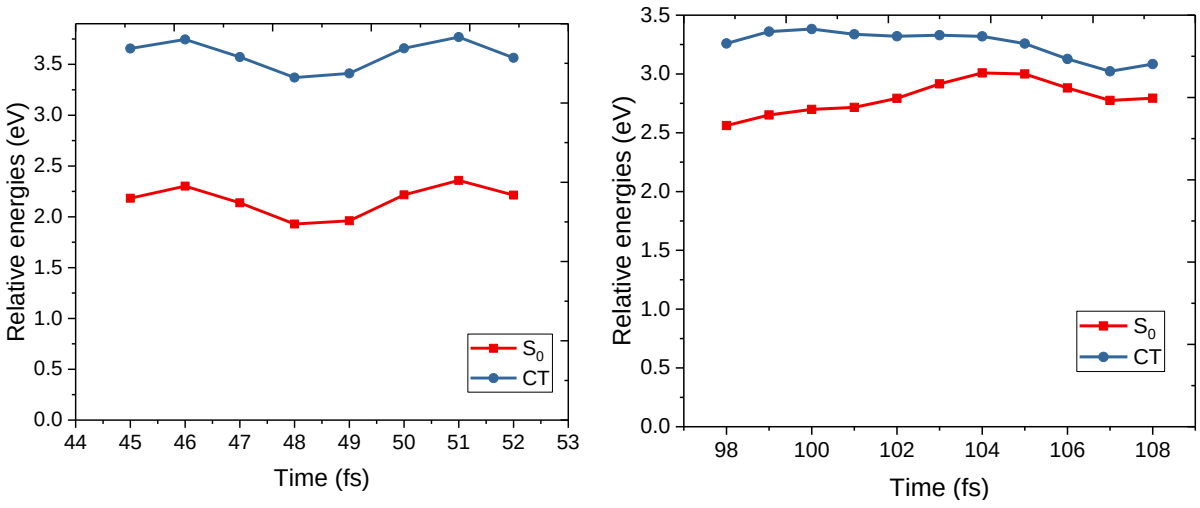


FIG. S12: Ground and excited state during the first two steps of the reaction in CT state (on-A-HSE-GLUP structure). Left: 1st step — proton abstraction by chromophore's N from protonated Glu222. Right: 2nd step — proton transfer from Tyr203 to deprotonated Glu222.

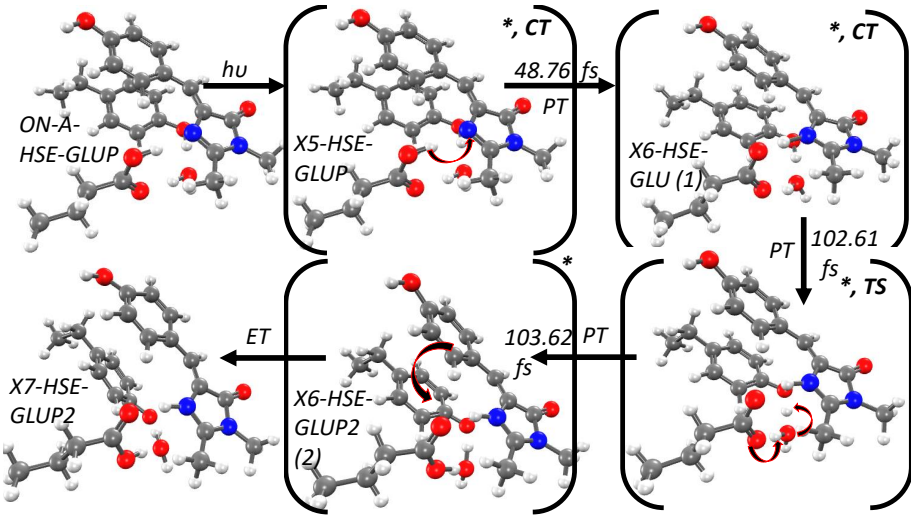


FIG. S13: Analysis of the AIMD trajectory on the CT state (on-A-HSE-GLUP structure).

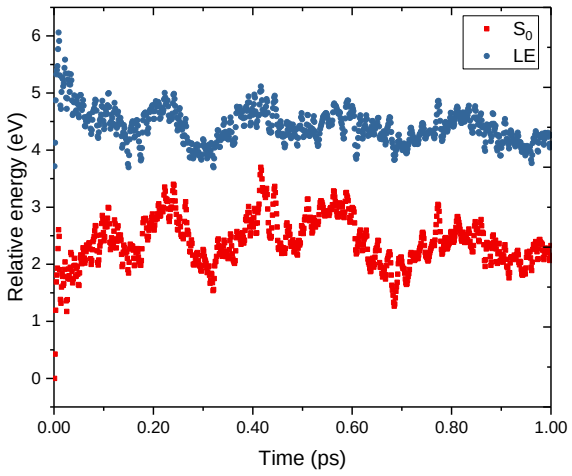


FIG. S14: Energies of the Kohn-Sham reference state (S_0) and the LE state (2nd TD-DFT state) along the AIMD trajectory on the LE potential energy surface.

