A photoelectron imaging and quantum chemistry study of the deprotonated cyan fluorescent protein chromophore anion

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1 Velocity map imaging images

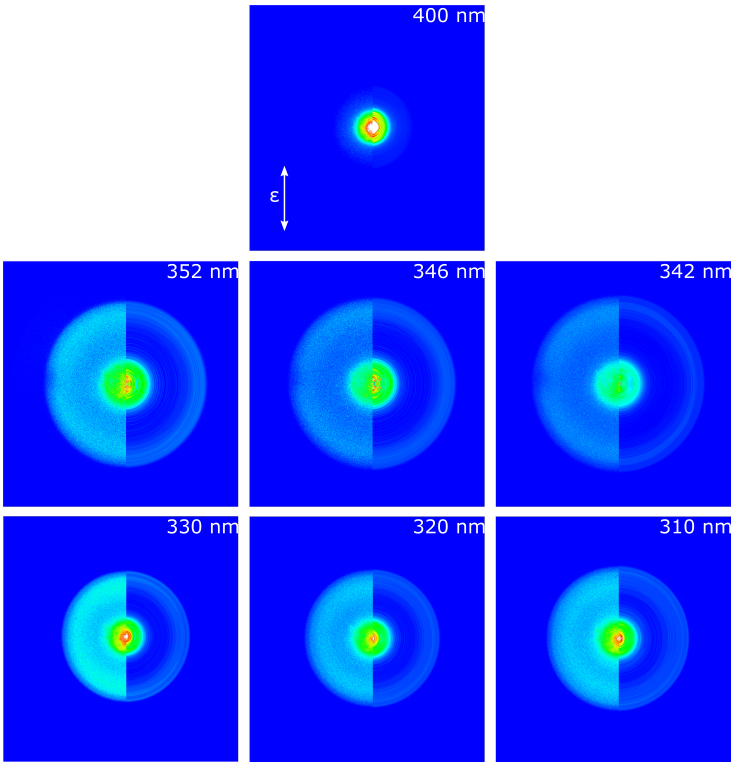

Figure S1: Photoelectron images recorded following photodetachment from the deprotonated CFP anion. The left side of each image is the raw image while the right-hand side has the inverted image. Wavelengths of the detaching laser are indicated for each image and the direction of the electric field of the laser is shown in the 400 nm image.

2 Optimised geometries

Atom	Anion			Neutral		
	x	y	z	x	y	z
C	-4.443797	-1.576752	0.00037	4.471694	-1.544013	0.000005
C	-3.053333	-1.510568	0.000495	3.074502	-1.515511	-0.000061
C	-2.434001	-0.256446	0.000255	2.443211	-0.277032	-0.000031
C	-3.225669	0.924463	-0.000139	3.196679	0.913386	0.000066
C	-4.619837	0.848658	-0.000287	4.578398	0.890647	0.000134
C	-5.222622	-0.406239	-0.000019	5.210522	-0.359532	0.000101
H	-4.938239	-2.549533	0.000512	4.991646	-2.501082	-0.00001
H	-2.462889	-2.428224	0.000698	2.506005	-2.445161	-0.000121
H	-5.214534	1.762837	-0.000622	5.149336	1.817482	0.00021
H	-6.311008	-0.485657	-0.000155	6.29907	-0.409299	0.000155
C	-1.060992	0.206266	-0.000012	1.050376	0.149723	-0.000033
C	-1.186547	1.637371	0.000507	1.138599	1.621172	-0.000093
H	-0.3464	2.328355	0.000469	0.280196	2.287188	-0.000096
N	-2.43459	2.069497	-0.000442	2.356253	2.058348	0.000091
C	0.086401	-0.587778	-0.000243	-0.069148	-0.656822	-0.000057
H	-0.070365	-1.670266	-0.000308	0.074134	-1.738547	-0.000059
C	2.505658	-1.204349	-0.000516	-2.530529	-1.212964	-0.000204
C	3.241989	0.933625	-0.000033	-3.208851	0.921089	-0.000074
C	1.42583	-0.238588	-0.000299	-1.407442	-0.25183	-0.000111
N	3.653089	-0.390928	0.000087	-3.654379	-0.37816	0.000024
N	1.954967	1.062553	-0.000227	-1.900083	1.028419	-0.00018
O	2.54647	-2.440591	-0.000351	-2.54871	-2.430652	-0.000028
C	4.218543	2.058751	0.000339	-4.143458	2.073581	0.000015
H	3.659745	2.998833	0.00003	-3.564896	2.999959	-0.000038
H	4.869506	2.030924	0.887108	-4.793235	2.050764	-0.885492
H	4.870319	2.030817	-0.885828	-4.793076	2.050764	0.885639
C	4.993197	-0.909584	0.000263	-5.018965	-0.857638	0.000263
H	5.552795	-0.596249	-0.893172	-5.556099	-0.521037	0.895141
H	5.552559	-0.596319	0.893868	-5.556416	-0.52098	-0.894402
H	4.903559	-2.001628	0.000198	-4.973047	-1.95062	0.00022

