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

Impacts of retinal polyene (de)methylation on the photoisomerization mechanism and photon energy storage of rhodopsin

Elżbieta Walczak and Tadeusz Andruniów*

Department of Chemistry, Wroclaw University of Technology,
Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland

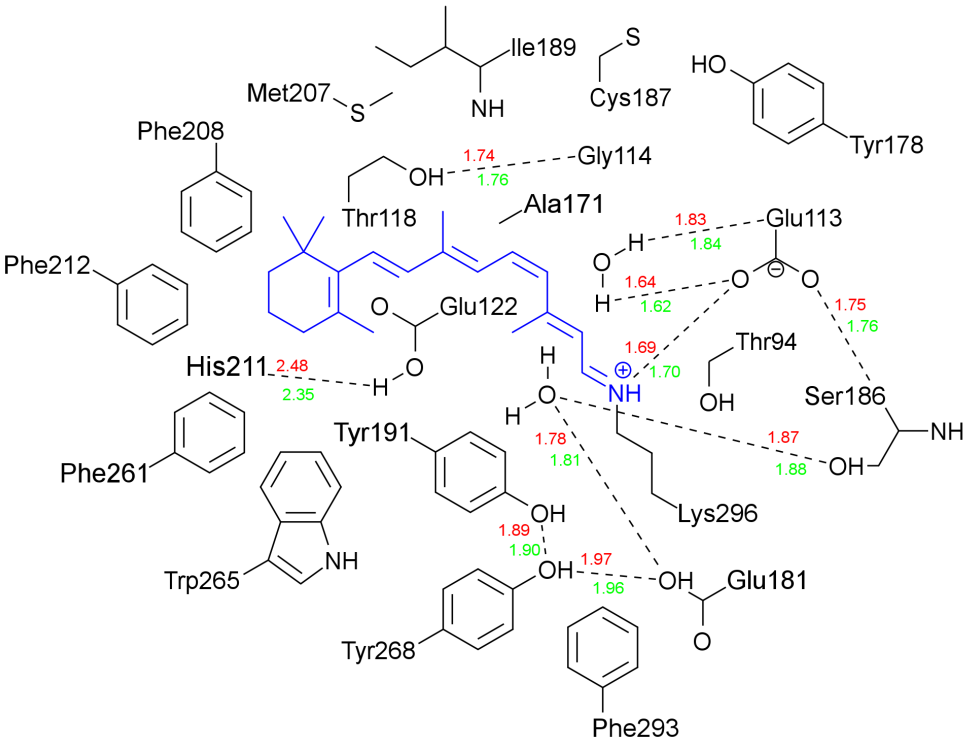

Figure S1: Hydrogen bond network surrounding native RPSB chromophore within opsin, resulting from CASSCF/MM relaxation. In red distances (in Å) for dark state Rh, in green for Batho.

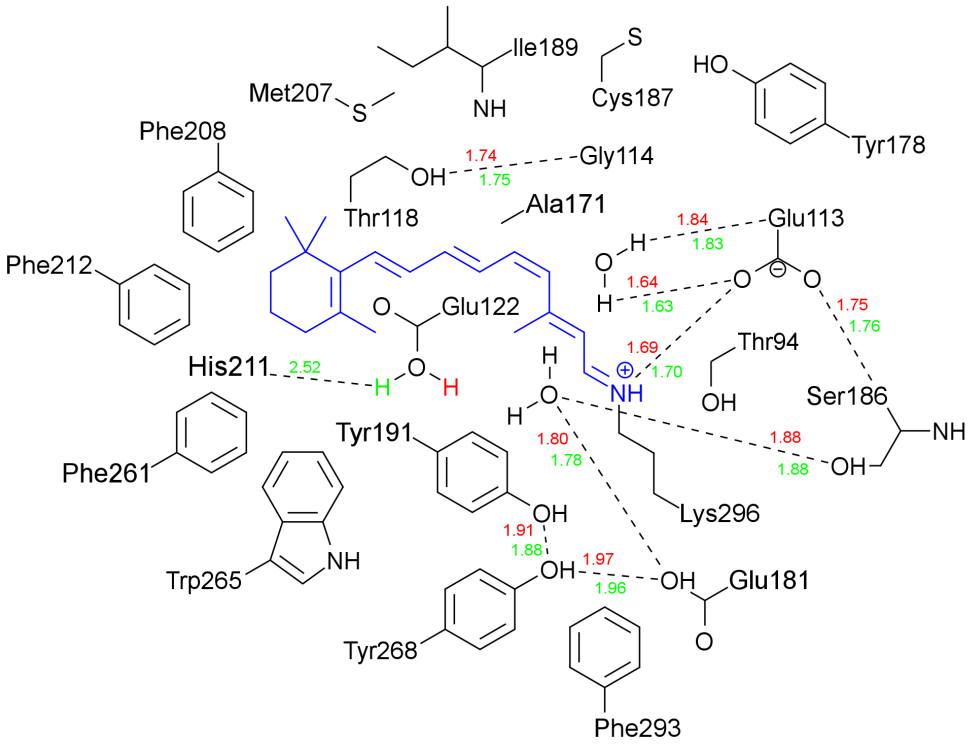

Figure S2: Hydrogen bond network surrounding 9-dm-RPSB chromophore within opsin, resulting from CASSCF/MM relaxation. In red distances (in Å) for dark state 9-dm-Rh, in green for 9-dm-Batho.

