SUPPLEMENTARY INFORMATION - Electronic Circular Dichroism of Fluorescent Proteins: a Computational Study

Anna Pikulska,[†] Arnfinn Hykkerud Steindal,[‡] Maarten T. P. Beerepoot,[‡] and Magdalena Pecul[*,†]

Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warszawa, Poland, and Centre for Theoretical and Computational Chemistry, Department of Chemistry, University of Tromsø—The Arctic University of Norway, N-9037 Tromsø, Norway

E-mail: mpecul@chem.uw.edu.pl

[*]To whom correspondence should be addressed
[†]University of Warsaw
[‡]University of Tromsø

Table 1: Absorption wavelengths (λ, in nm), rotatory (R, in 10^{-40} esu $\cdot$ cm $\cdot$ erg $\cdot$ G^{-1}) and oscillator strengths (*f*, dimensionless) calculated for the neutral and anionic isolated chromophores with the geometry optimized in different ways. For the calculations in which the chromophore is optimized with QM/QM/PCM and QM/QM/MM, the results for the intermediate form are shown, since the different surrounding here influences the geometry of the anionic chromophore. ECD calculations have been performed at the B3LYP/6-31+G* level of theory.

Structures	Optimization method	λ	R	f
neutral	QM/vacuum	370	-32.14	0.71
	QM/PCM, ε=4	372	-32.50	0.69
	QM/QM/MM	376	-62.91	0.47
	QM/QM/PCM	367	-31.22	0.56
anionic	QM/vacuum	417	-47.06	0.98
	QM/PCM, ε=4	420	-82.34	0.04
		420	44.41	0.93
	QM/QM/MM	432	-86.83	0.82
		439	19.56	0.00
	QM/QM/PCM	437	-55.39	0.77
intermediate	QM/QM/MM	439	-15.93	0.00
		432	-70.95	0.86
	QM/QM/PCM	439	-15.93	0.00
		436	-47.14	0.74

Table 2: Absorption wavelengths (λ, in nm), rotatory (R, in 10^{-40} esu $\cdot$ cm $\cdot$ erg $\cdot$ G^{-1}) and oscillator strengths (*f*, dimensionless) calculated with PCM in both the structure optimization and ECD calculation. Two different values for the dielectric constant (ϵ) are used in both the structure optimization and ECD calculation. ECD calculations have been performed at the B3LYP/6-31+G* level of theory.

Structure	ϵ	λ	R	f
neutral	4	384	-30.22	0.91
	15	384	-27.37	0.93
anionic	4	433	-27.98	1.16
	15	429	-23.11	1.16

Table 3: Absorption wavelengths (λ, in nm), rotatory (R, in 10^{-40} esu $\cdot$ cm $\cdot$ erg $\cdot$ G^{-1}) and oscillator strengths (*f*, dimensionless) for the GFP chromophores optimized with QM/MM scheeme and ECD calculations performed with the rest of the protein included through polarizable embedding (PE-QM/MM). ECD calculations have been performed at the B3LYP/6-31+G* level of theory.

Structure	λ	R	f
neutral	393	-97.94	0.65
	372	12.95	0.13
	361	5.85	0.24
anionic	424	-92.42	1.16
	340	31.68	0.03
intermediate	429	-78.87	1.16

Table 4: Absorption wavelengths (λ, in nm), rotatory (R, in 10^{-40} esu $\cdot$ cm $\cdot$ erg $\cdot$ G^{-1}) and oscillator strengths (f, dimensionless) calculated for the QM/QM/PCM-optimized system (a cluster containing the GFP chromophore, 9 amino acid residues and 5 water molecules) at the B3LYP/6-31+G* level of theory with and without PCM in the ECD calculations.

Structure	Method	λ	R	f
neutral	QM/QM	388	-48.92	0.69
	QM/QM/PCM	391	-54.59	0.84
anionic	QM/QM	420	-53.24	0.92
	QM/QM/PCM	426	-12.99	0.33
		424	-48.87	0.71
intermediate	QM/QM	417	-57.33	0.91
	QM/QM/PCM	423	-59.42	1.03

Table 5: Absorption wavelengths (λ, in nm), rotatory (R, in 10^{-40} esu $\cdot$ cm $\cdot$ erg $\cdot$ G^{-1}) and oscillator strengths (f, dimensionless) calculated for the fluorescent proteins. The B3LYP/6-31+G* calculations were performed on the chromophore only (vacuum) and using the polarizable embedding (PE-QM/MM) scheme. Chromophore geometries were optimized with QM/MM scheeme. λ_{max} is the experimental absorption maximum.

	QM/vacuum			PE-QM/MM			Experiment
Protein	λ	R	f	λ	R	f	λ_{max}
BFP	355	-22.30	0.17	372	-27.61	0.40	382[1]
	325	-11.32	0.46	358	-28.87	0.32	
eCFP	401	-20.54	0.53	482	-19.42	0.04	435[2]
				418	1.13	0.60	
eGFP	431	-133.47	0.82	434	-69.52	1.01	488[3]
	428	78.88	0.04				
YFP neutral	376	-23.83	0.28	390	-62.05	0.91	392[4]
	360	15.24	0.06				
	355	-15.59	0.50				
YFP anionic	406	-112.78	0,85	439	-96.51	0.78	514[4]
	406	47.69	0.01				
DsRed	534	-23.28	0.00	474	-122.56	1.17	558[5]
	524	-87.59	0.95				

Table 6: Absorption wavelengths (λ, in nm), rotatory (R, in 10^{-40} esu $\cdot$ cm $\cdot$ erg $\cdot$ G^{-1}) and oscillator strengths (*f*, dimensionless) calculated for the PCM-optimized structures of the fluorescent proteins using DFT with B3LYP/6-31+G* and PCM. $\lambda_{\mathbf{max}}$ is the experimental absorption maximum.

Protein	ϵ	λ	R	f	$\lambda_{\max}$
BFP	4	359	-16.30	0.71	382[1]
	15	358	-17.39	0.75	
eCFP	4	410	-25.79	0.72	435[2]
	15	411	-24.64	0.74	
eGFP	4	433	-10.80	1.16	488[3]
	15	430	-10.19	1.16	
YFP neutral	4	383	-37.47	0.93	392[4]
	15	383	-35.23	0.94	
YFP anionic	4	432	-40.11	1.17	514[4]
	15	429	-42.60	1.17	
DsRed	4	539	-62.75	1.08	558[5]
	15	533	-63.88	1.07	