Table S1: CAM-B3LYP/aug-cc-pVDZ optimised geometries of the *cis-trans* deprotonated CFP chromophore anion and corresponding neutral radical.

Atom	Anion			Neutral		
	x	y	z	x	y	z
C	2.949946	2.206404	-0.000009	4.471694	-1.544013	0.000005
C	1.838334	1.368394	-0.000016	3.074502	-1.515511	-0.000061
C	2.037644	-0.017732	-0.000004	2.443211	-0.277032	-0.000031
C	3.362913	-0.539379	0.000017	3.196679	0.913386	0.000066
C	4.470209	0.311793	0.000025	4.578398	0.890647	0.000134
C	4.255549	1.685985	0.000012	5.210522	-0.359532	0.000101
H	2.80611	3.288336	-0.000018	4.991646	-2.501082	-0.00001
H	0.826697	1.767483	-0.000029	2.506005	-2.445161	-0.000121
H	5.477019	-0.107145	0.000043	5.149336	1.817482	0.00021
H	5.107758	2.368125	0.000019	6.29907	-0.409299	0.000155
C	1.176848	-1.186535	-0.000003	1.050376	0.149723	-0.000033
C	2.107413	-2.281118	0.000004	1.138599	1.621172	-0.000093
H	1.81373	-3.333484	0.000007	0.280196	2.287188	-0.000096
N	3.377061	-1.932576	0.000031	2.356253	2.058348	0.000091
C	-0.203166	-1.401066	-0.000016	-0.069148	-0.656822	-0.000057
H	-0.494788	-2.455965	-0.000014	0.074134	-1.738547	-0.000059
C	-2.664441	-1.076661	-0.000065	-2.530529	-1.212964	-0.000204
C	-2.582613	1.18181	-0.000021	-3.208851	0.921089	-0.000074
C	-1.306757	-0.565205	-0.000039	-1.407442	-0.25183	-0.000111
N	-3.442101	0.092946	0.000006	-3.654379	-0.37816	0.000024
N	-1.33595	0.839152	-0.000061	-1.900083	1.028419	-0.00018
O	-3.14592	-2.216338	-0.00001	-2.54871	-2.430652	-0.000028
C	-3.088786	2.58298	0.000008	-4.143458	2.073581	0.000015
H	-2.229494	3.259469	-0.000008	-3.564896	2.999959	-0.000038
H	-3.706157	2.792185	-0.886561	-4.793235	2.050764	-0.885492
H	-3.706112	2.792168	0.886611	-4.793076	2.050764	0.885639
C	-4.879086	0.089928	0.000086	-5.018965	-0.857638	0.000263
H	-5.288433	0.583333	0.893609	-5.556099	-0.521037	0.895141
H	-5.288526	0.583413	-0.89335	-5.556416	-0.52098	-0.894402
H	-5.18755	-0.961439	0.000056	-4.973047	-1.95062	0.00022

Table S2: CAM-B3LYP/aug-cc-pVDZ optimised geometries of the *cis-cis* deprotonated CFP chromophore anion and corresponding neutral radical.

