Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Electronic Supplementary Information for "A polarizable embedding DFT study of one-photon absorption in fluorescent proteins"

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Table SI–I: Excitation energies (in eV) of the fluorescent protein crystal structures. Excitation energy calculations have been performed with no embedding (NE), non-polarizable embedding (NPE) and polarizable embedding (PE) at the CAM-B3LYP/6-31+G* level of theory. The vacuum calculations (vac) have been performed on chromophores optimized in vacuum, where the imidazolidone ring has been methyl-terminated. These results are plotted in Figure 2 in the main article.

			Non-opt			QM/MM-opt			
FPs	Q	vac	NE	NPE	PE	NE	NPE	PE	Exp
BFP	0	3.89	3.33	3.14	3.10	3.84	3.67	3.60	3.25
YFP	0	3.69	3.35	3.29	3.16	3.61	3.51	3.39	3.16
wtGFP	0	3.69	3.53	3.46	3.36	3.64	3.55	3.45	3.14
eCFP	0	3.55	3.34	3.14	3.09	3.33	3.15	3.10	2.85
wtGFP	-1	3.13	2.83	2.98	3.01	3.03	3.09	2.99	2.61
eGFP	-1	3.13	2.63	2.77	2.74	3.00	3.05	2.96	2.54
YFP	-1	3.13	3.02	3.05	2.90	3.09	3.13	2.93	2.41
DsRed[†]	-1	2.39				2.53	2.89	2.83	2.22

[†] Data from List *et al.* J. Phys. Chem. Lett. 2012, 3, 3513-3521

Table SI–II: Excitation energies (in eV) of the fluorescent proteins after MD. The results are averages of 50 snapshots and excitation energy calculations have been performed with no embedding (NE), non-polarizable embedding (NPE) and polarizable embedding (PE) at the CAM-B3LYP/6-31+G* level of theory. Standard deviations are shown between brackets. The vacuum calculations (vac) have been performed on chromophores optimized in vacuum, where the imidazolidone ring has been methyl-terminated. These results are plotted in Figure 4 in the main article.

			Non-opt			QM/MM-opt			
Name	Q	vac	NE	NPE	PE	NE	NPE	PE	Exp
BFP	0	3.89	3.72 (0.104)	3.67 (0.124)	3.60 (0.132)	3.80 (0.034)	3.80 (0.044)	3.70 (0.026)	3.25
YFP	0	3.69	3.63 (0.132)	3.59 (0.140)	3.47 (0.142)	3.61 (0.020)	3.60 (0.035)	3.48 (0.037)	3.16
wtGFP	0	3.69	3.65 (0.118)	3.62 (0.124)	3.51 (0.129)	3.63 (0.025)	3.62 (0.037)	3.51 (0.038)	3.14
eCFP	0	3.55	3.48 (0.122)	3.49 (0.134)	3.40 (0.145)	3.40 (0.024)	3.43 (0.048)	3.34 (0.056)	2.85
wtGFP	-1	3.13	2.99 (0.061)	3.07 (0.069)	2.92 (0.079)	3.03 (0.014)	3.13 (0.025)	3.00 (0.034)	2.61
eGFP	-1	3.13	2.99 (0.073)	3.07 (0.080)	2.94 (0.086)	3.01 (0.017)	3.10 (0.031)	2.98 (0.043)	2.54
YFP	-1	3.13	3.00 (0.064)	3.05 (0.068)	2.89 (0.075)	3.05 (0.027)	3.10 (0.028)	2.95 (0.030)	2.41

Table SI–III: Excitation energies (in eV) for neutral (E_n) and anionic (E_a) wtGFP and YFP and the magnitude of the neutral–anionic shift (ΔE). For YFP, data are shown for excitation energy calculations with only the chromophore in the QM region (YFP) and those with the chromophore and Tyr203 in the QM region (YFP extended). For the QM/MM geometry optimized structures, both the YFP chromophore and Tyr203 were present in the QM region of the geometry optimization. The MD results are averages of 50 snapshots. Excitation energy calculations have been performed with no embedding (NE), non-polarizable embedding (NPE) and polarizable embedding (PE) at the CAM-B3LYP/6-31+G* level of theory.

	wtGFP			YFP			YFP extended		
	E_n	E_a	ΔE	E_n	E_a	ΔE	E_n	E_a	ΔE
CS QM/MM-opt									
NE	3.64	3.03	0.61	3.61	3.09	0.52	3.51	2.98	0.53
NPE	3.55	3.09	0.46	3.51	3.13	0.38	3.43	3.02	0.41
PE	3.45	2.99	0.46	3.39	2.93	0.46	3.33	2.84	0.49
MD Non-opt									
NE	3.65	2.99	0.66	3.63	3.00	0.63	3.59	2.97	0.62
NPE	3.62	3.07	0.55	3.59	3.05	0.54	3.55	3.01	0.54
PE	3.51	2.92	0.59	3.47	2.89	0.58	3.45	2.88	0.57
MD QM/MM-opt									
NE	3.63	3.03	0.60	3.61	3.05	0.56	3.56	3.01	0.55
NPE	3.62	3.13	0.49	3.60	3.10	0.50	3.55	3.06	0.49
PE	3.51	3.00	0.51	3.48	2.95	0.53	3.45	2.93	0.52
Exp.	3.14	2.61	0.53	3.16	2.41	0.75	3.16	2.41	0.75

