Supplementary Material (ESI) for *PCCP*

The 559-to-600 nm shift observed in red fluorescent protein eqFP611 is attributed to *cis–trans* isomerization of the chromophore in an anionic protein pocket

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Supporting Materials

Table 1: atomic coordinates (in Å) of the trans and cis isomers of the eqFP611 chromophore at the neutral and anionic protonated states optimised at the ONIOM(B3LYP/6-31G(d):Amber) level. Atoms are labelled in Figure 1.

	neutral/trans			neutral/cis			anionic/trans			anionic/cis		
	X	**Y**	**Z**	**X**	**Y**	**Z**	**X**	**Y**	**Z**	**X**	**Y**	**Z**
C1	1.036	4.343	0.561	1.076	4.031	0.583	0.910	4.179	0.491	0.943	4.075	0.660
O2	0.266	5.318	0.757	0.258	4.986	0.625	0.130	5.145	0.698	0.130	5.029	0.777
N3	0.517	3.116	0.168	0.630	2.749	0.311	0.412	2.973	0.098	0.490	2.829	0.338
C4	0.522	2.424	-0.903	0.546	1.987	-0.706	0.432	2.142	-0.888	0.474	2.007	-0.654
C5	1.014	2.894	-2.261	0.928	2.384	-2.116	0.905	2.511	-2.290	0.889	2.408	-2.062
C6	-0.123	3.550	-3.062	-0.242	3.140	-2.773	-0.234	3.143	-3.104	-0.279	3.154	-2.727
C7	0.033	1.028	-0.869	-0.023	0.630	-0.524	-0.084	0.798	-0.754	-0.059	0.673	-0.493
N8	0.307	0.234	-1.872	0.102	-0.277	-1.468	0.120	-0.089	-1.722	0.078	-0.257	-1.440
N9	-0.632	0.387	0.175	-0.681	0.186	0.619	-0.743	0.214	0.336	-0.726	0.180	0.636
C10	-0.761	-0.966	-0.163	-1.003	-1.164	0.408	-0.897	-1.159	0.067	-1.013	-1.169	0.400
O11	-1.251	-1.840	0.549	-1.554	-1.926	1.191	-1.415	-1.990	0.828	-1.593	-1.955	1.158
C12	-0.141	-1.036	-1.511	-0.466	-1.437	-0.943	-0.312	-1.302	-1.272	-0.457	-1.406	-0.929
C13	-1.084	0.915	1.456	-1.063	0.871	1.850	-1.156	0.809	1.604	-1.155	0.848	1.858
C14	0.131	-2.075	-2.358	-0.527	-2.685	-1.484	-0.045	-2.416	-2.096	-0.493	-2.691	-1.487
C15	-0.129	-3.487	-2.350	0.037	-3.177	-2.711	-0.240	-3.787	-1.999	0.099	-3.202	-2.635
C16	0.373	-4.201	-3.459	-0.415	-4.402	-3.242	0.227	-4.591	-3.098	-0.214	-4.537	-3.059
C17	-0.829	-4.208	-1.351	1.062	-2.500	-3.399	-0.848	-4.485	-0.892	1.039	-2.471	-3.437
C18	0.193	-5.564	-3.573	0.073	-4.891	-4.442	0.084	-5.940	-3.122	0.258	-5.051	-4.227
C19	-1.013	-5.577	-1.463	1.562	-2.995	-4.589	-1.002	-5.837	-0.912	1.528	-2.984	-4.595
C20	-0.499	-6.261	-2.577	1.042	-4.167	-5.149	-0.564	-6.667	-2.036	1.114	-4.278	-5.123
O21	-0.665	-7.607	-2.640	1.440	-4.650	-6.356	-0.727	-7.902	-2.065	1.482	-4.690	-6.242
S22	0.292	3.933	-4.808	-0.032	3.500	-4.562	0.162	3.421	-4.876	-0.067	3.579	-4.511
C23	2.013	4.521	-4.686	1.727	3.971	-4.673	1.687	4.405	-4.763	1.722	3.902	-4.659
H24	0.583	-6.096	-4.439	-0.297	-5.829	-4.835	0.466	-6.524	-3.953	0.000	-6.060	-4.524
H25	0.909	-3.675	-4.243	-1.184	-4.966	-2.719	0.726	-4.088	-3.927	-0.862	-5.139	-2.421
H26	-1.214	-3.670	-0.495	1.463	-1.585	-2.985	-1.145	-3.895	-0.033	1.372	-1.503	-3.079
H27	-1.552	-6.137	-0.705	2.388	-2.488	-5.070	-1.430	-6.361	-0.060	2.281	-2.450	-5.157
H28	0.683	-1.755	-3.238	-1.091	-3.402	-0.892	0.481	-2.111	-3.001	-1.071	-3.393	-0.887
H29	1.817	3.615	-2.112	1.813	3.026	-2.065	1.741	3.211	-2.210	1.765	3.062	-2.000
H30	1.426	2.044	-2.805	1.193	1.495	-2.689	1.265	1.615	-2.796	1.173	1.523	-2.631
H31	-0.456	4.453	-2.548	-0.427	4.060	-2.219	-0.548	4.089	-2.658	-0.491	4.061	-2.160
H32	-0.980	2.873	-3.130	-1.147	2.524	-2.737	-1.099	2.474	-3.131	-1.170	2.521	-2.718
H33	2.305	4.796	-5.701	1.910	4.191	-5.727	1.928	4.726	-5.780	1.894	4.131	-5.714
H34	2.110	5.393	-4.037	1.962	4.854	-4.075	1.543	5.288	-4.138	2.049	4.752	-4.052
H35	2.677	3.737	-4.318	2.365	3.142	-4.355	2.518	3.822	-4.360	2.296	3.017	-4.377
H36	-0.274	1.423	1.937	-0.243	1.463	2.198	-0.351	1.390	2.004	-0.361	1.466	2.224
H37	-1.419	0.110	2.075	-1.324	0.148	2.595	-1.414	0.033	2.294	-1.405	0.115	2.596
H38	-0.290	-7.892	-3.521	2.149	-4.103	-6.748						