Atom	Anion			Neutral		
	x	y	z	x	y	z
C	4.41538	-1.680967	0.00009	-4.43585	-1.651852	-0.000194
C	3.024884	-1.603508	0.00005	-3.039056	-1.602656	-0.00018
C	2.415039	-0.345678	0.000041	-2.425278	-0.356019	-0.000065
C	3.214183	0.828398	0.000077	-3.195343	0.821746	0.000026
C	4.607537	0.743037	0.000118	-4.576924	0.780035	0.000018
C	5.201835	-0.516301	0.000123	-5.191712	-0.478378	-0.000098
H	4.902433	-2.657436	0.000101	-4.941486	-2.616594	-0.000262
H	2.428252	-2.517368	0.000033	-2.458607	-2.525011	-0.000218
H	5.208145	1.653254	0.000145	-5.16012	1.699209	0.000098
H	6.289646	-0.603063	0.000157	-6.27945	-0.543509	-0.000103
C	1.041881	0.127116	0.000032	-1.033437	0.089766	0.000095
C	1.17857	1.561885	-0.000024	-1.146015	1.562505	-0.000068
H	0.340344	2.254285	-0.000048	-0.299409	2.242423	0.000001
N	2.430941	1.978957	0.000066	-2.371348	1.977418	0.000166
C	-0.08392	-0.6929	0.000042	0.061866	-0.745153	0.000169
H	0.130343	-1.766171	0.000063	-0.147182	-1.816759	0.000155
C	-2.193254	0.797218	0.000011	2.195198	0.797918	0.00028
C	-3.547578	-1.01817	-0.00005	3.538988	-0.998258	0.000088
C	-1.452172	-0.44624	0.000027	1.44254	-0.479389	0.000221
N	-3.532267	0.367545	-0.000101	3.52803	0.373919	-0.000067
N	-2.358895	-1.526136	0.000037	2.335779	-1.522392	0.000302
O	-1.873233	1.992419	-0.000053	1.845076	1.965848	0.000066
C	-4.819216	-1.794917	-0.000105	4.798731	-1.782277	-0.00006
H	-4.570663	-2.860187	-0.000071	4.552499	-2.846525	0.000097
H	-5.43293	-1.573429	0.886221	5.405891	-1.550283	-0.885835
H	-5.432836	-1.573461	-0.886504	5.406212	-1.550095	0.885445
C	-4.648661	1.27227	-0.000243	4.657732	1.277471	-0.000427
H	-5.276843	1.145104	-0.893931	5.275577	1.135569	0.894315
H	-5.277065	1.145098	0.893286	5.275197	1.135358	-0.895397
H	-4.228473	2.283996	-0.000191	4.254409	2.294075	-0.000459

Table S3: CAM-B3LYP/aug-cc-pVDZ optimised geometries of the *trans-trans* deprotonated CFP chromophore anion and corresponding neutral radical.

Atom	Anion			Neutral		
	x	y	z	x	y	z
C	3.100026	2.126781	0.000051	3.076766	2.140838	0.0001
C	1.944428	1.350785	0.000093	1.918477	1.356445	0.000063
C	2.066699	-0.043805	0.000009	2.05329	-0.029391	-0.000111
C	3.363391	-0.631444	-0.000119	3.34737	-0.603404	-0.000263
C	4.516133	0.156911	-0.000161	4.492104	0.168824	-0.000231
C	4.376453	1.53983	-0.000074	4.343178	1.560702	-0.000044
H	3.011658	3.214453	0.000113	2.983101	3.226072	0.000245
H	0.957892	1.80898	0.000184	0.937737	1.818773	0.000175
H	5.497384	-0.318619	-0.000259	5.473123	-0.30279	-0.000348
H	5.26383	2.175648	-0.000106	5.228317	2.196564	-0.000013
C	1.14386	-1.172483	-0.000023	1.135535	-1.171891	-0.000129
C	2.030371	-2.309989	-0.000025	2.05472	-2.329713	-0.000532
H	1.684331	-3.346611	-0.000048	1.715139	-3.36667	-0.000634
N	3.311508	-2.025065	-0.000186	3.304708	-2.020892	-0.000432
C	-0.232876	-1.402493	-0.000003	-0.218522	-1.441305	0.000029
H	-0.460903	-2.473515	-0.00002	-0.441948	-2.510857	-0.00008
C	-1.760924	0.725834	0.000074	-1.762147	0.727418	0.000609
C	-3.605548	-0.591733	0.000056	-3.588109	-0.574879	0.000277
C	-1.428085	-0.685577	0.000041	-1.427617	-0.715973	0.000326
N	-3.166821	0.721916	0.000036	-3.160707	0.72769	0.000065
N	-2.628662	-1.434962	0.000046	-2.599617	-1.436413	0.000496
O	-1.111469	1.778502	0.000031	-1.086211	1.741956	0.000203
C	-5.053548	-0.943793	0.000069	-5.025934	-0.941493	0.000139
H	-5.141514	-2.034109	0	-5.112626	-2.030391	-0.000016
H	-5.570453	-0.545875	0.886475	-5.534826	-0.537207	0.885921
H	-5.570503	-0.545754	-0.886252	-5.534778	-0.536928	-0.885538
C	-3.954282	1.924028	0.00005	-3.962067	1.931942	-0.000369
H	-4.591608	1.993781	-0.893464	-4.593689	1.98393	-0.895207
H	-4.591387	1.993917	0.893715	-4.593735	1.984533	0.894405
H	-3.245737	2.759359	-0.000092	-3.269084	2.777923	-0.000626