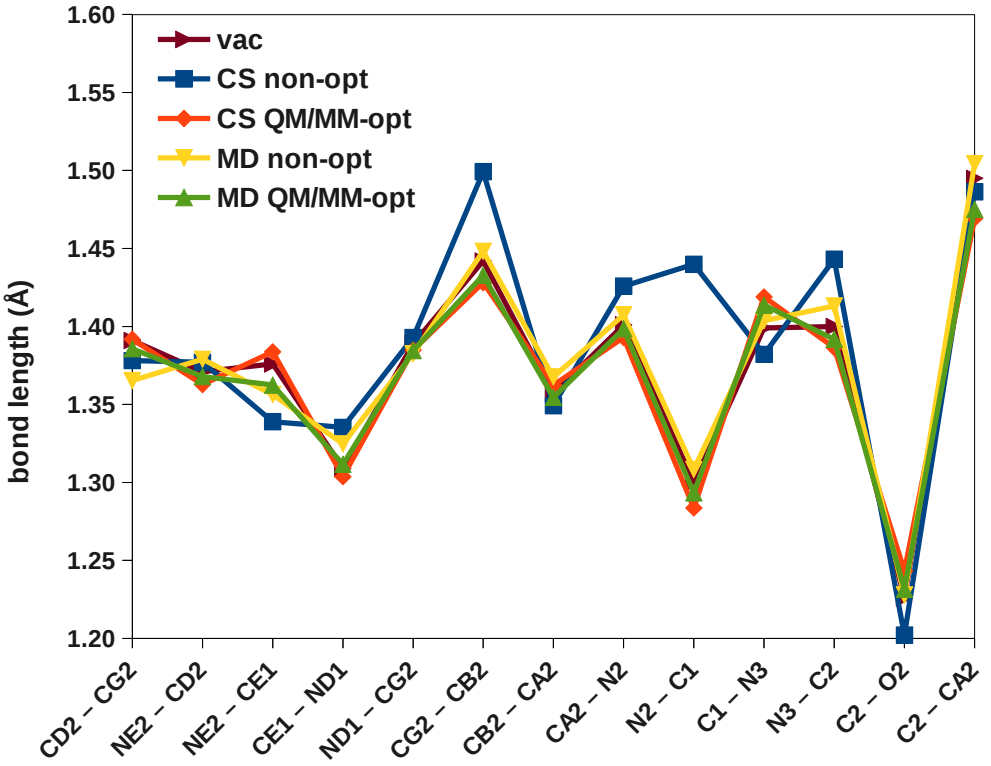


Figure SI–1: A selection of calculated bond lengths of the BFP chromophore obtained using different models: the optimized vacuum chromophore (vac), the non-optimized crystal structure (CS non-opt), the QM/MM geometry-optimized crystal structure (CS QM/MM-opt), the average over 50 non-optimized MD snapshots (MD non-opt) and the average over 50 QM/MM geometry-optimized MD snapshots (MD QM/MM-opt). The atom labeling of the chromophore is shown in the inset of Figure 3 in the main article.

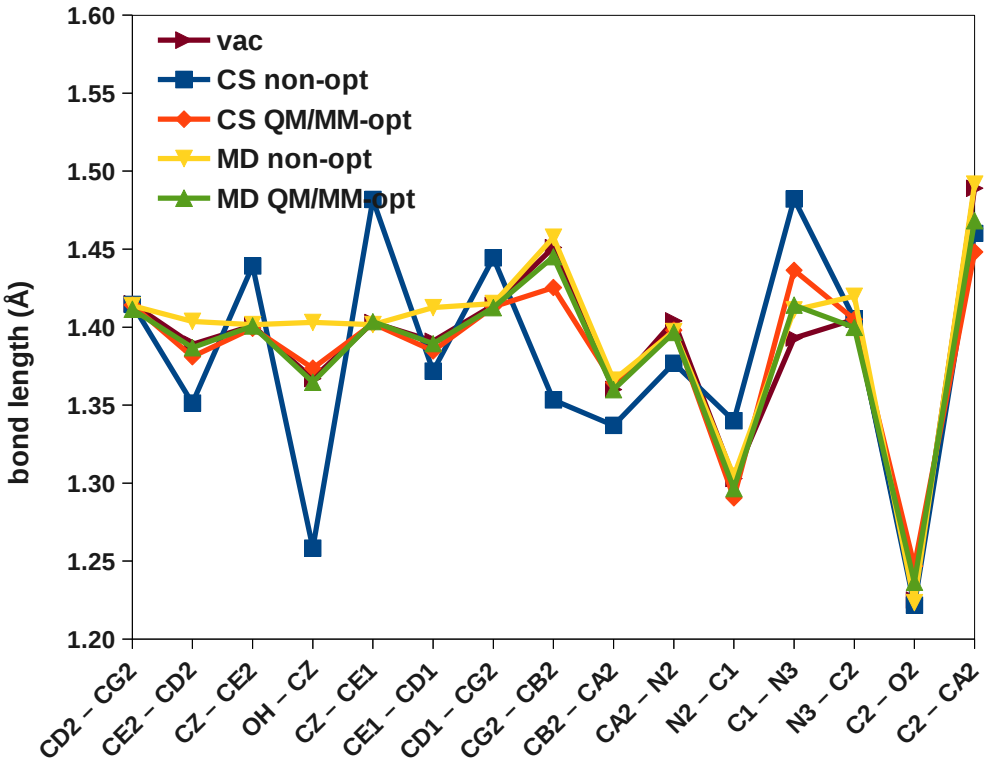


Figure SI–2: A selection of calculated bond lengths of the neutral YFP chromophore obtained using different models: the optimized vacuum chromophore (vac), the non-optimized crystal structure (CS non-opt), the QM/MM geometry-optimized crystal structure (CS QM/MM-opt), the average over 50 non-optimized MD snapshots (MD non-opt) and the average over 50 QM/MM geometry-optimized MD snapshots (MD QM/MM-opt). The atom labeling of the chromophore is shown in Figure SI–8a.