FP chromophores - different methods of optimization

40
! GFP NEUTRAL - VACUUM

C	11.725362	67.913137	-12.075590
C	10.208584	68.136844	-10.144233
C	9.351297	69.287066	-9.694306
C	10.771125	67.352739	-8.948888
C	8.405956	70.782881	-8.447595
C	7.886067	70.992949	-9.817296
C	8.030668	71.555447	-7.406628
C	8.438342	71.521412	-6.015252
C	9.422490	70.655695	-5.509514
C	7.819414	72.410279	-5.118322
C	9.761915	70.673983	-4.166894
C	8.150352	72.435109	-3.775767
C	9.125940	71.561582	-3.296201
C	8.360213	69.840921	-11.991905
C	7.418670	68.735681	-12.467016
O	9.757877	66.874488	-8.096309
O	7.103646	71.808031	-10.266141
O	9.418655	71.621422	-1.971442
O	7.309495	68.521444	-13.660349
O	11.207649	66.894296	-12.483804
N	11.281945	68.626958	-10.999291
N	9.305272	69.703385	-8.468720
N	8.525112	69.988187	-10.560533
N	6.761468	68.027940	-11.514241
H	11.858833	69.387659	-10.662248
H	9.610279	67.455574	-10.757497
H	11.303796	66.482201	-9.337111
H	11.486232	67.984091	-8.404017
H	9.923195	69.970561	-6.182183
H	7.059632	73.092620	-5.489591
H	10.526075	69.997603	-3.790749
H	7.669207	73.119607	-3.085626
H	10.102049	70.974119	-1.744087
H	7.304705	72.323522	-7.668698
H	7.956950	70.787510	-12.361651
H	9.325710	69.673438	-12.471680
H	6.743258	68.316678	-10.548189
H	9.364948	67.651927	-7.666049
H	12.603814	68.371524	-12.560094
H	6.089476	67.339207	-11.823308

39
! GFP ANIONIC - VACUUM
C 11.784952 67.838760 -12.040057
C 10.160852 68.082456 -10.180856
C 9.311037 69.232346 -9.727121
C 10.735810 67.310499 -8.982700
C 8.341347 70.710191 -8.468603
C 7.848293 70.958514 -9.801752
C 7.962525 71.479647 -7.372681
C 8.340628 71.457998 -6.029698
C 9.309507 70.560600 -5.466443
C 7.739715 72.396608 -5.126374
C 9.634015 70.593171 -4.146060
C 8.050295 72.444541 -3.803102
C 9.026452 71.537839 -3.215140
C 8.322397 69.859312 -12.001431
C 7.400307 68.745296 -12.487229
O 9.731645 66.837980 -8.116172
O 7.066618 71.786752 -10.279667
O 9.325246 71.567883 -2.005492
O 7.290441 68.513172 -13.682561
O 11.423251 66.724053 -12.373093
N 11.244048 68.570052 -11.036437
N 9.234940 69.634462 -8.495481
N 8.496506 69.965979 -10.576473
N 6.741964 68.048945 -11.530841
H 11.677818 69.452447 -10.797074
H 9.571020 67.390716 -10.791025
H 11.274355 66.439274 -9.362607
H 11.445415 67.958014 -8.449943
H 9.791049 69.848932 -6.128351
H 7.004967 73.090796 -5.532916
H 10.370628 69.910495 -3.730838
H 7.582575 73.162567 -3.135105
H 7.233970 72.243909 -7.644848
H 7.882139 70.805973 -12.331067
H 9.279898 69.729266 -12.511185
H 9.325420 67.632945 -7.724835
H 6.767191 68.331258 -10.561944
H 12.602847 68.369967 -12.558601
H 6.089805 67.336384 -11.822836

40
! GFP NEUTRAL - PCM, EPSILON=15
C 11.744876 67.891816 -12.058367
C 10.266700 68.101593 -10.075102
C 9.409825 69.263255 -9.635168
C 10.862837 67.344420 -8.868350
C 8.467818 70.778525 -8.396606
C 7.940033 70.974125 -9.762936
C 8.103715 71.570200 -7.346307
C 8.521647 71.557352 -5.963384
C 9.490319 70.673120 -5.430041
C 7.929005 72.496112 -5.082880
C 9.840890 70.721623 -4.087520
C 8.271867 72.551066 -3.739327
C 9.231914 71.659834 -3.236088
C 8.418036 69.814673 -11.942043
C 7.470230 68.714326 -12.434350
O 9.861581 66.812755 -8.009392
O 7.148369 71.796327 -10.225378
O 9.537195 71.750283 -1.913412
O 7.413586 68.485936 -13.648433
O 11.223611 66.860242 -12.485554
N 11.334557 68.571747 -10.959186
N 9.366678 69.699458 -8.408400
N 8.570239 69.960979 -10.504734
N 6.745076 68.050320 -11.516348
H 11.892157 69.369098 -10.663724
H 9.658970 67.396727 -10.657139
H 11.434190 66.494678 -9.252127
H 11.544726 68.005992 -8.317857
H 9.968352 69.949693 -6.080276
H 7.183726 73.189922 -5.472089
H 10.589410 70.035434 -3.689014
H 7.809784 73.274341 -3.069335
H 10.211104 71.091333 -1.666754
H 7.375606 72.343438 -7.601658
H 8.027550 70.766421 -12.320206
H 9.391363 69.648698 -12.410607
H 6.745155 68.297747 -10.534546
H 9.426587 67.568793 -7.573825
H 12.603788 68.371520 -12.560091
H 6.089502 67.339211 -11.823311

39
! GFP ANIONIC - PCM, EPSILON=15

C	11.755835	67.874096	-12.052197
C	10.260130	68.081145	-10.075587
C	9.399746	69.235504	-9.627921
C	10.867879	67.329088	-8.870842
C	8.435066	70.731577	-8.375490
C	7.917523	70.943912	-9.719930
C	8.065068	71.515192	-7.293098
C	8.466427	71.504159	-5.935175
C	9.430670	70.606136	-5.373829
C	7.882018	72.459872	-5.043049
C	9.773565	70.656997	-4.044118
C	8.216070	72.521553	-3.711034
C	9.186885	71.618688	-3.130809
C	8.405281	69.822413	-11.919000
C	7.461239	68.726201	-12.425866
O	9.875721	66.814224	-7.990746
O	7.115720	71.769904	-10.191567
O	9.509940	71.665024	-1.898246
O	7.397368	68.511270	-13.643417
O	11.261972	66.824775	-12.473364
N	11.329889	68.552512	-10.961236
N	9.341419	69.660603	-8.395005
N	8.558209	69.944409	-10.482795
N	6.741692	68.049265	-11.513155
H	11.862472	69.371437	-10.679249
H	9.659043	67.371202	-10.657995
H	11.430381	66.471929	-9.251462
H	11.559871	67.993566	-8.336201
H	9.898925	69.873997	-6.023930
H	7.145954	73.157948	-5.446244
H	10.511328	69.965182	-3.637059
H	7.754692	73.259630	-3.054994
H	7.331986	72.283345	-7.551678
H	8.006672	70.777497	-12.280596
H	9.376915	69.668280	-12.395741
H	9.436186	67.587405	-7.588006
H	6.758818	68.280328	-10.527425
H	12.602888	68.369974	-12.558606
H	6.089763	67.336377	-11.822831

40
! GFP NEUTRAL - QM/MM
C 11.675 67.990 -12.104
C 10.334 67.930 -10.023
C 9.523 69.141 -9.585
C 10.927 67.085 -8.865
C 8.620 70.709 -8.392
C 8.071 70.869 -9.751
C 8.393 71.589 -7.376
C 8.963 71.659 -6.051
C 9.909 70.747 -5.517
C 8.578 72.758 -5.248
C 10.424 70.932 -4.239
C 9.075 72.934 -3.966
C 9.985 72.005 -3.445
C 8.545 69.738 -11.906
C 7.353 68.895 -12.389
N 11.418 68.390 -10.851
N 9.491 69.617 -8.377
N 8.653 69.825 -10.456
N 6.861 67.960 -11.592
O 10.933 67.241 -12.738
O 9.955 66.211 -8.345
O 7.362 71.767 -10.225
O 10.394 72.174 -2.150
O 6.828 69.142 -13.459
H 12.602 68.372 -12.559
H 12.114 68.985 -10.400
H 9.679 67.277 -10.609
H 11.724 66.450 -9.254
H 11.372 67.686 -8.069
H 9.777 66.420 -7.412
H 10.240 69.886 -6.089
H 7.873 73.479 -5.650
H 11.135 70.208 -3.856
H 8.748 73.767 -3.351
H 10.730 71.316 -1.748
H 7.691 72.383 -7.622
H 8.484 70.727 -12.366
H 9.433 69.313 -12.363
H 6.099 67.340 -11.823
H 7.319 67.822 -10.702