Table S4: CAM-B3LYP/aug-cc-pVDZ optimised geometries of the *trans-cis* deprotonated CFP chromophore anion and corresponding neutral radical.

3 Vibrational frequencies used in the ezSpectrum calculation

ν	Anion	Neutral	ν	Anion	Neutral
1	41.6733	41.7726	43	1051.2879	1050.5956
2	53.5146	51.0945	44	1095.0158	1081.408
3	66.8288	71.9685	45	1123.2063	1119.4579
4	105.9377	106.1101	46	1149.6218	1151.2995
5	129.1618	116.0395	47	1155.7096	1166.1533
6	153.0717	141.1119	48	1196.9301	1192.4886
7	166.9598	169.5014	49	1204.1454	1196.8168
8	182.2887	171.2616	50	1226.8041	1211.8884
9	203.0391	210.8027	51	1246.8613	1243.9223
10	226.0138	221.6715	52	1298.9125	1257.956
11	233.511	225.1227	53	1325.7054	1311.6332
12	269.2234	270.4018	54	1345.7756	1332.7576
13	279.4905	273.5631	55	1380.0635	1367.6722
14	329.6371	330.9747	56	1391.5831	1379.2512
15	356.29	349.0263	57	1398.9885	1396.991
16	445.1974	433.2379	58	1418.1338	1433.8824
17	468.6923	465.3304	59	1440.2672	1440.6276
18	503.3753	490.9585	60	1456.4839	1457.0298
19	560.227	558.5385	61	1462.8554	1462.1664
20	575.9789	573.1437	62	1486.87	1487.7715
21	605.0036	596.3018	63	1490.7421	1490.1178
22	618.2665	602.9533	64	1499.8229	1496.7367
23	628.6121	627.0849	65	1501.1388	1507.1438
24	667.5761	663.7888	66	1516.253	1519.2344
25	694.9407	690.187	67	1542.5246	1540.9165
26	723.223	708.82	68	1616.4017	1580.1233
27	757.2955	739.4447	69	1636.4884	1622.5226
28	761.5858	770.3366	70	1670.6379	1663.474
29	781.3137	777.825	71	1681.6699	1680.7385
30	787.0941	790.4472	72	1731.4451	1772.7399
31	811.4626	812.1471	73	3031.2214	3061.2919
32	837.9921	821.0697	74	3037.477	3067.6484
33	878.108	877.4503	75	3085.3911	3123.0539
34	888.5531	900.4098	76	3088.7656	3138.635
35	935.2689	948.2844	77	3148.0827	3176.2422
36	955.6419	970.8193	78	3164.4968	3190.2583
37	966.1023	972.2409	79	3166.0002	3195.3823
38	991.8745	985.9133	80	3168.1428	3196.8068
39	1003.8541	1017.2127	81	3176.4521	3205.4221
40	1015.2084	1024.9605	82	3189.4833	3219.21
41	1039.7544	1032.5453	83	3200.9077	3228.1205
42	1050.0266	1045.1757	84	3236.3874	3257.7823

Table S5: Harmonic wavenumbers for the *cis-trans* deprotonated CFP anion and neutral radical calculated using the CAM-B3LYP/aug-cc-pVDZ method.

