Supplementary Material (ESI) for PCCP

Supporting information

QM/MM Studies of the Far-red Fluorescent Protein HcRed

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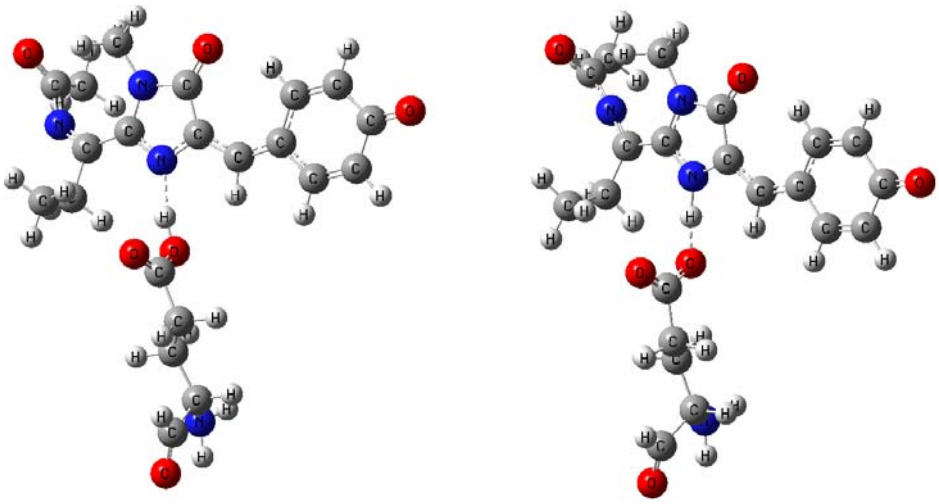

	complex (a)	complex (b)
total energy:	-1446.4923509 a.u.	-1446.4846623 a.u.
relative energy:	0 kcal/mol	4.8 kcal/mol

Figure S1. Gas-phase B3LYP/6-31+G* energies of hydrogen bonded complexes between (a) anionic chromophore and protonated Glu214, and (b) zwitterionic chromophore and deprotonated Glu214.

Table S1. Geometry of the QM region for model A of HcRed (*cis*-chromophore): Average values from SCC-DFTB/MM MD simulation and experimental data (see Tables 2 and 3 for notation). Δm is the rms deviation between the average MD values and the X-ray data.

Bond lengths (Å)	MD	Δm	Exp [15]
OH_CZ	1.263	-0.104	1.367
CZ_CE1	1.459	0.030	1.429
CE1_CD1	1.367	-0.075	1.442
CD1_CG2	1.439	0.004	1.435
CG2_CD2	1.441	-0.001	1.442
CD2_CE2	1.365	-0.060	1.425
CE2_CZ	1.459	0.025	1.434
CG2-CB2	1.400	-0.095	1.495
CB2-CA2	1.413	0.024	1.389
CA2-N2	1.387	0.015	1.372
N2-C1	1.341	-0.009	1.350
C1-N3	1.413	-0.024	1.437
N3-C2	1.383	-0.031	1.414
C2-CA2	1.447	-0.019	1.466
O2-C2	1.287	0.050	1.237
C1-CA1	1.463	0.006	1.457
CA1-CB1	1.514	-0.030	1.544
CB1-CG1	1.533	-0.008	1.541
CG1-CD3	1.588	0.089	1.499
CD3-OE2	1.285	-0.087	1.372
CD3-OE1	1.266	0.029	1.237
CA1-N	1.299	0.029	1.270
N3-CA3	1.443	-0.126	1.569
CA3-C	1.522	0.334	1.188
C-O	1.260	-0.112	1.372
N64-63C	1.371	0.100	1.271
63C-63O	1.234	-0.002	1.236
63C-63CA	1.534	0.009	1.525
64C-65N	1.352	0.007	1.345
65N-65CA	1.459	-0.012	1.471
65CA-65CB	1.524	-0.001	1.525
65CB-65OG	1.426	0.015	1.411
65CA-65C	1.483	-0.047	1.530
Dihedral angle (º)			
N2_CA2_CB2_CG2	7.8	7.8	0.0
CA2_CB2_CG2_CD1	8.6	0.2	8.4
Distance (Å)			
O_NE2(Gln107)	3.140	0.121	3.019
O2_NH2(Arg93)	2.689	-0.501	3.190
OH_OG(Ser144)	2.888	0.287	2.601
N2_OE2(Glu214)	3.626	0.660	2.966
N2_NE2(Gln40)	2.993	-0.190	3.183

Table S2. Relevant dihedral angles (º) and hydrogen bond distances (Å) for the *cis*- and *trans*-chromophore in model A of HcRed: DFT/MM optimized values for snapshots 1-4 and experimental data (see Figure 1a for atom labels).