39
! GFP anionic - QM/MM
C 11.671 67.998 -12.109
C 10.313 67.905 -10.062
C 9.502 69.094 -9.619
C 10.913 67.040 -8.932
C 8.620 70.670 -8.417
C 8.110 70.872 -9.767
C 8.388 71.559 -7.393
C 8.918 71.666 -6.077
C 9.882 70.794 -5.480
C 8.525 72.809 -5.326
C 10.398 71.057 -4.226
C 9.039 73.078 -4.081
C 9.988 72.204 -3.464
C 8.557 69.723 -11.928
C 7.330 68.915 -12.379
N 11.395 68.407 -10.865
N 9.464 69.556 -8.400
N 8.676 69.826 -10.487
N 6.861 67.959 -11.592
O 10.933 67.241 -12.738
O 9.966 66.109 -8.477
O 7.417 71.798 -10.235
O 10.400 72.431 -2.256
O 6.781 69.182 -13.432
H 12.602 68.374 -12.561
H 12.071 69.018 -10.407
H 9.659 67.265 -10.662
H 11.730 66.441 -9.337
H 11.337 67.623 -8.111
H 9.604 66.405 -7.637
H 10.230 69.925 -6.030
H 7.811 73.498 -5.771
H 11.143 70.399 -3.789
H 8.735 73.962 -3.527
H 7.700 72.355 -7.671
H 8.535 70.714 -12.387
H 9.429 69.260 -12.370
H 6.099 67.340 -11.823
H 7.319 67.822 -10.702

39
! GFP INTERMEDIATE - QM/MM
C 11.669 68.000 -12.109
C 10.312 67.909 -10.058
C 9.518 69.100 -9.588
C 10.913 67.026 -8.943
C 8.671 70.679 -8.359
C 8.128 70.883 -9.701
C 8.459 71.548 -7.309
C 9.014 71.621 -5.995
C 10.023 70.762 -5.460
C 8.593 72.700 -5.168
C 10.538 70.970 -4.195
C 9.084 72.895 -3.894
C 10.061 72.016 -3.331
C 8.547 69.743 -11.880
C 7.319 68.943 -12.348
N 11.393 68.410 -10.865
N 9.512 69.562 -8.369
N 8.670 69.832 -10.436
N 6.860 67.962 -11.588
O 10.933 67.241 -12.738
O 9.960 66.101 -8.488
O 7.431 71.809 -10.162
O 10.469 72.157 -2.101
O 6.760 69.239 -13.388
H 12.601 68.374 -12.560
H 12.070 69.021 -10.407
H 9.647 67.284 -10.661
H 11.721 66.422 -9.360
H 11.348 67.596 -8.120
H 9.585 66.408 -7.658
H 10.402 69.944 -6.062
H 7.863 73.398 -5.571
H 11.318 70.314 -3.820
H 8.727 73.723 -3.288
H 7.751 72.340 -7.549
H 8.531 70.738 -12.330
H 9.418 69.279 -12.327
H 6.100 67.341 -11.822
H 7.319 67.822 -10.702

40
! GFP NEUTRAL - QM/QM/PCM
C -3.5449 -3.8687 -3.7891
O -4.2662 -4.6275 -3.1384
N -2.3336 -3.4363 -3.3211
C -1.8596 -3.6988 -1.9608
C -1.6544 -2.3737 -1.2706
C -0.5899 -4.5662 -1.9621
O -0.2557 -4.9676 -0.6400
H -1.7860 -2.8054 -3.8942
H -2.6591 -4.2634 -1.4711
H -0.8019 -5.4572 -2.5641
H 0.2423 -4.0220 -2.4201
H 0.6179 -4.5769 -0.3609
N -0.5039 -1.7943 -1.1238
C -0.7607 -0.5371 -0.5615
C -2.2137 -0.3774 -0.4031
O -2.8882 0.6025 -0.0389
C 0.1354 0.4483 -0.2549
C 1.5649 0.4901 -0.4234
C 2.3575 -0.6396 -0.7375
C 2.2128 1.7462 -0.3169
C 3.7182 -0.5074 -0.9782
C 3.5678 1.8887 -0.5716
C 4.3289 0.7602 -0.9267
O 5.6411 0.9396 -1.1989
H 1.9133 -1.6272 -0.7809
H 1.6274 2.6235 -0.0509
H 4.2949 -1.3942 -1.2187
H 4.0514 2.8586 -0.5026
H 6.1255 0.0830 -1.4037
H -0.3139 1.3610 0.1331
N -2.7369 -1.5935 -0.8227
C -4.1641 -1.8334 -0.9922
C -4.9373 -2.5012 0.1504
O -6.1766 -2.4803 0.0769
H -4.6447 -0.8695 -1.1694
H -4.3308 -2.4494 -1.8764
N -4.2605 -3.0958 1.1396
H -3.2463 -3.1918 1.1155
H -4.7550 -3.4648 1.9392
H -3.8964 -3.5450 -4.7576

39
! GFP ANIONIC - QM/QM/PCM
C -3.3746 -4.1091 -3.8084
O -4.1552 -4.8253 -3.1792
N -2.2405 -3.5919 -3.2478
C -1.9064 -3.7246 -1.8305
C -1.7597 -2.3403 -1.2553
C -0.6440 -4.5846 -1.6561
O -0.4312 -4.8908 -0.2790
H -1.6475 -2.9955 -3.8119
H -2.7489 -4.2464 -1.3663
H -0.8013 -5.5149 -2.2144
H 0.2241 -4.0644 -2.0735
H 0.4853 -4.6555 -0.0260
N -0.6243 -1.7295 -1.1052
C -0.9198 -0.4469 -0.6263
C -2.3677 -0.3039 -0.5191
O -3.0839 0.6726 -0.2033
C -0.0130 0.5371 -0.2908
C 1.4086 0.5408 -0.3919
C 2.1684 -0.5269 -0.9590
C 2.1330 1.6821 0.0634
C 3.5383 -0.4575 -1.0535
C 3.5075 1.7547 -0.0199
C 4.2714 0.6759 -0.5759
O 5.5667 0.7118 -0.6347
H 1.6490 -1.4077 -1.3163
H 1.5776 2.5156 0.4908
H 4.0958 -1.2837 -1.4831
H 4.0403 2.6311 0.3403
H -0.4581 1.4447 0.1154
N -2.8603 -1.5449 -0.9129
C -4.2764 -1.8206 -1.0975
C -4.9927 -2.6159 -0.0025
O -6.2129 -2.8079 -0.1420
H -4.7840 -0.8567 -1.1837
H -4.4323 -2.3606 -2.0339
N -4.2709 -3.0813 1.0212
H -3.2707 -2.8902 1.1149
H -4.7116 -3.6426 1.7359
H -3.6137 -3.8942 -4.8394

