Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information for

Protomers of the green and cyan fluorescent protein chromophores investigated using action spectroscopy

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Cyan ^{1}H NMR spectrum

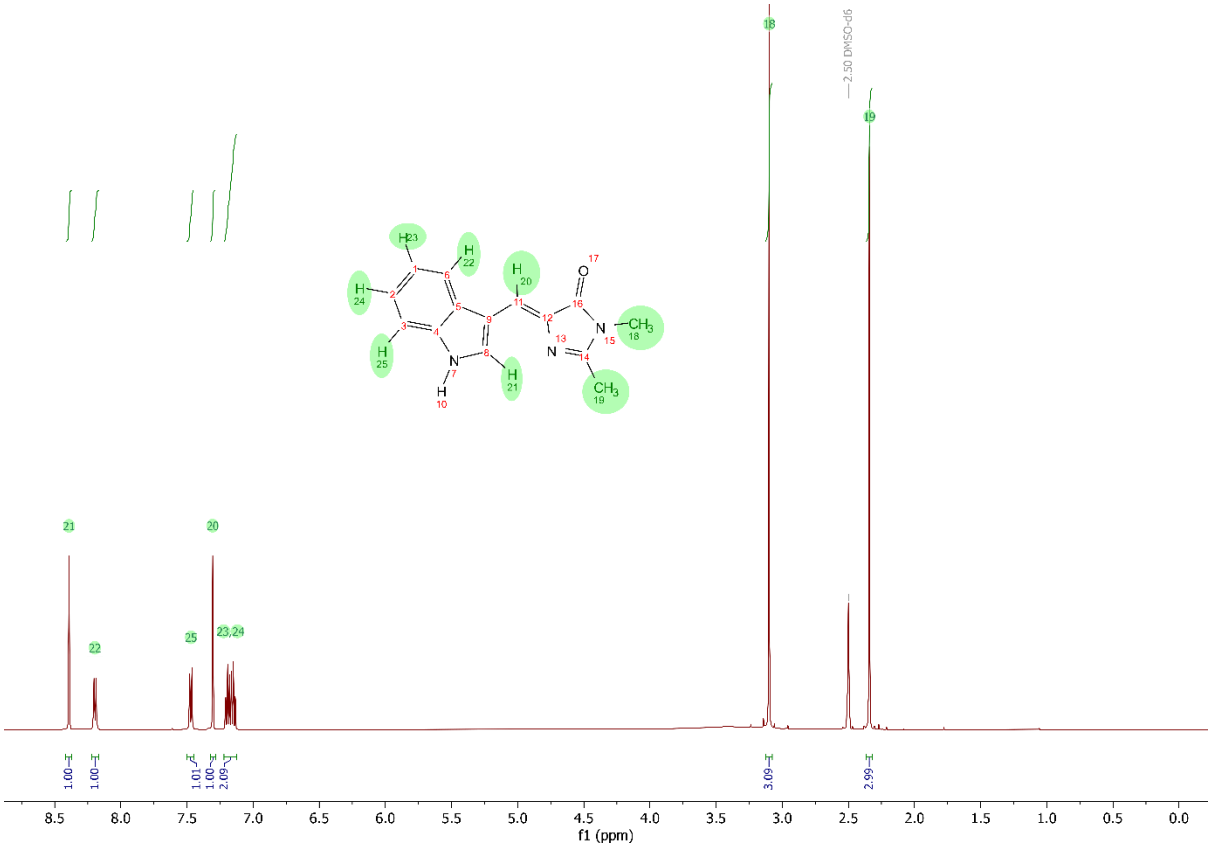

Figure 1 ^{1}H NMR spectrum of Cyan in DMSO, with proton assignments highlighted in green.

Optimised geometries

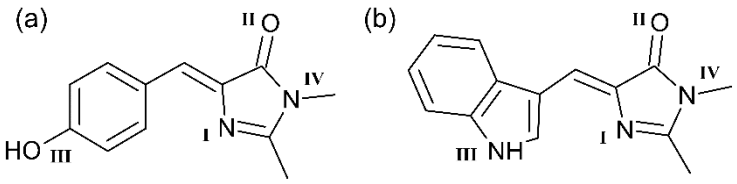

Figure 2 Protonation sites for (a) pHBDI and (b) Cyan. The numbering corresponds to the spectra in the main manuscript and the optimised geometries given below.