Table 2: Atomic ESP charges of the trans and cis isomers of the eqFP611 chromophore at the neutral and anionic states obtained at the ONIOM(B3LYP/6-311++G(d,p):Amber) level. Atoms are labelled in Figure 1

	neutral/trans	**neutral/cis**	**anionic/trans**	**anionic/cis**
C1	0.7774	0.7614	0.7864	0.7772
O2	-0.6877	-0.6871	-0.7392	-0.7298
N3	-0.4681	-0.4054	-0.5283	-0.4869
C4	0.2524	0.1431	0.3430	0.2057
C5	-0.0153	0.1280	-0.1351	0.0263
C6	-0.0852	-0.0477	-0.0905	0.0718
C7	0.5062	0.4990	0.3875	0.4537
N8	-0.7199	-0.7276	-0.6534	-0.8198
N9	-0.2396	-0.1867	-0.2848	-0.1670
C10	0.6073	0.5490	0.6762	0.5098
O11	-0.6831	-0.7082	-0.8434	-0.8033
C12	0.1770	0.2460	0.0998	0.3082
C13	-0.0291	-0.0774	-0.0633	-0.1086
C14	-0.1125	-0.1963	-0.2035	-0.3077
C15	0.0964	0.2570	0.1859	0.3485
C16	-0.0686	-0.1929	-0.1751	-0.2383
C17	-0.1039	-0.2303	-0.2460	-0.3207
C18	-0.3175	-0.1460	-0.3065	-0.2925
C19	-0.2575	-0.2683	-0.3297	-0.2630
C20	0.5445	0.4679	0.7794	0.7519
O21	-0.7405	-0.6985	-0.8402	-0.8421
S22	-0.2519	-0.2512	-0.2210	-0.2902
C23	-0.1429	-0.1321	-0.1444	-0.1194
H24	0.1827	0.1749	0.1692	0.1392
H25	0.1070	0.1624	0.1214	0.1420
H26	0.1075	0.1977	0.2038	0.2392
H27	0.1738	0.1195	0.1362	0.1234
H28	0.0995	0.1002	0.0944	0.0754
H29	0.1006	0.0755	0.1233	0.0934
H30	0.0736	0.0293	0.1246	0.0574
H31	0.0756	0.0610	0.0831	0.0310
H32	0.0647	0.0337	0.0043	-0.0169
H33	0.1130	0.1047	0.1060	0.1025
H34	0.0801	0.0775	0.0771	0.0693
H35	0.0647	0.0483	0.0654	0.0491
H36	0.1156	0.1193	0.1350	0.1206
H37	0.0744	0.1082	0.1023	0.1100
H38	0.5286	0.4912		

Supplementary Material (ESI) for *PCCP*

Table 3: ESP charges for the ground state (GS) and excited state (ES) of the anionic chromophore at the absorption and fluorescence electronic states obtained at ONIOM(CASSCF(12,11)/6-31G(d):Amber) level. Atoms are labelled in Figure 1