39
! GFP INTERMEDIATE - QM/QM/PCM
C -3.2903 -4.4642 -3.4657
O -3.9365 -5.2598 -2.7810
N -2.1934 -3.8078 -2.9789
C -1.7138 -3.9515 -1.6028
C -1.5217 -2.5734 -1.0239
C -0.4382 -4.8125 -1.5649
O -0.0934 -5.1698 -0.2277
H -1.6899 -3.1750 -3.5885
H -2.5025 -4.4857 -1.0639
H -0.6477 -5.7295 -2.1259
H 0.3879 -4.2833 -2.0505
H 0.7627 -4.7675 0.0238
N -0.3775 -1.9548 -0.9633
C -0.6474 -0.6643 -0.4769
C -2.0835 -0.5306 -0.2548
O -2.7807 0.4392 0.0936
C 0.2443 0.3749 -0.2875
C 1.6359 0.4678 -0.5603
C 2.4006 -0.5725 -1.1678
C 2.3174 1.6829 -0.2588
C 3.7308 -0.4007 -1.4682
C 3.6557 1.8602 -0.5434
C 4.4224 0.8233 -1.1787
O 5.6705 0.9786 -1.4702
H 1.9150 -1.5134 -1.3975
H 1.7584 2.4928 0.2069
H 4.2904 -1.2023 -1.9409
H 4.1511 2.7993 -0.3105
H -0.2182 1.2732 0.1209
N -2.5944 -1.7912 -0.5889
C -4.0218 -2.0370 -0.7255
C -4.7811 -2.7180 0.4204
O -6.0250 -2.6812 0.3725
H -4.4979 -1.0666 -0.8792
H -4.2058 -2.6350 -1.6214
N -4.1037 -3.3526 1.3832
H -3.0803 -3.3924 1.4033
H -3.6159 -4.2925 -4.4810
H -4.6018 -3.8172 2.1289

36
! BFP, OPTIMIZATION IN PCM, EPSILON=15

C	23.685973	27.278185	35.226813
C	26.788585	27.059162	37.123356
C	28.408941	27.724314	38.403199
C	27.255737	27.471625	39.293897
C	25.999908	26.668326	35.897653
C	26.903671	26.576784	34.647111
C	24.906758	26.702833	38.842691
C	24.586598	25.222518	39.087770
C	29.611714	28.158195	38.875900
C	30.835409	28.450673	38.191632
C	32.321969	28.710101	36.654368
C	31.993714	28.898384	38.827230
O	27.119661	27.595707	40.512359
O	27.977299	25.659719	34.808077
O	23.421493	24.911079	39.362956
O	23.291898	26.119584	35.079916
N	25.591777	24.333928	38.991311
N	28.030603	27.445604	37.080680
N	26.257185	27.024184	38.416104
N	24.920770	27.630056	35.661750
N	31.072550	28.341464	36.823381
N	32.917654	29.055824	37.841491
H	25.527801	25.690444	36.060902
H	25.151688	28.620357	35.666080
H	26.293501	26.213063	33.815520
H	27.277400	27.578450	34.395425
H	24.733148	27.226177	39.789879
H	24.182363	27.083971	38.118982
H	29.650214	28.308368	39.957005
H	32.855338	28.750051	35.709616
H	33.875446	29.372066	37.960674
H	32.208371	29.105421	39.870118
H	26.553455	24.609329	38.833382
H	28.583077	26.055829	35.463833
H	23.040442	28.152139	35.025932
H	25.408524	23.355486	39.190020

46
! eCFP, optimization in PCM, epsilon=15
C 21.511326 27.276308 36.041912
C 23.820592 26.553370 36.609492
C 24.626149 26.297333 35.298059
C 25.183855 27.545807 34.610922
C 24.679793 26.913065 37.798947
C 25.227308 27.272983 39.965911
C 26.352711 27.505511 39.052576
C 22.852911 26.551051 39.575437
C 22.549089 25.067615 39.825159
C 27.593837 27.911343 39.467353
C 28.757368 28.195752 38.697249
C 28.911155 28.163304 37.305927
C 30.052250 28.599434 39.230414
C 30.915798 28.786779 38.121855
C 30.553523 28.812794 40.525646
C 32.251661 29.177243 38.258230
C 31.884017 29.201868 40.674141
C 32.724360 29.382420 39.553579
O 21.069733 26.138356 35.869576
O 25.640897 25.318228 35.540516
O 25.122784 27.372870 41.192597
O 21.390729 24.743901 40.114401
N 23.563145 24.189747 39.720946
N 22.778768 27.567491 36.423476
N 25.934681 27.261637 37.735114
N 24.186911 26.877294 39.105807
N 30.179036 28.511255 36.975450
H 20.888000 28.179000 35.908000
H 23.394000 23.210000 39.926000
H 24.519559 24.475355 39.551520
H 23.032394 28.549366 36.486866
H 23.297793 25.614286 36.834433
H 23.923339 25.807477 34.613165
H 25.701883 27.242516 33.694539
H 24.378654 28.232963 34.326845
H 25.888689 28.081573 35.253118
H 26.282886 25.712728 36.161052
H 22.710834 27.068267 40.531389
H 22.101412 26.933128 38.880918
H 27.692268 28.034326 40.547960
H 28.185449 27.915056 36.546123
H 30.533711 28.564672 36.026451
H 29.919080 28.677138 41.401196

H	32.893762	29.314156	37.388963
H	32.284930	29.370301	41.672740
H	33.759218	29.686838	39.703456

42
! eGFP, optimization in PCM, epsilon=15
C 21.758185 26.911876 35.944378
C 24.118470 26.374204 36.528187
C 25.127653 26.221915 35.357310
C 24.477164 25.705648 34.082092
C 24.812944 26.819294 37.796481
C 25.136666 27.292550 39.987626
C 26.294328 27.634676 39.174223
C 22.877986 26.351025 39.419867
C 22.638825 24.854646 39.645957
C 27.452726 28.178267 39.708137
C 28.688312 28.547485 39.123155
C 29.690743 29.135030 39.960646
C 29.026795 28.374067 37.742753
C 30.914866 29.529787 39.475622
C 30.247146 28.761774 37.244664
C 31.265148 29.368096 38.080878
O 25.757202 27.471543 35.054171
O 24.904869 27.398950 41.204963
O 21.493626 24.468400 39.913835
O 32.394564 29.731360 37.614626
O 21.335432 25.753824 36.012599
N 23.693927 24.028093 39.535180
N 23.027824 27.297306 36.203048
N 26.019009 27.320289 37.834823
N 24.222040 26.755673 39.056498
H 23.260565 28.276481 36.063901
H 23.651205 25.395029 36.691152
H 25.888614 25.500725 35.694933
H 22.632330 26.858101 40.359642
H 22.160140 26.686046 38.667880
H 29.461626 29.272746 41.019031
H 28.298456 27.917656 37.080862
H 31.657284 29.977582 40.136167
H 30.484400 28.617609 36.190447
H 27.384449 28.348690 40.785449
H 24.635484 24.364475 39.375306
H 26.201734 27.749446 35.882961
H 25.240557 25.586044 33.305978
H 24.001238 24.733811 34.253404
H 23.719390 26.405372 33.713235
H 21.103518 27.758840 35.676068
H 23.561422 23.034483 39.688223