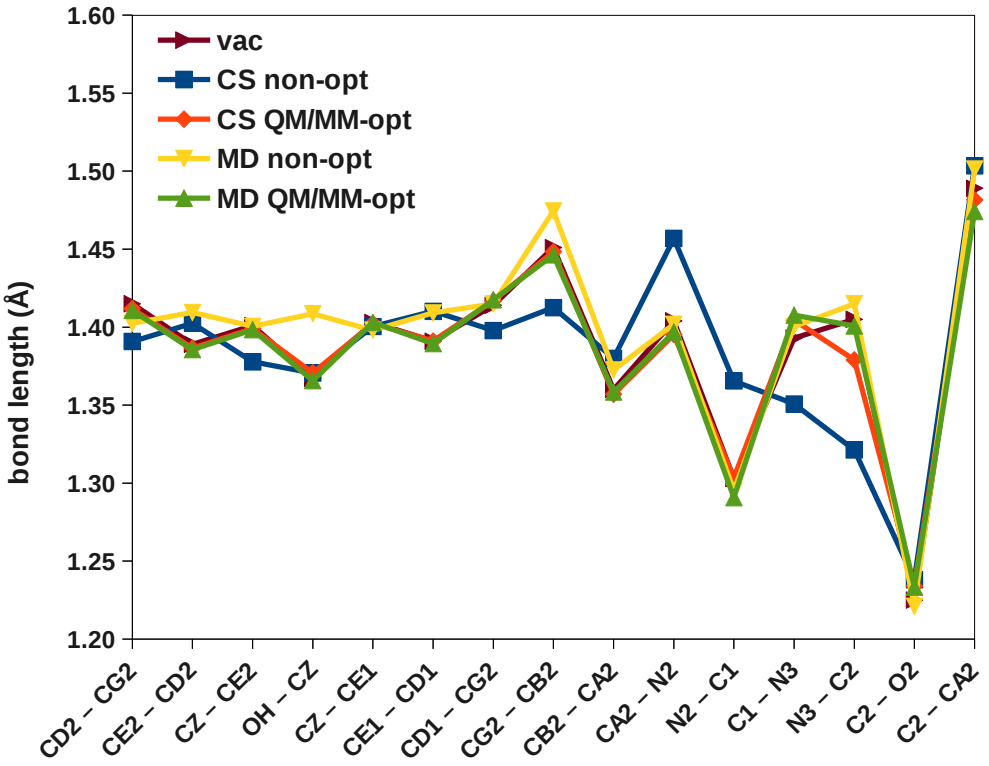


Figure SI–3: A selection of calculated bond lengths of the neutral wtGFP chromophore obtained using different models: the optimized vacuum chromophore (vac), the non-optimized crystal structure (CS non-opt), the QM/MM geometry-optimized crystal structure (CS QM/MM-opt), the average over 50 non-optimized MD snapshots (MD non-opt) and the average over 50 QM/MM geometry-optimized MD snapshots (MD QM/MM-opt). The atom labeling of the chromophore is shown in Figure SI–8a.

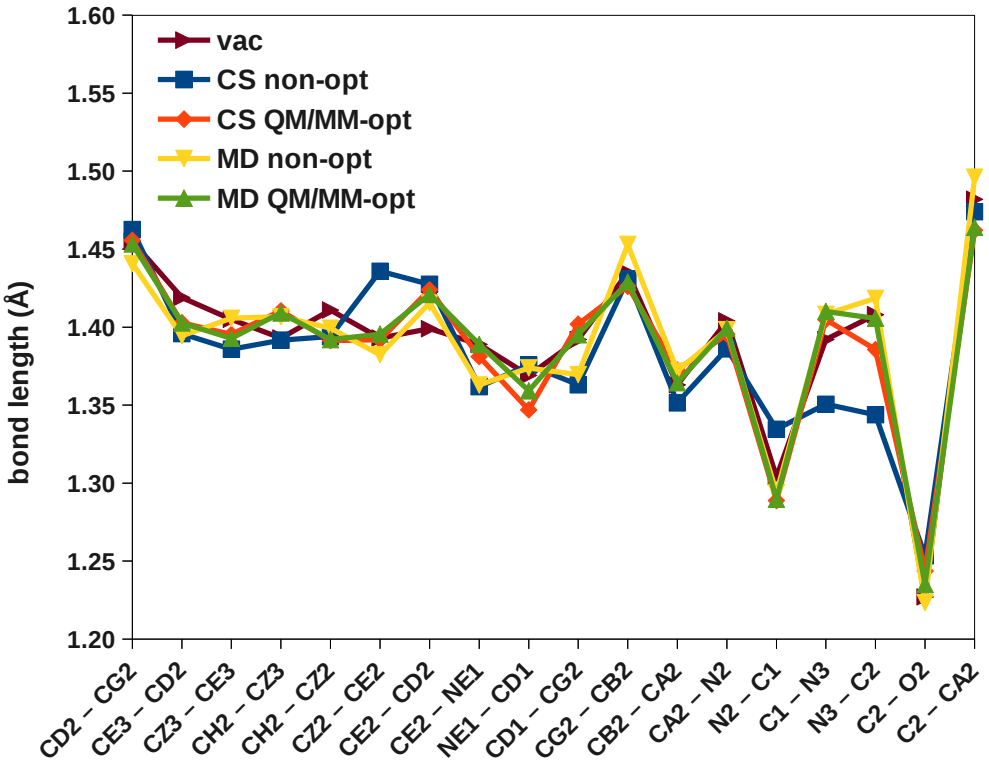


Figure SI–4: A selection of calculated bond lengths of the eCFP chromophore obtained using different models: the optimized vacuum chromophore (vac), the non-optimized crystal structure (CS non-opt), the QM/MM geometry-optimized crystal structure (CS QM/MM-opt), the average over 50 non-optimized MD snapshots (MD non-opt) and the average over 50 QM/MM geometry-optimized MD snapshots (MD QM/MM-opt). The atom labeling of the chromophore is shown in Figure SI–8b.