Z-*p*HBDI$^+$ – protomer **I** E_{HF} = -724.703206 au

C	4.151718	0.345784	0.018671
C	3.859824	-0.976629	0.379977
C	2.553589	-1.429808	0.314441
C	1.502253	-0.578965	-0.079283
C	1.826098	0.740494	-0.465819
C	3.126780	1.199894	-0.422331
H	2.334272	-2.461941	0.588324
H	1.066340	1.400861	-0.884467
C	0.161905	-1.111380	-0.084804
H	0.061062	-2.199731	-0.094214
O	5.390918	0.858342	0.047925
C	-1.031777	-0.473334	-0.050149
C	-2.340211	-1.136852	-0.105149
C	-2.632794	1.103510	0.132996
N	-1.320709	0.901787	0.086741
N	-3.281480	-0.055740	0.002857
C	-3.285403	2.420402	0.309569
H	-3.915643	2.404909	1.208699
H	-2.541944	3.217318	0.402378
H	-3.934071	2.630408	-0.551428
O	-2.648130	-2.292311	-0.219864
C	-4.724771	-0.250561	-0.016708
H	-5.168949	0.088109	0.925760
H	-5.170002	0.285672	-0.861884
H	-4.895188	-1.324497	-0.135130
H	4.657235	-1.647765	0.700170
H	3.385133	2.207332	-0.742700
H	6.032863	0.204618	0.344228
H	-0.641345	1.628793	0.260126

***Z-p*HBDI$^+$ – protomer II** E_{HF} = -724.687669 au

C	4.148069	0.342814	0.000000
C	3.876418	-1.038120	-0.000001
C	2.566241	-1.463117	-0.000001
C	1.490959	-0.538033	0.000000
C	1.793201	0.852279	0.000000
C	3.098237	1.282935	0.000000
H	2.355608	-2.533186	-0.000001
H	0.979190	1.572827	0.000001
C	0.169465	-1.052673	0.000000
H	0.110931	-2.145874	-0.000001
O	5.388565	0.833818	0.000000
C	-1.043121	-0.390347	0.000000
C	-2.324629	-1.019583	0.000000
C	-2.529859	1.174534	0.000000
N	-1.251353	0.990721	0.000001
N	-3.237484	-0.045829	0.000000
C	-3.243054	2.475813	0.000001
H	-3.881821	2.573704	0.888479
H	-2.504302	3.280886	0.000002
H	-3.881819	2.573705	-0.888479
O	-2.716900	-2.277096	0.000000
C	-4.686477	-0.205075	0.000000
H	-5.107752	0.262773	0.896179
H	-5.107752	0.262774	-0.896179
H	-4.924373	-1.271112	-0.000001
H	4.693992	-1.759154	-0.000001
H	3.347151	2.342250	0.000001
H	6.048124	0.131275	0.000000
H	-1.971339	-2.887443	0.000000

***Z*-*p*HBDI$^+$ – protomer III** E_{HF} = -724.621577 au

C	-3.811617	-1.066862	-0.000007
C	-2.490250	-1.502354	-0.000006
C	-1.417103	-0.590483	-0.000002
C	-1.693419	0.792212	0.000004
C	-3.005014	1.257488	0.000003
H	-2.288873	-2.572656	-0.000009
H	-0.864322	1.497295	0.000008
C	-0.056675	-1.122600	0.000000
H	0.051045	-2.208341	-0.000001
O	-5.398622	0.825184	0.000000
C	1.097149	-0.426616	0.000003
C	2.434578	-1.117522	0.000006
C	2.594518	1.119407	0.000007
N	1.299152	0.952076	0.000007
N	3.328430	-0.048572	0.000009
C	3.267149	2.442259	-0.000011
H	3.909244	2.544260	-0.885746
H	2.513989	3.233495	0.000108
H	3.909467	2.544190	0.885568
O	2.710215	-2.293139	-0.000001
C	4.771627	-0.188987	-0.000003
H	5.207737	0.267524	-0.896526
H	5.207750	0.267496	0.896528
H	4.991208	-1.261050	-0.000020
H	-4.640211	-1.774549	-0.000010
H	-3.234623	2.321209	0.000008
H	-5.900489	0.588889	-0.799027
H	-5.900475	0.588916	0.799044