36
! YFP NEUTRAL, OPTIMIZATION IN PCM, EPSILON=15
C 23.081186 26.573720 33.021810
C 25.287407 25.583733 33.561149
C 25.810858 26.713909 34.406882
C 26.139919 28.073761 36.182015
C 27.049501 28.373060 35.056865
C 24.265608 26.429463 36.458066
C 24.354371 24.939313 36.789297
C 27.980188 29.370208 35.105059
C 28.945021 29.808442 34.122328
C 29.100618 29.227844 32.840025
C 29.792299 30.891747 34.463531
C 30.052937 29.706588 31.949852
C 30.746493 31.376587 33.580144
C 30.879764 30.782793 32.315775
O 26.010448 28.594606 37.290786
O 23.322383 24.319049 37.068626
O 31.828364 31.290914 31.483223
O 21.869625 26.524166 32.795375
N 23.828559 25.533018 33.447955
N 26.774523 27.489140 34.002384
N 25.380809 26.988712 35.710341
N 25.575910 24.372157 36.756973
H 23.370472 24.627838 33.524382
H 25.602908 24.626967 33.991005
H 25.765497 25.677098 32.579270
H 24.226083 26.982111 37.405062
H 23.317562 26.593193 35.939491
H 27.993304 29.926234 36.045138
H 28.467436 28.397196 32.547665
H 29.690349 31.355125 35.444964
H 30.163040 29.250723 30.964901
H 31.393002 32.209802 33.850840
H 31.837472 30.812322 30.634426
H 26.425868 24.913311 36.650385
H 23.661770 27.508507 32.918455
H 25.666390 23.392762 36.999925

35
! YFP anionic, optimization in PCM, epsilon=15
C 23.058362 26.590189 32.823111
C 25.184699 25.424139 33.394006
C 25.711008 26.566141 34.215729
C 26.034170 27.973932 35.960178
C 26.946738 28.236510 34.855540
C 24.156354 26.328393 36.252874
C 24.295077 24.868954 36.688485
C 27.897466 29.245548 34.886344
C 28.863075 29.664651 33.938447
C 29.056917 29.071561 32.649080
C 29.717845 30.762786 34.276231
C 30.016660 29.534908 31.781082
C 30.681603 31.235350 33.417163
C 30.887614 30.645833 32.111992
O 25.879957 28.515320 37.069693
O 23.284812 24.231618 37.013523
O 31.776088 31.073495 31.303119
O 21.870545 26.619202 32.481901
N 23.737352 25.471644 33.144918
N 26.687521 27.332281 33.815892
N 25.269639 26.879019 35.504076
N 25.535805 24.348289 36.698570
H 23.246916 24.585623 33.045243
H 25.380958 24.470885 33.898116
H 25.748580 25.424837 32.453788
H 24.063402 26.939828 37.159221
H 23.217957 26.413683 35.698210
H 27.889173 29.815021 35.819049
H 28.425450 28.236991 32.359451
H 29.590429 31.233630 35.252846
H 30.147655 29.069754 30.803750
H 31.317958 32.073512 33.701081
H 26.360197 24.906843 36.514915
H 23.661377 27.509755 32.923401
H 25.666692 23.390111 36.997734

63
! DsRed, optimization in PCM, epsilon=15

C	18.200569	-19.849899	37.541209
C	20.237999	-20.872984	38.513174
C	20.739946	-20.380120	39.887324
C	21.417358	-21.466907	37.698953
C	20.978778	-22.242330	36.474106
C	21.033154	-21.670302	35.194585
C	20.512045	-23.561172	36.598986
C	20.628587	-22.393828	34.066653
C	20.106290	-24.287841	35.476068
C	20.162655	-23.705242	34.204108
C	21.037280	-18.133693	40.631911
C	22.543416	-18.083041	40.436987
C	23.309509	-18.454754	41.720569
C	20.409851	-16.957559	41.218183
C	18.881035	-15.529935	42.082172
C	20.234977	-14.982072	42.107255
C	17.905827	-17.639221	41.181310
C	20.528531	-13.709370	42.610952
C	21.739618	-13.004814	42.711636
C	23.014105	-13.500345	42.267362
C	21.721784	-11.691992	43.297632
C	24.156471	-12.756419	42.399100
C	22.857321	-10.938181	43.435868
C	24.149273	-11.428111	42.992063
C	17.560340	-18.765243	42.158011
C	24.820594	-18.437794	41.509093
N	19.531603	-19.847337	37.766183
N	20.301862	-19.142651	40.291453
N	21.111364	-15.895157	41.566707
N	19.046725	-16.804665	41.518031
N	25.558390	-18.135335	42.600920
N	18.286103	-18.865334	43.285832
O	17.409236	-20.723107	37.911031
O	21.396013	-21.134411	40.607177
O	17.787663	-15.054942	42.437840
O	25.209435	-10.742408	43.111425
O	25.340015	-18.708063	40.416261
O	16.621465	-19.524822	41.884897
H	17.865600	-18.962599	36.974792
H	18.073400	-19.603401	43.947708
H	20.063663	-19.052496	37.397080
H	19.512089	-21.668738	38.725504
H	22.090055	-20.650069	37.403763

H	21.979341	-22.119542	38.374891
H	21.402464	-20.651153	35.077370
H	20.470048	-24.024578	37.585742
H	20.681259	-21.932411	33.080833
H	19.750398	-25.311050	35.593606
H	19.849784	-24.271331	33.327361
H	19.039445	-18.226736	43.504796
H	22.826355	-18.774259	39.639033
H	22.829849	-17.074559	40.126206
H	23.037521	-17.778573	42.538005
H	23.035374	-19.471473	42.031814
H	17.032875	-16.977984	41.140300
H	18.045038	-18.093095	40.200616
H	19.647980	-13.184022	42.988368
H	23.055803	-14.487615	41.818960
H	20.764176	-11.296076	43.640329
H	25.115499	-13.144646	42.055959
H	22.819984	-9.945264	43.883359
H	26.570903	-18.166213	42.546782
H	25.137598	-17.913044	43.494915

155
! GFP neutral, optimization with QM/QM/PCM scheme, epsilon=4
C 0.3923 1.9903 -3.7228
O -0.4632 3.0695 -3.2725
C 0.3664 1.8218 -5.2359
H 1.4141 2.1610 -3.3623
H -0.1221 3.8977 -3.6476
H 0.7551 2.7138 -5.7434
H 0.9947 0.9719 -5.5300
H -0.6536 1.6388 -5.5925
H 0.0059 1.0996 -3.2222
C -3.5449 -3.8687 -3.7891
O -4.2662 -4.6275 -3.1384
N -2.3336 -3.4363 -3.3211
C -1.8596 -3.6988 -1.9608
C -1.6544 -2.3737 -1.2706
C -0.5899 -4.5662 -1.9621
O -0.2557 -4.9676 -0.6400
H -1.7860 -2.8054 -3.8942
H -2.6591 -4.2634 -1.4711
H -0.8019 -5.4572 -2.5641
H 0.2423 -4.0220 -2.4201
H 0.6179 -4.5769 -0.3609
N -0.5039 -1.7943 -1.1238
C -0.7607 -0.5371 -0.5615
C -2.2137 -0.3774 -0.4031
O -2.8882 0.6025 -0.0389
C 0.1354 0.4483 -0.2549
C 1.5649 0.4901 -0.4234
C 2.3575 -0.6396 -0.7375
C 2.2128 1.7462 -0.3169
C 3.7182 -0.5074 -0.9782
C 3.5678 1.8887 -0.5716
C 4.3289 0.7602 -0.9267
O 5.6411 0.9396 -1.1989
H 1.9133 -1.6272 -0.7809
H 1.6274 2.6235 -0.0509
H 4.2949 -1.3942 -1.2187
H 4.0514 2.8586 -0.5026
H 6.1255 0.0830 -1.4037
H -0.3139 1.3610 0.1331
N -2.7369 -1.5935 -0.8227
C -4.1641 -1.8334 -0.9922
C -4.9373 -2.5012 0.1504
O -6.1766 -2.4803 0.0769