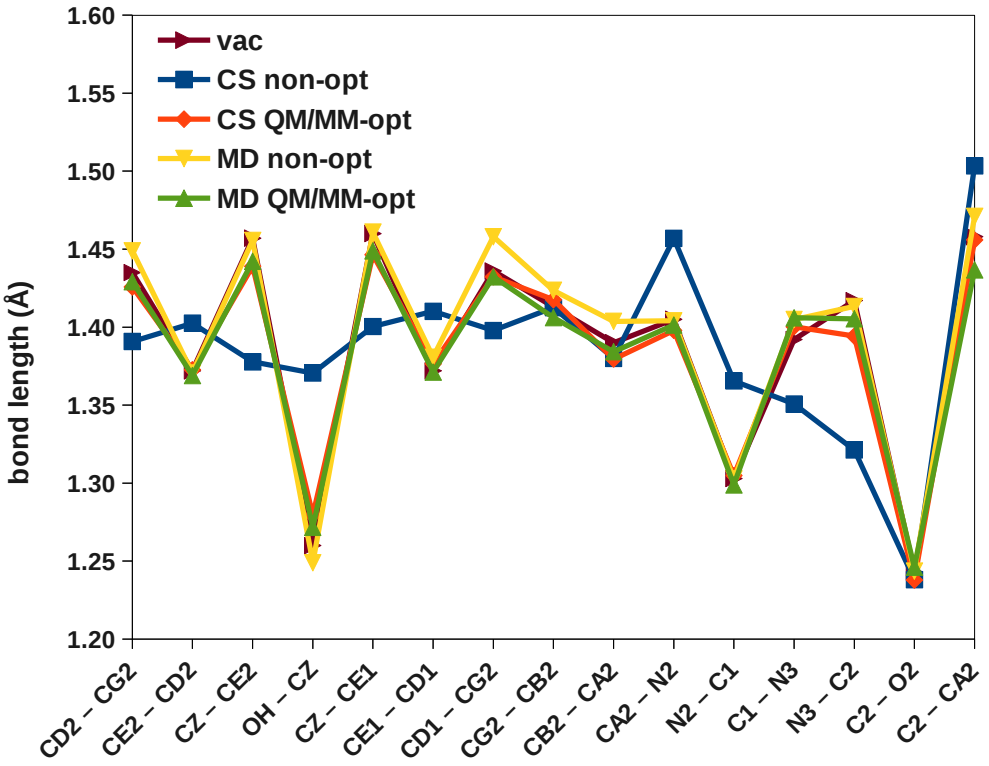


Figure SI–5: A selection of calculated bond lengths of the anionic wtGFP chromophore obtained using different models: the optimized vacuum chromophore (vac), the non-optimized crystal structure (CS non-opt), the QM/MM geometry-optimized crystal structure (CS QM/MM-opt), the average over 50 non-optimized MD snapshots (MD non-opt) and the average over 50 QM/MM geometry-optimized MD snapshots (MD QM/MM-opt). The atom labeling of the chromophore is shown in Figure SI–8a.

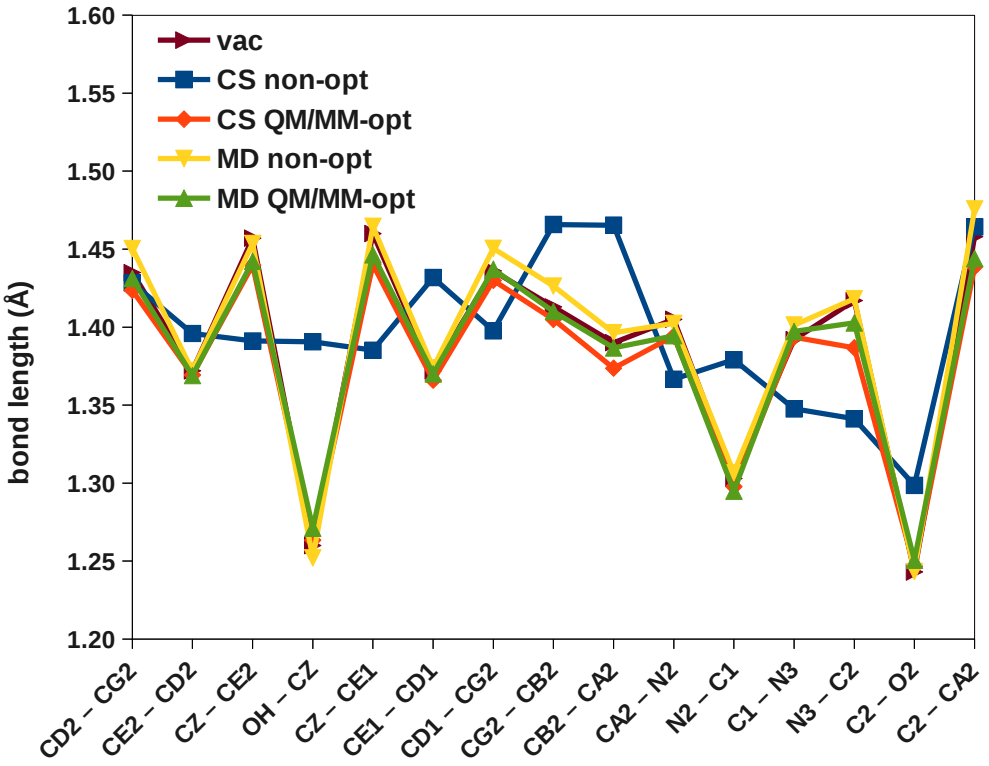


Figure SI–6: A selection of calculated bond lengths of the eGFP chromophore obtained using different models: the optimized vacuum chromophore (vac), the non-optimized crystal structure (CS non-opt), the QM/MM geometry-optimized crystal structure (CS QM/MM-opt), the average over 50 non-optimized MD snapshots (MD non-opt) and the average over 50 QM/MM geometry-optimized MD snapshots (MD QM/MM-opt). The atom labeling of the chromophore is shown in Figure SI–8a.