***Z*-*p*HBDI$^+$ – protomer IV** E_{HF} = -724.651374 au

C	-4.133844	0.354089	-0.080032
C	-3.869729	-1.025106	-0.051087
C	-2.560755	-1.458775	0.006919
C	-1.482410	-0.543206	0.037716
C	-1.776264	0.844712	0.007471
C	-3.078755	1.285598	-0.050426
H	-2.355782	-2.529437	0.029610
H	-0.965181	1.567692	0.030589
C	-0.162308	-1.079171	0.097651
H	-0.092768	-2.170115	0.115650
O	-5.373282	0.854511	-0.136019
C	1.059192	-0.454481	0.138139
C	2.284841	-1.206728	0.198761
C	2.559232	1.171759	0.174373
N	1.315499	0.929399	0.129019
N	3.379533	-0.071305	0.222470
C	3.238410	2.490889	0.185842
H	3.836648	2.636912	-0.724434
H	2.476827	3.273532	0.237384
H	3.909801	2.582561	1.051526
O	2.627338	-2.345975	0.221831
C	4.369798	-0.243494	-0.881115
H	3.848194	-0.095846	-1.831747
H	5.173366	0.488593	-0.764723
H	4.763053	-1.262338	-0.817330
H	-4.689791	-1.743117	-0.074030
H	-3.318733	2.346768	-0.073900
H	-6.034127	0.153906	-0.152738
H	3.866365	-0.140581	1.121974

***E*-*p*HBDI$^+$ – protomer I** E_{HF} = -724.869393 au

C	4.136764	0.246957	-0.000004
C	3.846270	-1.120673	0.000011
C	2.537492	-1.529464	0.000015
C	1.475305	-0.603058	0.000004
C	1.795018	0.771111	-0.000010
C	3.100477	1.185916	-0.000014
H	2.320554	-2.589945	0.000026
H	1.005877	1.505808	-0.000018
C	0.158075	-1.143413	0.000005
H	0.142105	-2.230640	0.000009
O	5.383332	0.719588	-0.000010
C	-1.081585	-0.585930	-0.000003
C	-1.583371	0.785272	0.000008
C	-3.338046	-0.654348	-0.000005
N	-2.246779	-1.388704	-0.000008
N	-3.006034	0.635070	0.000010
C	-4.721326	-1.170571	-0.000013
H	-5.252446	-0.808306	-0.880591
H	-4.733297	-2.256952	-0.000009
H	-5.252457	-0.808299	0.880557
O	-1.042942	1.857231	0.000016
C	-3.919348	1.764889	0.000004
H	-4.542035	1.750493	-0.891825
H	-4.542074	1.750472	0.891805
H	-3.310033	2.664113	0.000029
H	4.647762	-1.847665	0.000018
H	3.351458	2.236400	-0.000027
H	6.029382	0.009422	0.000000
H	-2.252565	-2.394142	-0.000024

***E*-*p*HBDI$^+$ – protomer II** E_{HF} = -724.846210 au

C	-3.951478	0.291774	0.027408
C	-3.686607	-0.927001	-0.601285
C	-2.424803	-1.469610	-0.537697
C	-1.378396	-0.796314	0.110709
C	-1.677936	0.406497	0.778383
C	-2.939785	0.945852	0.740206
H	-2.230293	-2.418929	-1.018666
H	-0.931612	0.872433	1.410915
C	-0.069875	-1.380638	0.104707
H	-0.012516	-2.463669	0.058449
O	-5.151128	0.871717	0.015962
C	1.141540	-0.760791	0.126920
C	1.487187	0.610292	-0.135016
C	3.291846	-0.629252	0.129130
N	2.337990	-1.470998	0.261786
N	2.809120	0.678455	-0.129333
C	4.743316	-0.898877	0.217424
H	5.195372	-0.335102	1.034644
H	4.890725	-1.959289	0.395146
H	5.251117	-0.616615	-0.705666
O	0.782207	1.664782	-0.438923
C	3.618817	1.857566	-0.394803
H	4.327464	1.999057	0.417288
H	4.157120	1.732233	-1.331726
H	2.970416	2.724744	-0.463977
H	-4.476546	-1.449051	-1.125178
H	-3.182143	1.850378	1.278665
H	-5.794435	0.349970	-0.469999
H	-0.166014	1.472680	-0.419902