4 ezSpectrum calculations

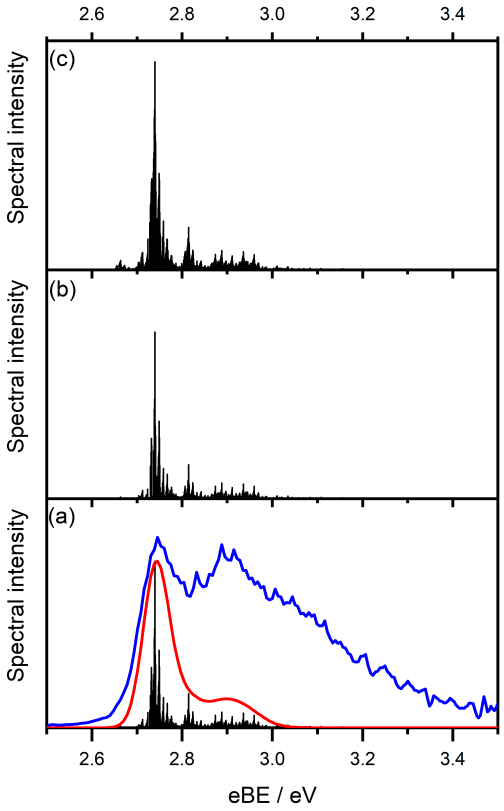

Figure S2: Comparison between ezSpectrum calculations at different vibrational temperatures and quanta for the *cis-trans* isomer. (a) 300 K with maximum numbers of vibrational quanta in the anion and neutral set to 3 and 4 respectively. The blue line is the experimental spectrum recorded at 330 nm and the red line is a convolution of the stick spectrum with a Gaussian instrument function. (b) 300 K with maximum numbers of vibrational quanta in the anion and neutral set to 2 and 4 respectively. (c) 600 K with maximum numbers of vibrational quanta in the anion and neutral set to 3 and 4 respectively. The equilibrium geometries, harmonic frequencies and normal mode vectors of the deprotonated CFP chromophore anions and corresponding neutral radicals were calculated using the CAM-B3LYP/aug-cc-pVDZ method.

5 Excited State Calculations

		VEE (f)	Main configuration
S_1	*cis-trans*	3.46 (1.31)	0.98 ($\pi_{63} \rightarrow \pi^*_{65}$)
	trans-trans	3.53 (1.25)	0.97 ($\pi_{63} \rightarrow \pi^*_{65}$)
S_2	*cis-trans*	4.22 (0.002)	$0.85(\pi_{63} \rightarrow \pi^*_{71}) - 0.65(\pi_{62} \rightarrow \pi^*_{65})$
	trans-trans	4.22 (0.003)	$0.70(\pi_{63} \rightarrow \pi^*_{71}) - 0.65(\pi_{62} \rightarrow \pi^*_{65})$

Table S6: TDDFT CAM-B3LYP/6-31+G* calculated vertical excitation energies (VEEs) of the lowest two singlet states of the deprotonated CFP chromophore anions with non-negligible oscillator strengths, *f*. All values are in eV.

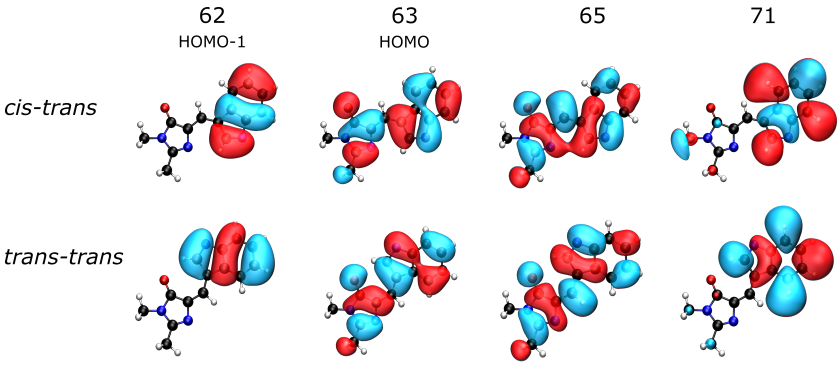

Figure S3: TD-DFT CAM-B3LYP/6-31+G* molecular orbtials of the *cis-trans* (top) and *trans-trans* (bottom) isomers of the deprotonated CFP chromophore anion.