H	-4.6447	-0.8695	-1.1694
H	-4.3308	-2.4494	-1.8764
N	-4.2605	-3.0958	1.1396
H	-3.2463	-3.1918	1.1155
C	-2.6336	-0.8227	5.0999
C	-2.3191	-0.4791	3.6254
C	-1.6560	0.8817	3.4317
O	-2.3166	1.9052	3.1590
N	-0.3166	0.9243	3.5841
H	-3.2911	-0.0691	5.5473
H	-1.7192	-0.8859	5.6997
H	-1.6708	-1.2579	3.2063
H	-3.2457	-0.4638	3.0451
H	0.1418	1.8217	3.4827
H	0.2875	0.1033	3.7157
H	-3.1431	-1.7907	5.1516
C	-8.6110	3.0466	0.7605
C	-8.1402	1.7706	0.0459
C	-6.7861	1.3287	0.5743
O	-6.6510	0.8875	1.7249
N	-5.7297	1.4862	-0.2577
H	-7.9302	3.8849	0.5696
H	-8.6634	2.8870	1.8420
H	-8.8470	0.9558	0.2372
H	-8.1062	1.9318	-1.0381
H	-4.8050	1.1556	0.0149
H	-5.8641	1.7706	-1.2175
H	-9.6079	3.3345	0.4091
C	-5.0911	7.3267	-3.5435
C	-4.8200	6.8543	-2.1040
C	-3.7130	5.7922	-2.0000
N	-3.4833	5.4339	-0.5892
C	-3.0969	4.2354	-0.1117
N	-2.5771	3.3035	-0.9188
N	-3.2510	3.9677	1.1940
H	-5.4152	6.4964	-4.1825
H	-4.1955	7.7718	-3.9935
H	-4.5378	7.7206	-1.4894
H	-5.7405	6.4444	-1.6689
H	-4.0033	4.8831	-2.5372
H	-2.7832	6.1668	-2.4479
H	-3.6683	6.1561	0.0970
H	-2.5106	2.3378	-0.5806
H	-2.0900	3.5273	-1.7825
H	-2.8534	3.1409	1.6500

H	-3.8980	4.5095	1.7514
H	-5.8819	8.0836	-3.5492
C	7.8179	3.2935	0.5323
C	7.7723	3.9453	-0.8126
N	7.0757	3.4082	-1.8782
C	8.3356	5.0997	-1.3129
C	7.2442	4.2383	-2.9472
N	8.0025	5.2768	-2.6436
H	6.8168	3.1746	0.9648
H	8.4106	3.9039	1.2206
H	6.5298	2.5477	-1.8393
H	8.9625	5.8100	-0.7890
H	6.7989	4.0397	-3.9131
H	8.2766	2.2975	0.4882
C	3.0624	4.0314	4.5806
C	3.1964	4.3612	6.0721
C	1.9977	4.8808	3.8756
H	2.8188	2.9666	4.4640
H	2.2487	4.1923	6.5999
H	3.9611	3.7393	6.5530
H	3.4768	5.4124	6.2208
H	1.0086	4.7293	4.3277
H	2.2341	5.9507	3.9481
H	1.9225	4.6278	2.8105
H	4.0324	4.1792	4.0855
C	5.7277	-0.8341	2.7288
O	4.2994	-0.7317	2.5524
C	6.0353	-0.7067	4.2104
H	6.0787	-1.8011	2.3435
H	4.0895	-0.8135	1.6062
H	5.5435	-1.5037	4.7790
H	7.1163	-0.7799	4.3777
H	5.6904	0.2586	4.5978
H	6.2238	-0.0357	2.1602
C	6.4710	-4.5133	-2.5482
O	6.3230	-3.6804	-1.3997
H	7.5150	-4.4597	-2.8706
H	5.8269	-4.1797	-3.3728
H	5.3576	-3.7267	-1.1229
H	6.2303	-5.5566	-2.3078
C	3.5325	-6.8100	0.2694
C	3.9487	-5.4377	0.8319
C	3.1940	-4.3317	0.0956
O	1.9946	-4.1045	0.4606
O	3.7561	-3.7359	-0.8663

H	3.7684	-6.8857	-0.7988
H	2.4566	-6.9728	0.3946
H	3.7064	-5.3914	1.8996
H	5.0287	-5.2974	0.7157
H	4.0612	-7.6178	0.7891
O	7.1438	-1.1679	-1.7734
H	6.8326	-2.1109	-1.6293
H	8.0001	-1.0971	-1.3233
O	1.6629	-1.1997	3.8729
H	2.5584	-1.0455	3.5113
H	1.3908	-2.0928	3.5428
O	-1.6198	-4.2921	1.6369
H	-1.1714	-4.5748	0.7971
H	-1.9001	-5.1198	2.0599
O	0.8894	-3.6994	2.9414
H	1.3946	-3.8828	2.1108
H	-0.0429	-3.7815	2.6509
O	-8.0416	-1.6106	2.0948
H	-7.4761	-2.0421	1.4231
H	-7.6723	-0.7060	2.1416
H	-4.7550	-3.4648	1.9392
H	-3.8964	-3.5450	-4.7576

155
! GFP anionic, optimization with QM/QM/PCM scheme, epsilon=4
C 0.6063 1.7185 -3.9225
O -0.2926 2.8058 -3.5935
C 0.9056 1.6442 -5.4135
H 1.5296 1.8188 -3.3389
H 0.1430 3.6374 -3.8416
H 1.4228 2.5478 -5.7609
H 1.5579 0.7875 -5.6239
H -0.0168 1.5289 -5.9938
H 0.0913 0.8180 -3.5788
C -3.3746 -4.1091 -3.8084
O -4.1552 -4.8253 -3.1792
N -2.2405 -3.5919 -3.2478
C -1.9064 -3.7246 -1.8305
C -1.7597 -2.3403 -1.2553
C -0.6440 -4.5846 -1.6561
O -0.4312 -4.8908 -0.2790
H -1.6475 -2.9955 -3.8119
H -2.7489 -4.2464 -1.3663
H -0.8013 -5.5149 -2.2144
H 0.2241 -4.0644 -2.0735
H 0.4853 -4.6555 -0.0260
N -0.6243 -1.7295 -1.1052
C -0.9198 -0.4469 -0.6263
C -2.3677 -0.3039 -0.5191
O -3.0839 0.6726 -0.2033
C -0.0130 0.5371 -0.2908
C 1.4086 0.5408 -0.3919
C 2.1684 -0.5269 -0.9590
C 2.1330 1.6821 0.0634
C 3.5383 -0.4575 -1.0535
C 3.5075 1.7547 -0.0199
C 4.2714 0.6759 -0.5759
O 5.5667 0.7118 -0.6347
H 1.6490 -1.4077 -1.3163
H 1.5776 2.5156 0.4908
H 4.0958 -1.2837 -1.4831
H 4.0403 2.6311 0.3403
H -0.4581 1.4447 0.1154
N -2.8603 -1.5449 -0.9129
C -4.2764 -1.8206 -1.0975
C -4.9927 -2.6159 -0.0025
O -6.2129 -2.8079 -0.1420
H -4.7840 -0.8567 -1.1837