***Z*-Cyan$^+$ – protomer I** E_{HF} = -781.045317 au

C	1.176526	-1.298217	-0.059317
C	-0.215264	-1.563656	-0.208850
H	-0.514725	-2.601316	-0.375709
C	-1.263875	-0.705945	-0.118985
C	-2.673313	-1.065494	-0.278470
C	-2.502726	1.117639	0.333712
N	-1.263671	0.653999	0.252776
N	-3.374045	0.153398	0.019531
C	-2.872451	2.498828	0.718576
H	-3.523966	2.478016	1.602079
H	-1.981322	3.092954	0.940764
H	-3.430128	2.975489	-0.098646
O	-3.211102	-2.099324	-0.578737
C	-4.824487	0.267905	-0.019144
H	-5.214216	0.533482	0.969791
H	-5.129730	1.014515	-0.760564
H	-5.208952	-0.713860	-0.310553
C	2.070231	-2.281336	0.359247
H	1.880917	-3.331704	0.558184
C	1.949757	-0.070780	-0.173709
C	3.262043	-0.386757	0.237873
N	3.287766	-1.738063	0.557305
H	4.100039	-2.244780	0.877363
C	4.303734	0.541018	0.244168
C	1.705093	1.216970	-0.683721
C	4.015177	1.821696	-0.203305
C	2.733217	2.149589	-0.680252
H	5.306585	0.269670	0.571054
H	4.800733	2.575580	-0.215732
H	2.555197	3.146989	-1.079833
H	0.754854	1.473824	-1.151750
H	-0.438794	1.191649	0.489219

***Z*-Cyan$^+$ – protomer II** E_{HF} = -781.027437 au

C	-1.164913	-1.181560	-0.000001
C	0.210037	-1.421748	0.000006
H	0.492348	-2.477803	0.000000
C	1.297357	-0.551983	0.000019
C	2.640405	-0.986300	0.000012
C	2.530660	1.216448	-0.000001
N	1.293918	0.840478	0.000018
N	3.411191	0.116932	-0.000016
C	3.037540	2.613118	-0.000002
H	3.650678	2.814351	-0.889288
H	2.181931	3.292885	0.000012
H	3.650702	2.814344	0.889270
O	3.060151	-2.239312	0.000046
C	4.862309	0.206421	-0.000030
H	5.205542	0.734080	-0.896466
H	5.205561	0.734095	0.896389
H	5.308321	-0.794021	-0.000028
C	-2.051100	-2.283006	-0.000014
H	-1.810773	-3.342372	-0.000018
C	-2.015205	0.011009	0.000000
C	-3.344438	-0.452658	-0.000011
N	-3.309229	-1.853578	-0.000018
H	-4.123280	-2.452705	-0.000026
C	-4.455279	0.384182	-0.000013
C	-1.789457	1.392837	0.000008
C	-4.204558	1.750742	-0.000004
C	-2.889724	2.243014	0.000006
H	-5.471025	-0.008505	-0.000021
H	-5.040835	2.448339	-0.000005
H	-2.729058	3.320226	0.000013
H	-0.774936	1.779607	0.000017
H	4.020654	-2.317938	0.000040

***Z*-Cyan$^+$ – protomer III** E_{HF} = -780.990340 au

C	-1.160805	-1.223777	-0.000002
C	0.274983	-1.444742	-0.000001
H	0.579145	-2.493307	0.000000
C	1.303408	-0.574333	-0.000002
C	2.722718	-1.094600	0.000000
C	2.602997	1.140559	-0.000004
N	1.337013	0.813468	-0.000003
N	3.476590	0.075016	-0.000003
C	3.103431	2.536931	-0.000002
H	3.728149	2.717605	-0.885581
H	2.258562	3.229143	-0.000027
H	3.728101	2.717615	0.885609
O	3.136027	-2.227891	0.000003
C	4.927197	0.116010	0.000005
H	5.301406	0.623583	-0.896755
H	5.301396	0.623586	0.896767
H	5.279423	-0.919940	0.000008
C	-2.007482	-2.277125	-0.000001
H	-1.822230	-3.345070	0.000000
C	-1.974888	0.017327	0.000000
C	-3.319807	-0.347102	-0.000001
N	-3.409539	-1.819690	0.000000
H	-3.913495	-2.161557	-0.825860
C	-4.383778	0.530708	0.000000
C	-1.663453	1.376017	0.000002
C	-4.052672	1.888374	0.000002
C	-2.715951	2.294318	0.000002
H	-5.421330	0.198330	0.000000
H	-4.847885	2.631983	0.000003
H	-2.487781	3.359251	0.000004
H	-0.622024	1.690509	0.000002
H	-3.913493	-2.161556	0.825862