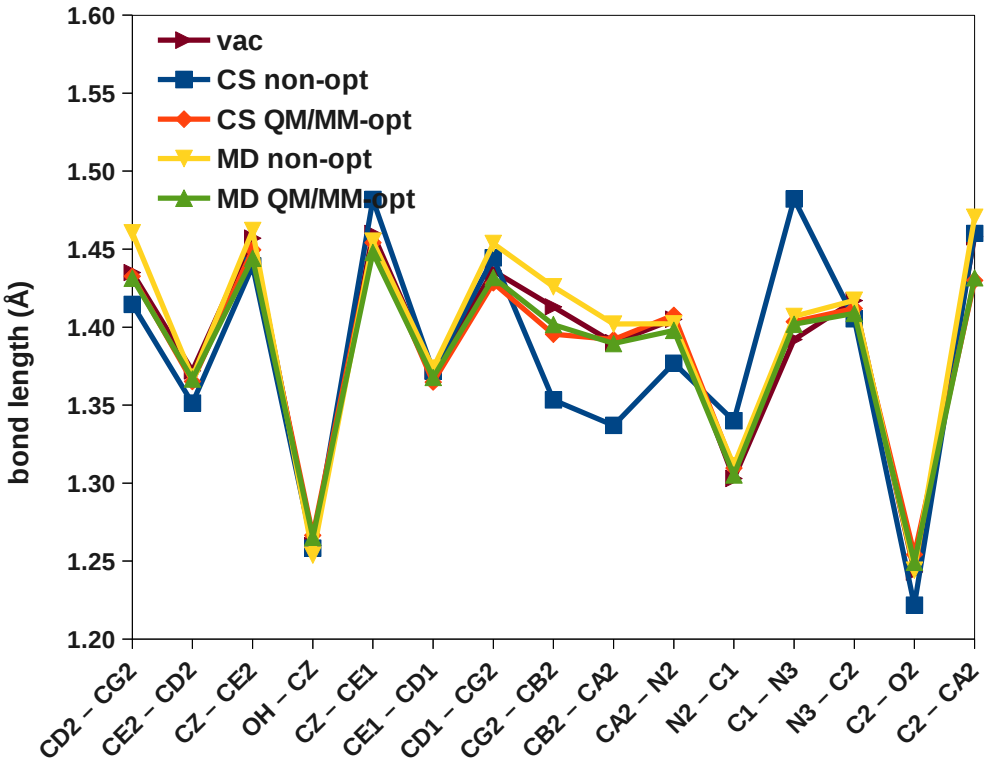
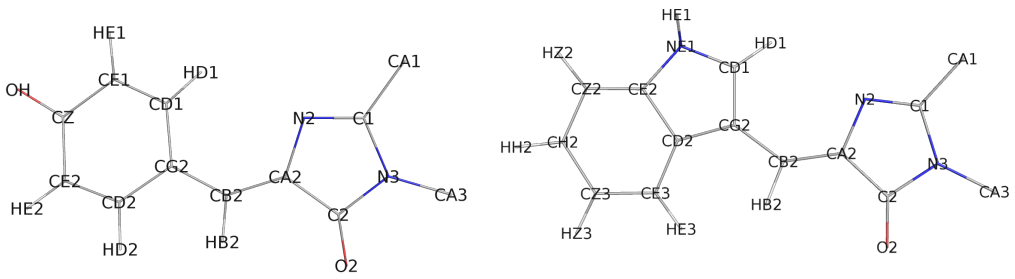


Figure SI–7: A selection of calculated bond lengths of the anionic YFP chromophore obtained using different models: the optimized vacuum chromophore (vac), the non-optimized crystal structure (CS non-opt), the QM/MM geometry-optimized crystal structure (CS QM/MM-opt), the average over 50 non-optimized MD snapshots (MD non-opt) and the average over 50 QM/MM geometry-optimized MD snapshots (MD QM/MM-opt). The atom labeling of the chromophore is shown in Figure SI–8a.



(a) (b)

Figure SI–8: Atom labeling for the chromophores of SI–8a wtGFP, YFP and eGFP and SI–8b eCFP. The atom labeling for the BFP chromophore is shown in the inset of Figure 3 in the main article.

Table SI–IV: A selection of bond lengths, angles and dihedral angles in the BFP chromophore. The vacuum calculation (vac) has been performed on the chromophore optimized in vacuum, where the imidazolidone ring has been methyl-terminated. The MD results are averages of 50 snapshots with standard deviations in parentheses.

	vac	CS Non-opt	CS QM/MM-opt	MD Non-opt	MD QM/MM-opt
Bond lengths					
CD2 - CG2	1.391	1.378	1.392	1.365 (0.025)	1.386 (0.003)
NE2 - CD2	1.371	1.377	1.363	1.379 (0.028)	1.368 (0.003)
NE2 - CE1	1.376	1.339	1.384	1.356 (0.025)	1.363 (0.003)
CE1 - ND1	1.309	1.335	1.304	1.325 (0.024)	1.312 (0.002)
ND1 - CG2	1.389	1.393	1.385	1.383 (0.029)	1.385 (0.003)
CG2 - CB2	1.442	1.499	1.428	1.448 (0.026)	1.433 (0.005)
CB2 - CA2	1.357	1.349	1.362	1.368 (0.021)	1.355 (0.002)
CA2 - N2	1.401	1.426	1.393	1.407 (0.027)	1.399 (0.002)
N2 - C1	1.300	1.440	1.284	1.308 (0.023)	1.293 (0.002)
C1 - N3	1.399	1.382	1.419	1.404 (0.024)	1.414 (0.004)
N3 - C2	1.400	1.443	1.386	1.413 (0.024)	1.392 (0.006)
C2 - O2	1.226	1.202	1.243	1.228 (0.020)	1.232 (0.004)
C2 - CA2	1.495	1.486	1.469	1.504 (0.024)	1.475 (0.007)
C1 - CA1	1.493	1.484	1.513	1.527 (0.026)	1.520 (0.004)
N3 - CA3	1.450	1.450	1.455	1.493 (0.030)	1.456 (0.003)
Angles					
ND1 - CG2 - CB2	126.2	122.9	123.9	120.6 (2.9)	124.9 (0.9)
CD2 - CG2 - CB2	124.3	129.3	126.2	129.1 (2.7)	126.0 (0.9)
CG2 - CB2 - CA2	130.4	122.9	125.4	131.4 (2.0)	128.7 (1.1)
CB2 - CA2 - C2	121.3	127.1	125.0	122.4 (2.7)	120.4 (1.2)
CB2 - CA2 - N2	129.4	123.6	125.6	130.1 (2.7)	130.0 (1.3)
Dihedral angles					
CD2 CG2 CB2 CA2	180.0	176.8	195.5	179.7 (9.9)	183.0 (8.4)
CG2 CB2 CA2 C2	180.0	183.7	178.8	180.4 (4.8)	181.8 (4.1)