H	-4.4323	-2.3606	-2.0339
N	-4.2709	-3.0813	1.0212
H	-3.2707	-2.8902	1.1149
C	-2.6909	-0.7609	4.9033
C	-3.0427	0.3753	3.9311
C	-1.9483	1.3566	3.5286
O	-2.2279	2.3814	2.8732
N	-0.6801	1.0927	3.8988
H	-2.3003	-0.3762	5.8527
H	-1.9542	-1.4520	4.4808
H	-3.4411	-0.0372	2.9957
H	-3.8537	0.9886	4.3413
H	0.0383	1.7511	3.6222
H	-0.3677	0.2437	4.3723
H	-3.5938	-1.3395	5.1241
C	-8.5739	2.8042	0.1982
C	-8.2894	1.7078	1.2290
C	-6.8776	1.1375	1.3064
O	-6.5974	0.2837	2.1631
N	-5.9556	1.5882	0.4268
H	-8.4151	2.4544	-0.8293
H	-7.9541	3.6937	0.3651
H	-8.5240	2.0656	2.2390
H	-8.9503	0.8484	1.0600
H	-5.0199	1.1843	0.4050
H	-6.2016	2.2402	-0.3037
H	-9.6204	3.1191	0.2713
C	-4.9627	6.9666	-4.1819
C	-4.5935	6.6794	-2.7194
C	-3.4108	5.7096	-2.5781
N	-3.1044	5.4730	-1.1580
C	-2.8940	4.2784	-0.5718
N	-2.5045	3.2165	-1.2843
N	-3.1038	4.1523	0.7461
H	-5.2510	6.0485	-4.7079
H	-4.1236	7.4179	-4.7252
H	-4.3448	7.6238	-2.2150
H	-5.4590	6.2568	-2.1929
H	-3.6534	4.7450	-3.0338
H	-2.5248	6.1079	-3.0881
H	-3.1983	6.2684	-0.5369
H	-2.5920	2.2795	-0.8673
H	-1.9654	3.3037	-2.1427
H	-2.7613	3.3636	1.3036
H	-3.6353	4.8538	1.2434

H	-5.8071	7.6619	-4.2329
C	7.8980	3.3840	0.5583
C	7.6525	3.7488	-0.8708
N	6.8540	2.9866	-1.6980
C	8.0876	4.7909	-1.6631
C	6.8392	3.5835	-2.9220
N	7.5760	4.6826	-2.9435
H	6.9681	3.3622	1.1404
H	8.5656	4.1174	1.0218
H	6.3558	2.1340	-1.4011
H	8.7411	5.6079	-1.3837
H	6.2838	3.1801	-3.7586
H	8.3654	2.3964	0.6537
C	2.9455	4.1613	4.3718
C	3.0750	4.5195	5.8571
C	1.8568	4.9700	3.6558
H	2.7296	3.0883	4.2742
H	2.1335	4.3357	6.3911
H	3.8576	3.9267	6.3459
H	3.3283	5.5800	5.9863
H	0.8727	4.7992	4.1115
H	2.0652	6.0466	3.7112
H	1.7862	4.6992	2.5949
H	3.9100	4.3258	3.8718
C	5.9366	-0.7918	2.6201
O	6.8730	-0.2107	1.7011
C	5.3148	0.2383	3.5569
H	5.1532	-1.3287	2.0655
H	6.3763	0.3230	1.0404
H	4.7337	0.9802	2.9959
H	4.6375	-0.2524	4.2673
H	6.0914	0.7639	4.1240
H	6.5068	-1.5319	3.1913
C	6.7725	-4.6049	-2.2590
O	6.2337	-3.9754	-1.0915
H	6.4844	-4.0677	-3.1712
H	6.3725	-5.6212	-2.2991
H	6.6037	-3.0498	-1.0008
H	7.8654	-4.6539	-2.1965
C	3.7302	-6.8847	0.5681
C	4.1220	-5.4819	1.0749
C	3.2338	-4.4546	0.4132
O	2.0774	-4.2568	0.8080
O	3.6784	-3.8128	-0.6491
H	3.8811	-6.9760	-0.5133

H	2.6813	-7.1024	0.7917
H	3.9597	-5.4207	2.1553
H	5.1754	-5.2796	0.8636
H	4.6703	-3.9545	-0.8358
H	4.3497	-7.6420	1.0596
O	7.2162	-1.4988	-0.7982
H	7.4033	-1.3384	0.1516
H	6.5729	-0.7687	-0.9854
O	0.9341	-1.1811	4.7789
H	0.7144	-1.9022	5.3883
H	1.0312	-1.5976	3.8929
O	-1.5624	-3.3767	1.8437
H	-0.7911	-2.7922	1.9978
H	-1.2487	-3.9918	1.1456
O	0.9675	-2.0452	2.1197
H	1.4481	-2.7766	1.6717
H	1.0509	-1.2834	1.5207
O	-8.1001	-2.1605	1.9504
H	-7.5341	-2.5207	1.2374
H	-7.6668	-1.3107	2.1699
H	-4.7116	-3.6426	1.7359
H	-3.6137	-3.8942	-4.8394

155
! GFP intermediate, optimization with QM/QM/PCM scheme, epsilon=4
C 0.3046 1.5394 -3.8637
O -0.1117 2.9156 -3.8460
C 0.4327 0.9839 -5.2783
H 1.2481 1.4257 -3.3158
H 0.5893 3.4504 -4.2522
H 1.2047 1.5202 -5.8451
H 0.7193 -0.0751 -5.2471
H -0.5157 1.0723 -5.8208
H -0.4682 1.0040 -3.3050
C -3.2903 -4.4642 -3.4657
O -3.9365 -5.2598 -2.7810
N -2.1934 -3.8078 -2.9789
C -1.7138 -3.9515 -1.6028
C -1.5217 -2.5734 -1.0239
C -0.4382 -4.8125 -1.5649
O -0.0934 -5.1698 -0.2277
H -1.6899 -3.1750 -3.5885
H -2.5025 -4.4857 -1.0639
H -0.6477 -5.7295 -2.1259
H 0.3879 -4.2833 -2.0505
H 0.7627 -4.7675 0.0238
N -0.3775 -1.9548 -0.9633
C -0.6474 -0.6643 -0.4769
C -2.0835 -0.5306 -0.2548
O -2.7807 0.4392 0.0936
C 0.2443 0.3749 -0.2875
C 1.6359 0.4678 -0.5603
C 2.4006 -0.5725 -1.1678
C 2.3174 1.6829 -0.2588
C 3.7308 -0.4007 -1.4682
C 3.6557 1.8602 -0.5434
C 4.4224 0.8233 -1.1787
O 5.6705 0.9786 -1.4702
H 1.9150 -1.5134 -1.3975
H 1.7584 2.4928 0.2069
H 4.2904 -1.2023 -1.9409
H 4.1511 2.7993 -0.3105
H -0.2182 1.2732 0.1209
N -2.5944 -1.7912 -0.5889
C -4.0218 -2.0370 -0.7255
C -4.7811 -2.7180 0.4204
O -6.0250 -2.6812 0.3725
H -4.4979 -1.0666 -0.8792