Snapshots	1	2	3	4	Exp [15]
cis-conformer / dihedral angle					
N2_CA2_CB2_CG2	7.3	7.0	7.0	6.2	0.0
CA2_CB2_CG2_CD1	8.8	8.3	9.0	8.3	8.4
cis-conformer / distance					
O_NE2(Gln107)	3.355	3.356	3.407	3.415	3.091
O2_NH2(Arg93)	2.646	2.647	2.646	2.645	3.190
OH_OG(Ser144)	2.658	2.687	2.684	2.699	2.601
OH_OH(TIP3189)	2.728	2.721	2.747	2.719	
N2_OE2(Glu214)	3.041	2.961	2.980	3.001	2.966
OE1_NE2(Gln40)	2.788	2.791	2.802	2.802	3.183
trans-conformer / dihedral angle					
N2_CA2_CB2_CG2	-174.8	-174.3	-174.4	-173.5	175.7
CA2_CB2_CG2_CD1	26.3	26.5	27.2	24.9	48.2
trans-conformer / distance					
O_NE2(Gln107)	3.569	3.522	3.575	3.559	3.091
O2_NH2(Arg93)	2.695	2.688	2.692	2.690	3.190
N2_OE2(Glu214)	2.903	2.878	2.899	2.908	2.996
OE1_NE2(Gln40)	2.801	2.802	2.807	2.813	3.183
OH_OE2((Glu146)	2.568	2.571	2.566	2.574	3.062
OH_ND2(Asn159)	3.761	3.582	3.771	3.757	3.069

Table S3. QM energies (a.u.), MM energies (a.u.), total QM/MM energies (a.u.), and relative energies (kcal/mol) for *cis*- and *trans*-conformers in model A of HcRed: DFT(B3LYP/SV(P))/MM results for snapshots 1-4.

snapshot		$E_{(QM,MM)}$ (a.u.)	ΔE_Q (kcal/mol)	$E_{(MM,QM)}$ (a.u.)	ΔE_M (kcal/mol)	E_{total} (a.u.)	ΔE_t (kcal/mol)
1	*cis*	-1479.051467	0	-54.526707	0	-1533.571910	0
	trans	-1479.045203	-3.9	-54.522132	2.9	-1533.573600	-1.1
2	*cis*	-1479.061768	0	-53.910447	0	-1532.972215	0
	trans	-1479.064876	-2.0	-53.910819	-0.2	-1532.975695	-2.2
3	*cis*	-1479.066244	0	-52.530826	0	-1531.597069	0
	trans	-1479.072476	-3.9	-52.531640	-0.5	-1531.604116	-4.4
4	*cis*	-1479.036883	0	-54.809072	0	-1533.845955	0
	trans	-1479.046814	-6.2	-54.803605	3.4	-1533.850419	-2.8

Table S4. Geometry of the QM region for model C of HcRed (*cis*-chromophore): Average values from SCC-DFTB/MM MD simulation and experimental data (see Tables 2 and 3 for notation). Δm is the rms deviation between the average MD values and the X-ray data.