	Absorption		Fluorescence	
	GS	ES	GS	ES
C1	0.7955	0.8012	0.8094	0.7896
O2	-0.7182	-0.7274	-0.6528	-0.6946
N3	-0.4860	-0.5111	-0.6207	-0.5953
C4	0.3176	0.2649	0.3276	0.0765
C5	-0.0571	-0.0221	-0.0098	0.0942
C6	-0.0853	-0.0912	-0.1244	-0.0632
C7	0.3523	0.3565	0.4962	0.4913
N8	-0.5801	-0.6353	-0.6058	-0.7252
N9	-0.3437	-0.3345	-0.3832	-0.3149
C10	0.6885	0.6516	0.8073	0.7875
O11	-0.8171	-0.7729	-0.8076	-0.8076
C12	-0.0181	0.2773	-0.0686	0.1412
C13	-0.0401	-0.0491	-0.0928	-0.1247
C14	-0.1737	-0.3205	-0.0460	-0.1998
C15	0.0548	0.2493	-0.0681	0.1802
C16	-0.1048	-0.2144	-0.0742	-0.1965
C17	-0.1107	-0.2892	-0.0959	-0.2505
C18	-0.3716	-0.2618	-0.4998	-0.3359
C19	-0.4086	-0.3426	-0.4090	-0.3561
C20	0.8659	0.7214	0.8593	0.7404
O21	-0.8251	-0.8352	-0.8669	-0.7151
S22	-0.2577	-0.2613	-0.2418	-0.2946
C23	-0.1364	-0.1338	-0.1362	-0.1224
H24	0.1713	0.1615	0.1943	0.1863
H25	0.1161	0.1228	0.1114	0.1418
H26	0.1655	0.1839	0.1479	0.2025
H27	0.1462	0.1437	0.1369	0.1521
H28	0.1059	0.1040	0.0954	0.1116
H29	0.1018	0.1002	0.0853	0.0630
H30	0.0776	0.0699	0.0795	0.0410
H31	0.0809	0.0841	0.0891	0.0715
H32	0.0363	0.0336	0.0490	0.0241
H33	0.1146	0.1147	0.1179	0.1079
H34	0.0726	0.0729	0.0741	0.0689
H35	0.0647	0.0615	0.0667	0.0583
H36	0.1072	0.1221	0.1376	0.1482
H37	0.0990	0.1054	0.1186	0.1184

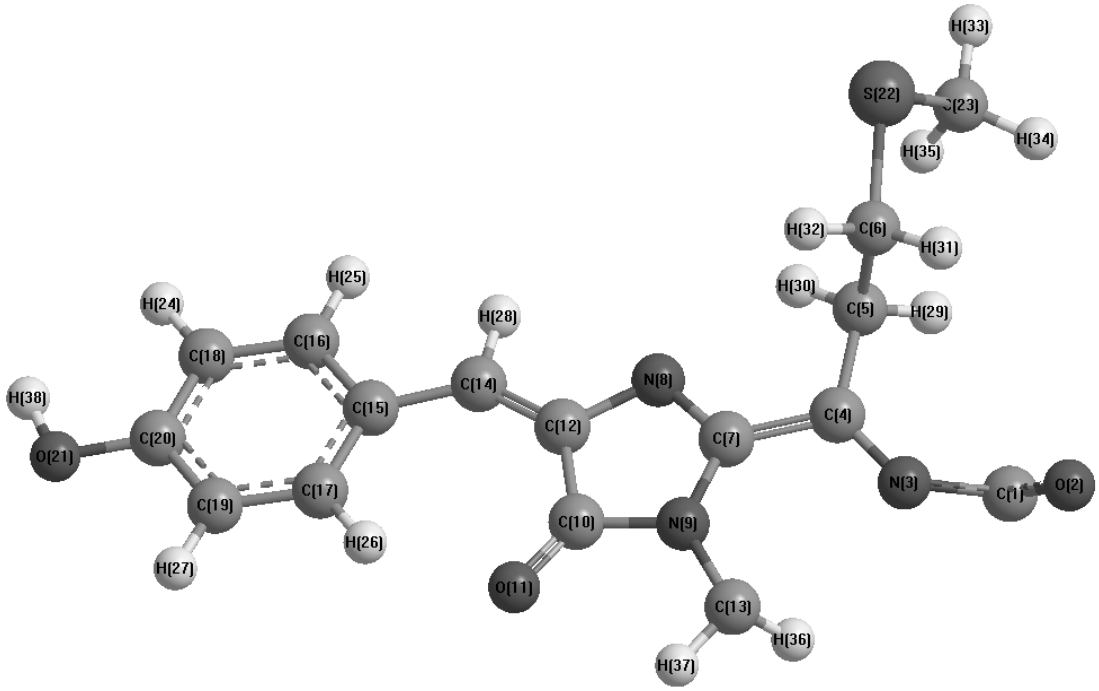

Figure 1: atomic labels of the chromophore in eqFP611