H	-4.2058	-2.6350	-1.6214
N	-4.1037	-3.3526	1.3832
H	-3.0803	-3.3924	1.4033
C	-2.7317	-0.7284	5.1115
C	-2.2773	-0.4052	3.6740
C	-2.0832	1.0856	3.4483
O	-3.0465	1.8491	3.2254
N	-0.8189	1.5480	3.5256
H	-3.6717	-0.2194	5.3514
H	-1.9766	-0.4234	5.8447
H	-1.3488	-0.9428	3.4565
H	-3.0357	-0.7428	2.9608
H	-0.6648	2.5426	3.4129
H	0.0051	0.9531	3.6581
H	-2.8905	-1.8066	5.2170
C	-8.8432	2.3608	0.3649
C	-8.2178	1.7623	1.6311
C	-6.6939	1.7159	1.5851
O	-6.0792	1.5271	0.5221
N	-6.0478	1.8756	2.7620
H	-8.5578	1.7899	-0.5220
H	-8.5280	3.4010	0.2208
H	-8.5494	2.3078	2.5230
H	-8.5606	0.7255	1.7560
H	-5.0348	1.7509	2.8340
H	-6.5653	1.9928	3.6224
H	-9.9357	2.3451	0.4443
C	-5.5057	6.4990	-4.2196
C	-5.4672	5.8557	-2.8244
C	-4.0454	5.4483	-2.4117
N	-3.9581	4.9565	-1.0277
C	-3.6612	3.7088	-0.6234
N	-3.6618	2.6759	-1.4758
N	-3.3083	3.4895	0.6496
H	-5.1416	5.8075	-4.9894
H	-4.8851	7.4028	-4.2585
H	-5.8607	6.5638	-2.0827
H	-6.1210	4.9747	-2.7941
H	-3.6423	4.6812	-3.0780
H	-3.3718	6.3101	-2.4895
H	-4.0081	5.6608	-0.3019
H	-3.4691	1.7507	-1.0819
H	-4.2278	2.7141	-2.3115
H	-3.2674	2.5473	1.0317
H	-3.1920	4.2501	1.3037

H	-6.5298	6.7835	-4.4835
C	7.5300	3.6830	0.3899
C	7.4905	4.1628	-1.0259
N	6.8788	3.4452	-2.0330
C	7.9838	5.2926	-1.6451
C	7.0238	4.1528	-3.1874
N	7.6892	5.2810	-2.9960
H	6.5242	3.5674	0.8132
H	8.0735	4.4008	1.0125
H	6.3945	2.5413	-1.8986
H	8.5338	6.1091	-1.1941
H	6.6315	3.8052	-4.1342
H	8.0349	2.7126	0.4780
C	2.6515	4.4308	4.2834
C	3.9168	4.8355	5.0489
C	1.6338	5.5725	4.1687
H	2.1838	3.5715	4.7833
H	3.6746	5.1525	6.0717
H	4.6269	4.0026	5.1160
H	4.4272	5.6727	4.5545
H	1.3122	5.9165	5.1605
H	2.0644	6.4346	3.6425
H	0.7371	5.2606	3.6180
H	2.9293	4.0859	3.2778
C	5.6417	-0.3636	2.9203
O	4.3728	0.3038	2.7464
C	5.7608	-0.7911	4.3723
H	5.6941	-1.2343	2.2530
H	4.2859	0.5966	1.8225
H	4.9523	-1.4788	4.6444
H	6.7171	-1.3017	4.5355
H	5.7155	0.0774	5.0389
H	6.4541	0.3246	2.6513
C	6.7938	-4.4242	-2.0625
O	6.4295	-3.5588	-0.9797
H	6.2980	-4.1266	-2.9944
H	6.4813	-5.4368	-1.7950
H	6.7294	-2.6338	-1.2016
H	7.8794	-4.4153	-2.2107
C	3.9066	-6.7249	0.9322
C	4.4003	-5.2970	1.2254
C	3.4595	-4.2945	0.5953
O	2.2769	-4.2260	0.9516
O	3.8908	-3.5182	-0.3768
H	3.9055	-6.9330	-0.1437

H	2.8922	-6.8714	1.3135
H	4.3904	-5.1201	2.3071
H	5.4235	-5.1525	0.8681
H	4.8852	-3.5922	-0.5750
H	4.5659	-7.4535	1.4155
O	7.2891	-1.1234	-1.7449
H	8.1243	-0.8333	-1.3474
H	6.6609	-0.3419	-1.6693
O	1.6398	-0.1306	3.7668
H	2.5651	0.0784	3.5221
H	1.4183	-0.9332	3.2450
O	-1.4352	-4.1003	2.0337
H	-0.7405	-3.4384	2.2375
H	-1.0563	-4.6078	1.2836
O	0.7286	-2.2357	2.1279
H	1.3800	-2.8719	1.7555
H	0.4412	-1.7187	1.3539
O	-7.0465	-0.5213	-1.2393
H	-6.8095	-1.3233	-0.7260
H	-6.7544	0.2244	-0.6737
H	-3.6159	-4.2925	-4.4810
H	-4.6018	-3.8172	2.1289

References

(1) Wachter, R. M.; King, B. A.; Heim, R.; Kallio, K.; Tsien, R. Y.; Boxer, S. G.; Remington, S. J. Crystal Structure and Photodynamic Behavior of the Blue Emission Variant Y66H/Y145F of Green Fluorescent Protein. *Biochemistry* **1997**, *36*, 9759–9765.

(2) Lelimousin, M.; Noirclerc-Savoye, M.; Lazareno-Saez, C.; Paetzold, B.; Le Vot, S.; Chazal, R.; Macheboeuf, P.; Field, M. J.; Bourgeois, D.; Royant, A. Intrinsic Dynamics in ECFP and Cerulean Control Fluorescence Quantum Yield. *Biochemistry* **2009**, *48*, 10038–10046.

(3) Cormack, B. P.; Valdivia, R. H.; Falkow, S. FACS-Optimized Mutants of the Green Fluorescent Protein (GFP). *Gene.* **1996**, *173*, 33–38.

(4) Wachter, R. M.; Yarbrough, D.; Kallio, K.; Remington, S. J. Crystallographic and Energetic Analysis of Binding of Selected Anions to the Yellow Variants of Green Fluorescent Protein. *J. Mol. Biol.* **2000**, *301*, 157–171.

(5) Matz, M. V.; Fradkov, A. F.; Labas, Y. A.; Savitsky, A. P.; Zaraisky, A. G.; Markelov, M. L.; Lukyanov, S. A. Fluorescent Proteins from Nonbioluminescent Anthozoa Species. *Nat. Biotechnol.* **1999**, *17*, 969–973.