Bond length (Å)	MD	Δm	Exp [15]
OH_CZ	1.268	-0.099	1.367
CZ_CE1	1.455	0.026	1.429
CE1_CD1	1.369	-0.073	1.442
CD1_CG2	1.439	0.004	1.435
CG2_CD2	1.440	-0.002	1.442
CD2_CE2	1.366	-0.059	1.425
CE2_CZ	1.456	0.022	1.434
CG2-CB2	1.400	-0.095	1.495
CB2-CA2	1.411	0.022	1.389
CA2-N2	1.381	0.009	1.372
N2-C1	1.342	-0.008	1.350
C1-N3	1.421	-0.016	1.437
N3-C2	1.380	-0.034	1.414
C2-CA2	1.450	-0.016	1.466
O2-C2	1.289	0.052	1.237
C1-CA1	1.453	-0.004	1.457
CA1-CB1	1.503	-0.041	1.544
CB1-CG1	1.537	-0.004	1.541
CG1-CD3	1.587	0.088	1.499
CD3-OE2	1.284	-0.088	1.372
CD3-OE1	1.267	0.030	1.237
CA1-N	1.318	0.048	1.270
N3-CA3	1.441	-0.128	1.569
CA3-C	1.520	0.332	1.188
C-O	1.262	-0.110	1.372
N64-63C	1.340	0.069	1.271
63C-63O	1.263	0.027	1.236
63C-63CA	1.536	0.011	1.525
64C-65N	1.355	0.010	1.345
65N-65CA	1.463	-0.008	1.471
65CA-65CB	1.522	-0.003	1.525
65CB-65OG	1.439	0.028	1.411
65CA-65C	1.481	-0.049	1.530
Dihedral angle (º)			
N2_CA2_CB2_CG2	3.7	3.7	0.0
CA2_CB2_CG2_CD1	5.4	5.4	8.4
Distance (Å)			
O_NE2(Gln107)	3.148	0.129	3.019
O2_NH2(Arg93)	2.670	-0.520	3.190
OH_OG(Ser144)	2.794	0.193	2.601
N2_OE2(Glu214)	3.087	0.121	2.966
N2_NE2(Gln40)	2.765	-0.418	3.183

Table S5. Relevant dihedral angles (º) and hydrogen bond distances (Å) for the *cis*- and *trans*-chromophore in model C of HcRed: DFT/MM optimized values for snapshots 1-4 and experimental data (see Figure 1a for atom labels).

Snapshots	1	2	3	4	Exp [15]
cis-conformer / dihedral angle					
N2_CA2_CB2_CG2	1.7	-2.4	-4.3	-0.5	0.0
CA2_CB2_CG2_CD1	5.0	-1.7	-4.8	0.5	8.4
cis-conformer / distance (Å)					
O_NE2(Gln107)	3.234	3.192	3.070	3.123	3.091
O2_NH2(Arg93)	2.657	2.652	2.621	2.663	3.190
OH_OG(Ser144)	2.690	2.636	2.628	2.680	2.601
OH_OH(TIP3)	2.659	2.672	2.667	2.675	
N2_OE2(Glu214)	3.192	3.281	3.252	3.181	2.966
OE1_NE2(Gln40)	2.750	2.740	2.749	2.757	3.183
trans-conformer / dihedral angle					
N2_CA2_CB2_CG2	-179.3	-175.0	-179.0	-179.8	175.7
CA2_CB2_CG2_CD1	11.3	14.3	11.9	11.7	48.2
trans-conformer / distance					
O_NE2(Gln107)	3.200	3.259	3.197	3.302	3.091
O2_NH2(Arg93)	2.709	2.732	2.712	2.768	3.190
N2_OE2(Glu214)	3.237	3.166	3.223	3.225	2.996
OE1_NE2(Gln40)	2.939	2.764	2.929	2.991	3.183
OH_OE2((Glu146)	3.872	3.897	3.863	3.941	3.062
OH_ND2(Asn159)	2.731	2.781	2.716	2.756	3.069

Table S6. QM energies (a.u.), MM energies (a.u.), total QM/MM energies (a.u.), and relative energies (kcal/mol) for *cis*- and *trans*-conformers in model C of HcRed: DFT(B3LYP/SV(P))/MM results for snapshots 1-4.

snapshot		$E_{(QM,MM)}$ (a.u.)	ΔE_Q (kcal/mol)	$E_{(MM,QM)}$ (a.u.)	ΔE_M (kcal/mol)	E_{total} (a.u.)	ΔE_t (kcal/mol)
1	*cis*	-1478.951590	0	-54.391207	0	-1533.342798	0
	trans	-1478.920977	19.2	-54.402107	-6.8	-1533.323084	12.4
2	*cis*	-1478.960166	0	-54.490546	0	-1533.450711	0
	trans	-1478.911922	30.1	-54.507035	-10.3	-1533.418958	19.9
3	*cis*	-1478.911795	0	-54.401293	0	-1533.313087	0
	trans	-1478.889611	13.9	-54.399474	1.1	-1533.289084	15.1
4	*cis*	-1478.940143	0	-54.019252	0	-1532.959394	0
	trans	-1478.910184	18.8	-54.020366	-0.7	-1532.930550	18.1