Table SI–V: A selection of bond lengths, angles and dihedral angles in the YFP neutral chromophore. The vacuum calculation (vac) has been performed on the chromophore optimized in vacuum, where the imidazolidone ring has been methyl-terminated. The MD results are averages of 50 snapshots with standard deviations in parentheses.

	vac	CS Non-opt	CS QM/MM-opt	MD Non-opt	MD QM/MM-opt
Bond lengths					
CD2 - CG2	1.415	1.415	1.414	1.414 (0.026)	1.411 (0.002)
CE2 - CD2	1.389	1.351	1.381	1.404 (0.028)	1.387 (0.002)
CZ - CE2	1.401	1.439	1.399	1.402 (0.027)	1.401 (0.002)
OH - CZ	1.367	1.258	1.374	1.403 (0.032)	1.365 (0.005)
CZ - CE1	1.403	1.482	1.402	1.402 (0.028)	1.404 (0.002)
CE1 - CD1	1.391	1.372	1.385	1.413 (0.030)	1.389 (0.003)
CD1 - CG2	1.414	1.444	1.413	1.415 (0.027)	1.413 (0.002)
CG2 - CB2	1.451	1.353	1.425	1.458 (0.027)	1.445 (0.004)
CB2 - CA2	1.360	1.337	1.364	1.366 (0.021)	1.360 (0.002)
CA2 - N2	1.404	1.377	1.398	1.397 (0.023)	1.397 (0.002)
N2 - C1	1.303	1.400	1.291	1.305 (0.030)	1.297 (0.002)
C1 - N3	1.393	1.482	1.436	1.411 (0.024)	1.414 (0.004)
N3 - C2	1.405	1.405	1.405	1.420 (0.025)	1.400 (0.006)
C2 - O2	1.225	1.222	1.247	1.223 (0.022)	1.236 (0.003)
C2 - CA2	1.489	1.460	1.448	1.492 (0.033)	1.468 (0.005)
Angles					
CD1 - CG2 - CB2	123.8	119.9	123.3	123.7 (2.4)	123.7 (0.8)
CD2 - CG2 - CB2	118.5	121.5	116.9	118.3 (2.5)	118.2 (0.8)
CG2 - CB2 - CA2	130.1	134.2	133.0	132.0 (2.3)	130.3 (1.3)
CB2 - CA2 - C2	122.3	118.0	116.8	121.3 (3.3)	121.2 (1.6)
CB2 - CA2 - N2	128.5	131.1	132.6	130.2 (3.1)	129.3 (1.6)
Dihedral angles					
CD2 CG2 CB2 CA2	180.0	191.1	181.4	171.1 (10.8)	174.0 (7.7)
CG2 CB2 CA2 C2	180.0	173.2	182.4	183.5 (5.8)	184.0 (3.5)

Table SI–VI: A selection of bond lengths, angles and dihedral angles in the wtGFP neutral chromophore. The vacuum calculation (vac) has been performed on the chromophore optimized in vacuum, where the imidazolidone ring has been methyl-terminated. The MD results are averages of 50 snapshots with standard deviations in parentheses.

	vac	CS Non-opt	CS QM/MM-opt	MD Non-opt	MD QM/MM-opt
Bond lengths					
CD2 - CG2	1.415	1.391	1.412	1.403 (0.025)	1.411 (0.003)
CE2 - CD2	1.389	1.403	1.387	1.409 (0.026)	1.386 (0.003)
CZ - CE2	1.401	1.378	1.399	1.401 (0.030)	1.398 (0.003)
OH - CZ	1.367	1.371	1.371	1.409 (0.028)	1.366 (0.008)
CZ - CE1	1.403	1.400	1.402	1.398 (0.027)	1.403 (0.002)
CE1 - CD1	1.391	1.410	1.391	1.409 (0.027)	1.389 (0.002)
CD1 - CG2	1.414	1.398	1.417	1.415 (0.030)	1.418 (0.002)
CG2 - CB2	1.451	1.413	1.448	1.475 (0.025)	1.446 (0.005)
CB2 - CA2	1.360	1.380	1.357	1.373 (0.020)	1.358 (0.003)
CA2 - N2	1.404	1.457	1.395	1.402 (0.023)	1.397 (0.003)
N2 - C1	1.303	1.365	1.303	1.295 (0.022)	1.291 (0.002)
C1 - N3	1.393	1.351	1.405	1.400 (0.023)	1.408 (0.004)
N3 - C2	1.405	1.321	1.379	1.415 (0.031)	1.401 (0.006)
C2 - O2	1.225	1.238	1.234	1.221 (0.023)	1.233 (0.004)
C2 - CA2	1.489	1.503	1.482	1.501 (0.027)	1.474 (0.005)
Angles					
CD1 - CG2 - CB2	123.8	121.8	124.7	123.0 (2.5)	124.7 (0.7)
CD2 - CG2 - CB2	118.5	120.1	117.1	118.5 (3.0)	117.4 (0.6)
CG2 - CB2 - CA2	130.1	129.3	129.7	131.9 (2.3)	129.0 (0.8)
CB2 - CA2 - C2	122.3	121.2	123.9	123.1 (3.2)	123.5 (1.4)
CB2 - CA2 - N2	128.5	130.6	127.3	129.0 (3.0)	126.9 (1.2)
Dihedral angles					
CD2 CG2 CB2 CA2	180.0	179.8	175.5	179.4 (8.6)	178.6 (5.0)
CG2 CB2 CA2 C2	180.0	181.9	185.5	180.4 (5.6)	180.4 (3.9)