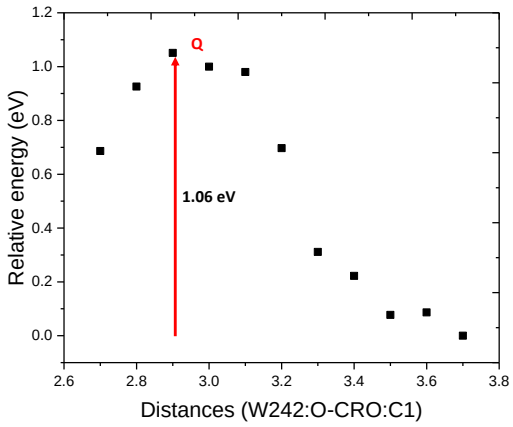


FIG. S15: Relaxed energy profile on the ground state surface (starting from X7 intermediate) along hydration reaction coordinate defined as W242:O-CRO:C1 distance. Zero energy corresponds to the energy of the reference state of the structure at t=248 fs, roughly corresponding to X7. ONIOM, ωB97X-D/aug-cc-pVDZ/CHARMM27.

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Cartesian coordinates

ωB97x-D/aug-cc-PVDZ optimized geometry of ON-A

C	3.3091392714	-2.1308271392	0.2134303414
C	2.5848741735	-0.8348662376	0.1239310625
N	1.2916641099	-0.7062162216	0.0942181618
N	3.2701059046	0.3635901151	0.0685756524
C	2.3357858127	1.3982844442	-0.0209219832
O	2.5968716423	2.5827704984	-0.0961886389
C	1.0371003294	0.6742859464	0.0103467948
C	4.7015770906	0.5603497681	0.0765287779
C	-0.1417695006	1.3303127748	-0.0352202594
C	-1.5055282597	0.8229202531	0.0235778174
C	-1.8302960776	-0.4988899432	0.3730205514
C	-2.5611520916	1.7062617066	-0.2673764360
C	-3.1521275670	-0.9212842253	0.4100735937
C	-3.8848542662	1.2940841740	-0.2387418156
C	-4.1826582533	-0.0285745246	0.0986161647
O	-5.4929560483	-0.3891408228	0.1157958982
O	0.1390370476	-3.2637870116	-0.4682586431
H	-4.6968740858	1.9812772426	-0.4697851985
H	-2.3334240442	2.7411406013	-0.5259990750
H	-1.0460681059	-1.2037186889	0.6327788152
H	-3.3820937267	-1.9523109421	0.6853201846
H	-5.5719772007	-1.3146707430	0.3630919490
H	-0.0367131841	2.4135082304	-0.1372189082
H	4.0264013014	-2.2305455081	-0.6126829750
H	2.5929853801	-2.9561462493	0.1733282001
H	-0.3313787551	-3.2391413644	-1.3043173035
H	0.4335890885	-2.3447530424	-0.3190811532
H	4.8761687873	1.6407366986	0.0448199919
H	5.1486624992	0.1481334333	0.9898458420
H	5.1693592899	0.0952169346	-0.8007436453
H	3.8772807657	-2.1811294827	1.1530660628

Nuclear Repulsion Energy = 1148.9571183284 hartrees

ωB97x-D/aug-cc-PVDZ optimized geometry of ON-B

C	3.3027564644	-2.1451620823	0.3323983921
C	2.5737735642	-0.8526074790	0.1981290730
N	1.2823706886	-0.7237066570	0.1642001048
N	3.2359788335	0.3523460149	0.0807602122
C	2.2771345443	1.3738511326	-0.0622696898
O	2.5628353477	2.5659494616	-0.1987306435
C	1.0204788214	0.6486532882	0.0065441900