***Z*-Cyan$^+$ – protomer IV** E_{HF} = -780.991886 au

C	-1.158982	-1.198311	-0.027389
C	0.221885	-1.455489	-0.084047
H	0.490226	-2.515475	-0.091517
C	1.323137	-0.626641	-0.133675
C	2.649233	-1.168610	-0.189134
C	2.534169	1.223102	-0.189943
N	1.347924	0.777295	-0.139222
N	3.545080	0.131196	-0.226925
C	2.985399	2.637003	-0.216520
H	3.626603	2.832915	-1.087783
H	2.103871	3.281503	-0.270041
H	3.555098	2.891154	0.688265
O	3.177347	-2.237686	-0.201724
C	4.548626	0.133027	0.876325
H	5.223797	0.983995	0.752579
H	4.008867	0.204566	1.825599
H	5.100917	-0.809863	0.823788
C	-2.049660	-2.286139	0.014455
H	-1.819407	-3.347575	0.009175
C	-1.994412	0.001982	0.002126
C	-3.328487	-0.448181	0.059879
N	-3.308699	-1.845045	0.065113
H	-4.126871	-2.436361	0.101424
C	-4.429495	0.403027	0.102088
C	-1.759435	1.383767	-0.014065
C	-4.167371	1.765959	0.085048
C	-2.848132	2.245588	0.027602
H	-5.448106	0.020211	0.146338
H	-4.996064	2.471828	0.116533
H	-2.677075	3.321194	0.015437
H	-0.745527	1.767861	-0.058645
H	4.035902	0.130910	-1.126622

***E*-Cyan^{+} – protomer I** E_{HF} = -781.213953 au

C	1.141933	-1.279560	-0.005247
C	-0.221188	-1.593617	-0.163421
H	-0.419285	-2.651835	-0.316959
C	-1.332220	-0.805847	-0.141842
C	-1.565346	0.534925	0.369879
C	-3.545963	-0.392296	-0.250650
N	-2.615756	-1.300704	-0.463728
N	-2.986557	0.708211	0.242971
C	-4.988408	-0.561925	-0.519370
H	-5.325969	0.192138	-1.230680
H	-5.197318	-1.549100	-0.922309
H	-5.556001	-0.427290	0.401719
O	-0.847406	1.356047	0.869382
C	-3.669817	1.922829	0.648861
H	-4.159704	2.388758	-0.203549
H	-4.399060	1.708819	1.427270
H	-2.911261	2.593804	1.042469
C	2.046785	-2.280444	0.350410
H	1.854226	-3.323917	0.540953
C	1.927416	-0.066722	-0.152396
C	3.248964	-0.411629	0.165306
N	3.268983	-1.765073	0.480618
H	4.087686	-2.283069	0.746649
C	4.308432	0.479049	0.100042
C	1.673735	1.223156	-0.616471
C	4.020829	1.759262	-0.325917
C	2.720490	2.119535	-0.691316
H	5.315622	0.182429	0.356815
H	4.814647	2.489150	-0.393402
H	2.532143	3.124250	-1.041685
H	0.683495	1.527421	-0.909807
H	-2.800531	-2.197274	-0.878777

***E*-Cyan^{+} – protomer II** E_{HF} = -781.192002 au

C	-1.111880	-1.292770	0.006961
C	0.235691	-1.614484	-0.129595
H	0.456555	-2.671010	-0.249078
C	1.382132	-0.834963	-0.135189
C	1.637954	0.451483	0.370382
C	3.503192	-0.486134	-0.337104
N	2.601905	-1.377953	-0.539058
N	2.953828	0.673328	0.235749
C	4.948436	-0.605336	-0.643528
H	5.557331	-0.495552	0.254688
H	5.125513	-1.589319	-1.066417
H	5.265704	0.147418	-1.366368
O	0.803114	1.276019	0.964835
C	3.654472	1.865450	0.674069
H	3.653055	1.938391	1.762124
H	4.684096	1.813639	0.336519
H	3.201434	2.755086	0.236516
C	-2.033057	-2.288177	0.368583
H	-1.845213	-3.330927	0.568695
C	-1.893142	-0.076168	-0.159540
C	-3.215922	-0.407379	0.160327
N	-3.243569	-1.761765	0.493005
H	-4.067384	-2.271089	0.763874
C	-4.270819	0.484537	0.073903
C	-1.633951	1.196237	-0.662266
C	-3.976433	1.754360	-0.381886
C	-2.676497	2.097285	-0.761466
H	-5.280590	0.198196	0.331772
H	-4.767262	2.485158	-0.469536
H	-2.487219	3.087013	-1.151960
H	-0.646549	1.475677	-0.992160
H	1.239402	2.050255	1.332869