Table SI–VII: A selection of bond lengths, angles and dihedral angles in the eCFP chromophore. The vacuum calculation (vac) has been performed on the chromophore optimized in vacuum, where the imidazolidone ring has been methyl-terminated. The MD results are averages of 50 snapshots with standard deviations in parentheses.

	vac	CS Non-opt	CS QM/MM-opt	MD Non-opt	MD QM/MM-opt
CD2 - CG2	1.455	1.463	1.456	1.441 (0.024)	1.453 (0.004)
CE3 - CD2	1.419	1.396	1.403	1.395 (0.023)	1.403 (0.002)
CZ3 - CE3	1.405	1.386	1.395	1.406 (0.024)	1.393 (0.002)
CH2 - CZ3	1.393	1.392	1.410	1.407 (0.030)	1.409 (0.002)
CH2 - CZ2	1.411	1.394	1.391	1.399 (0.022)	1.392 (0.002)
CZ2 - CE2	1.393	1.436	1.392	1.382 (0.030)	1.396 (0.002)
CE2 - CD2	1.399	1.427	1.424	1.416 (0.030)	1.421 (0.003)
CE2 - NE1	1.389	1.362	1.381	1.363 (0.028)	1.389 (0.003)
NE1 - CD1	1.369	1.376	1.347	1.374 (0.029)	1.359 (0.004)
CD1 - CG2	1.392	1.363	1.402	1.370 (0.031)	1.395 (0.003)
CG2 - CB2	1.435	1.431	1.426	1.453 (0.028)	1.429 (0.006)
CB2 - CA2	1.363	1.351	1.372	1.372 (0.025)	1.364 (0.004)
CA2 - N2	1.404	1.386	1.395	1.398 (0.026)	1.399 (0.003)
N2 - C1	1.304	1.335	1.289	1.294 (0.031)	1.290 (0.002)
C1 - N3	1.392	1.351	1.405	1.408 (0.026)	1.410 (0.005)
N3 - C2	1.408	1.344	1.386	1.419 (0.027)	1.406 (0.008)
C2 - O2	1.227	1.253	1.244	1.223 (0.022)	1.235 (0.005)
C2 - CA2	1.482	1.474	1.462	1.496 (0.027)	1.464 (0.007)
C1 - CA1	1.493	1.472	1.517	1.518 (0.028)	1.522 (0.005)
N3 - CA3	1.451	1.465	1.454	1.488 (0.027)	1.456 (0.004)
Angles					
CD1 - CG2 - CB2	127.8	126.3	125.0	127.4 (2.9)	126.6 (1.0)
CD2 - CG2 - CB2	126.1	130.6	129.5	126.1 (3.1)	127.2 (1.2)
CG2 - CB2 - CA2	128.1	126.7	125.1	130.5 (2.5)	126.2 (1.0)
CB2 - CA2 - C2	123.4	126.4	127.0	125.4 (2.9)	124.6 (1.3)
CB2 - CA2 - N2	127.1	124.3	124.0	126.6 (3.0)	125.6 (1.1)
Dihedral angles					
CD2 CG2 CB2 CA2	180.0	173.2	175.2	183.5 (9.0)	181.2 (5.6)
CG2 CB2 CA2 C2	180.0	186.6	183.1	180.2 (5.4)	175.5 (4.8)

Table SI–VIII: A selection of bond lengths, angles and dihedral angles in the wtGFP anionic chromophore. The vacuum calculation (vac) has been performed on the chromophore optimized in vacuum, where the imidazolidone ring has been methyl-terminated. The MD results are averages of 50 snapshots with standard deviations in parentheses.

	vac	CS Non-opt	CS QM/MM-opt	MD Non-opt	MD QM/MM-opt
Bond lengths					
CD2 - CG2	1.435	1.391	1.426	1.449 (0.030)	1.429 (0.003)
CE2 - CD2	1.372	1.403	1.373	1.371 (0.030)	1.369 (0.003)
CZ - CE2	1.457	1.378	1.439	1.456 (0.029)	1.442 (0.003)
OH - CZ	1.260	1.371	1.280	1.249 (0.017)	1.272 (0.005)
CZ - CE1	1.460	1.400	1.446	1.461 (0.029)	1.449 (0.004)
CE1 - CD1	1.372	1.410	1.376	1.381 (0.029)	1.371 (0.003)
CD1 - CG2	1.436	1.398	1.432	1.458 (0.025)	1.432 (0.003)
CG2 - CB2	1.413	1.413	1.418	1.424 (0.022)	1.406 (0.005)
CB2 - CA2	1.390	1.380	1.379	1.404 (0.023)	1.384 (0.005)
CA2 - N2	1.405	1.457	1.398	1.404 (0.027)	1.401 (0.003)
N2 - C1	1.303	1.365	1.305	1.303 (0.028)	1.299 (0.002)
C1 - N3	1.392	1.351	1.400	1.405 (0.028)	1.406 (0.004)
N3 - C2	1.417	1.321	1.394	1.413 (0.025)	1.405 (0.006)
C2 - O2	1.243	1.238	1.238	1.244 (0.018)	1.246 (0.005)
C2 - CA2	1.458	1.503	1.456	1.471 (0.023)	1.437 (0.006)
Angles					
CD1 - CG2 - CB2	124.7	121.8	125.5	123.7 (3.0)	124.8 (0.9)
CD2 - CG2 - CB2	118.9	120.1	117.5	119.2 (3.1)	117.5 (0.7)
CG2 - CB2 - CA2	132.2	129.3	132.4	134.9 (2.3)	132.2 (1.4)
CB2 - CA2 - C2	122.7	121.2	122.7	122.4 (3.3)	120.3 (1.5)
CB2 - CA2 - N2	127.6	130.6	127.8	128.9 (3.2)	129.6 (1.5)
Dihedral angles					
CD2 CG2 CB2 CA2	180.0	179.8	176.3	177.2 (7.4)	177.0 (4.6)
CG2 CB2 CA2 C2	180.0	181.9	188.1	183.5 (5.4)	181.2 (3.8)