C	4.6560018075	0.5786771117	0.0694292520
C	-0.2098616682	1.2735554584	-0.0897594095
C	-1.5267982581	0.7854494775	0.0152268322
C	-1.8674931179	-0.5506343601	0.4014120560
C	-2.6190902147	1.6650261570	-0.2671903500
C	-3.1656262017	-0.9737413462	0.4658381042
C	-3.9240287637	1.2604938988	-0.2163276932
C	-4.2917518616	-0.1019518288	0.1454115231
O	-5.4766677490	-0.4910057136	0.1929076623
O	0.1047780464	-3.0917160155	-0.9336598844
H	-4.7375854834	1.9513013766	-0.4442145162
H	-2.3866026056	2.6979301363	-0.5431817413
H	-1.0644472597	-1.2323274487	0.6768919484
H	-3.4065765254	-1.9921438854	0.7771094494
H	-0.1144289704	2.3457104150	-0.2899630401
H	3.9875292489	-2.3051938566	-0.5134348012
H	2.5762223272	-2.9628872630	0.3592889813
H	-0.8208027933	-2.8936533339	-1.0978892837
H	0.4522007713	-2.2588002241	-0.5527228569
H	4.8015463229	1.6613774886	-0.0221710796
H	5.1247605301	0.2267689077	0.9996986320
H	5.1375312464	0.0788741679	-0.7838656805
H	3.9031620573	-2.1655134287	1.2544962406

Nuclear Repulsion Energy = 1133.0413013516 hartrees

ωB97x-D/aug-cc-PVDZ optimized geometry of OFF-A

C	-2.4807177970	1.0232767613	0.1308360114
O	-2.9575249371	1.8995107343	-0.8723923920
C	-2.1323269991	-1.2872645732	-0.1924974857
N	-1.0801849973	0.7732152778	-0.1275990936
O	-2.3568405148	-2.4745147945	-0.3360957613
N	-3.0615871950	-0.3014194133	-0.0029591808
O	5.6031414480	0.8439237617	0.1268282906
C	4.3214475927	0.3872898896	0.0289212869
C	-2.7270767761	1.6202151601	1.5183336256
C	-0.8105412441	-0.5940795893	-0.1659835506
C	-4.4830424908	-0.5281887611	-0.0403433542
C	0.3629749230	-1.2461263359	-0.1533360145
C	2.7692971791	-1.4085175366	0.4296855879
C	1.9994783146	0.6230982282	-0.5920715635
C	4.0581582691	-0.8992174398	0.5044029532
C	3.2901808436	1.1436997141	-0.5279294208
C	1.7060014137	-0.6593237039	-0.1036964921
H	-3.7371306959	2.3587640496	-0.5486627594
H	-0.4270115993	1.3692379093	0.3632598529

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H        -2.2163270622    2.5879259505    1.6002136364
H        -3.7993064774    1.7790480792    1.6969912697
H        -4.6388500521   -1.5995257282   -0.2014971224
H        -4.9602558979   -0.2389510651    0.9070199404
H         0.2801677200   -2.3334025210   -0.1493499242
H         2.5749871045   -2.4167847654    0.7973674554
H         1.2225921952    1.2130570879   -1.0773184398
H         4.8731698950   -1.4864630146    0.9247161919
H         3.4938655846    2.1403074589   -0.9248345398
H        -4.9438196858    0.0293465198   -0.8673209125
H        -2.3459991877    0.9387961973    2.2875916131
H         5.6618094344    1.7237143586   -0.2547940897

Nuclear Repulsion Energy =  1177.1217891621 hartrees

$\omega$B97x-D/aug-cc-PVDZ optimized geometry of OFF-B

C        -2.4897413522    1.0086262888   -0.0061484603
O        -3.0275385296    1.7979414290   -1.0703043277
C        -2.0525438978   -1.3093135473   -0.1259711620
N        -1.0875483707    0.8019122878   -0.2624201273
O        -2.2843073959   -2.5139126969   -0.1502420472
N        -3.0287640951   -0.3335197974   -0.0242432060
O         5.6437420628    0.9036412038    0.1513203007
C         4.4757966385    0.4459307883    0.0709872965
C        -2.7523199588    1.7165776518    1.3288706891
C        -0.7829877331   -0.5872731447   -0.1813981111
C        -4.4334712312   -0.6194164977   -0.0105042068
C         0.4356983231   -1.1751990408   -0.0997753525
C         2.8580148234   -1.4145579346    0.2863896624
C         2.0576852248    0.7515375433   -0.4030158796
C         4.1449034165   -0.9349624289    0.3671034123
C         3.3398233134    1.2505226056   -0.3380324399
C         1.7540355369   -0.5992302782   -0.0790305270
H        -3.8520899241    2.1909315183   -0.7717843531
H        -0.4813016211    1.3895094210    0.2982754484
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H        -4.5402788389   -1.7067517300   -0.0947766645
H        -4.9119560171   -0.2897285010    0.9262706319
H         0.3762269517   -2.2613093489    0.0094762717
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H         4.9664523280   -1.5933021002    0.6576663323
H         3.5427232220    2.2893019097   -0.6081927226
H        -4.9444688612   -0.1428182141   -0.8611569444
H        -2.3322741380    1.1189468857    2.1468072929
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Nuclear Repulsion Energy =  1157.6033747204 hartrees
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