Table SI–IX: A selection of bond lengths, angles and dihedral angles in the eGFP chromophore. The vacuum calculation (vac) has been performed on the chromophore optimized in vacuum, where the imidazolidone ring has been methyl-terminated. The MD results are averages of 50 snapshots with standard deviations in parentheses.

	vac	CS Non-opt	CS QM/MM-opt	MD Non-opt	MD QM/MM-opt
Bond lengths					
CD2 - CG2	1.435	1.428	1.424	1.450 (0.032)	1.431 (0.003)
CE2 - CD2	1.372	1.396	1.369	1.372 (0.020)	1.369 (0.003)
CZ - CE2	1.457	1.391	1.440	1.454 (0.034)	1.442 (0.005)
OH - CZ	1.260	1.391	1.263	1.252 (0.017)	1.271 (0.004)
CZ - CE1	1.460	1.385	1.440	1.465 (0.031)	1.446 (0.003)
CE1 - CD1	1.372	1.432	1.366	1.374 (0.030)	1.370 (0.003)
CD1 - CG2	1.436	1.398	1.430	1.451 (0.024)	1.437 (0.003)
CG2 - CB2	1.413	1.466	1.405	1.426 (0.026)	1.410 (0.006)
CB2 - CA2	1.390	1.465	1.374	1.396 (0.028)	1.387 (0.005)
CA2 - N2	1.405	1.367	1.394	1.402 (0.025)	1.395 (0.005)
N2 - C1	1.303	1.379	1.298	1.307 (0.027)	1.295 (0.004)
C1 - N3	1.392	1.348	1.394	1.401 (0.024)	1.397 (0.006)
N3 - C2	1.417	1.341	1.387	1.418 (0.026)	1.403 (0.006)
C2 - O2	1.243	1.299	1.247	1.243 (0.022)	1.251 (0.005)
C2 - CA2	1.458	1.464	1.439	1.476 (0.029)	1.444 (0.006)
Angles					
CD1 - CG2 - CB2	124.7	124.9	123.2	123.1 (3.1)	125.1 (0.8)
CD2 - CG2 - CB2	118.9	113.2	118.7	119.9 (2.8)	117.8 (0.8)
CG2 - CB2 - CA2	132.2	128.1	130.0	134.7 (2.6)	130.5 (1.2)
CB2 - CA2 - C2	122.7	121.7	122.1	125.0 (2.9)	124.7 (1.7)
CB2 - CA2 - N2	127.6	127.9	128.4	126.5 (2.9)	125.8 (1.4)
Dihedral angles					
CD2 CG2 CB2 CA2	180.0	175.0	176.4	179.6 (6.1)	179.1 (4.8)
CG2 CB2 CA2 C2	180.0	185.5	184.3	185.7 (6.0)	182.2 (5.0)

Table SI–X: A selection of bond lengths, angles and dihedral angles in the YFP anionic chromophore. The vacuum calculation (vac) has been performed on the chromophore optimized in vacuum, where the imidazolidone ring has been methyl-terminated. The MD results are averages of 50 snapshots with standard deviations in parentheses.

	vac	CS Non-opt	CS QM/MM-opt	MD Non-opt	MD QM/MM-opt
Bond lengths					
CD2 - CG2	1.435	1.415	1.433	1.461 (0.026)	1.431 (0.003)
CE2 - CD2	1.372	1.351	1.365	1.368 (0.025)	1.367 (0.003)
CZ - CE2	1.457	1.439	1.450	1.462 (0.029)	1.444 (0.003)
OH - CZ	1.260	1.258	1.267	1.254 (0.018)	1.265 (0.004)
CZ - CE1	1.460	1.482	1.454	1.455 (0.027)	1.448 (0.005)
CE1 - CD1	1.372	1.372	1.365	1.374 (0.028)	1.368 (0.003)
CD1 - CG2	1.436	1.444	1.428	1.454 (0.031)	1.432 (0.003)
CG2 - CB2	1.413	1.353	1.395	1.426 (0.022)	1.402 (0.005)
CB2 - CA2	1.390	1.337	1.392	1.402 (0.026)	1.390 (0.004)
CA2 - N2	1.405	1.377	1.408	1.402 (0.028)	1.398 (0.004)
N2 - C1	1.303	1.340	1.310	1.311 (0.028)	1.305 (0.002)
C1 - N3	1.392	1.482	1.403	1.407 (0.029)	1.402 (0.004)
N3 - C2	1.417	1.405	1.412	1.417 (0.026)	1.409 (0.007)
C2 - O2	1.243	1.222	1.254	1.244 (0.019)	1.249 (0.004)
C2 - CA2	1.458	1.460	1.430	1.471 (0.026)	1.432 (0.006)
Angles					
CD1 - CG2 - CB2	124.7	119.9	124.8	123.7 (2.4)	124.5 (1.2)
CD2 - CG2 - CB2	118.9	121.5	117.9	119.4 (2.6)	117.9 (1.1)
CG2 - CB2 - CA2	132.2	134.2	135.6	135.5 (2.3)	132.5 (1.8)
CB2 - CA2 - C2	122.7	118.0	116.3	121.6 (2.9)	120.2 (1.8)
CB2 - CA2 - N2	127.6	131.1	133.2	130.0 (2.8)	130.1 (1.7)
Dihedral angles					
CD2 CG2 CB2 CA2	180.0	191.1	183.9	181.6 (5.5)	182.9 (3.6)
CG2 CB2 CA2 C2	180.0	173.2	184.8	188.0 (5.7)	188.2 (3.4)