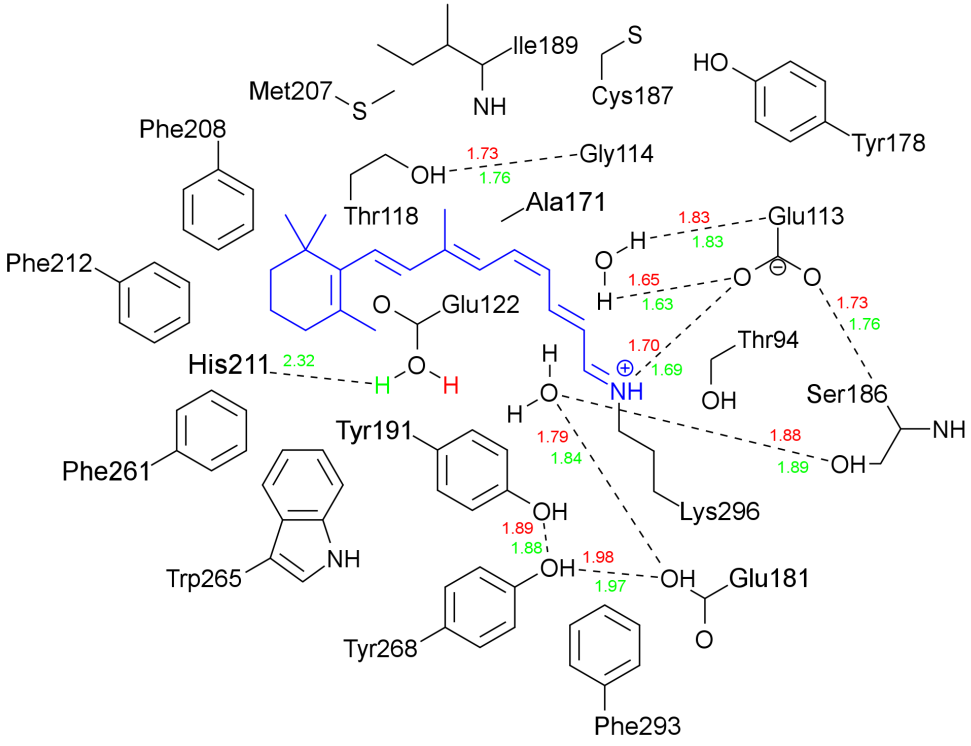

Figure S3: Hydrogen bond network surrounding 13-dm-RPSB chromophore within opsin, resulting from CASSCF/MM relaxation. In red distances (in Å) for dark state 13-dm-Rh, in green for 13-dm-Batho.

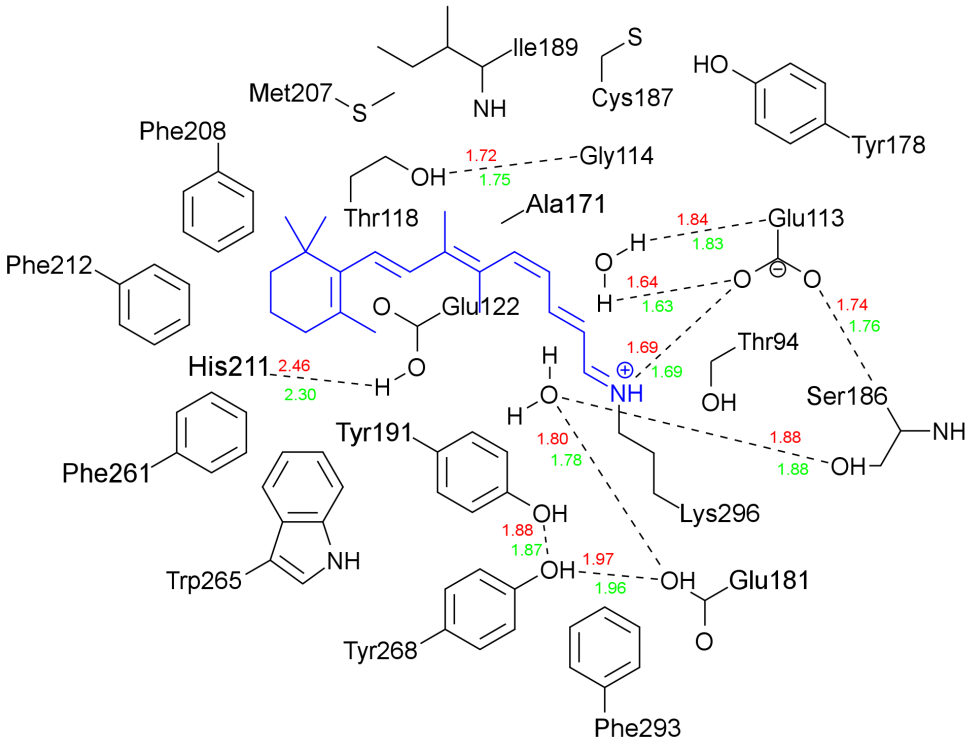

Figure S4: Hydrogen bond network surrounding 10-m-13-dm-RPSB chromophore within opsin, resulting from CASSCF/MM relaxation. In red distances (in Å) for dark state 10-m-13-dm-Rh, in green for 10-m-13-dm-Batho.

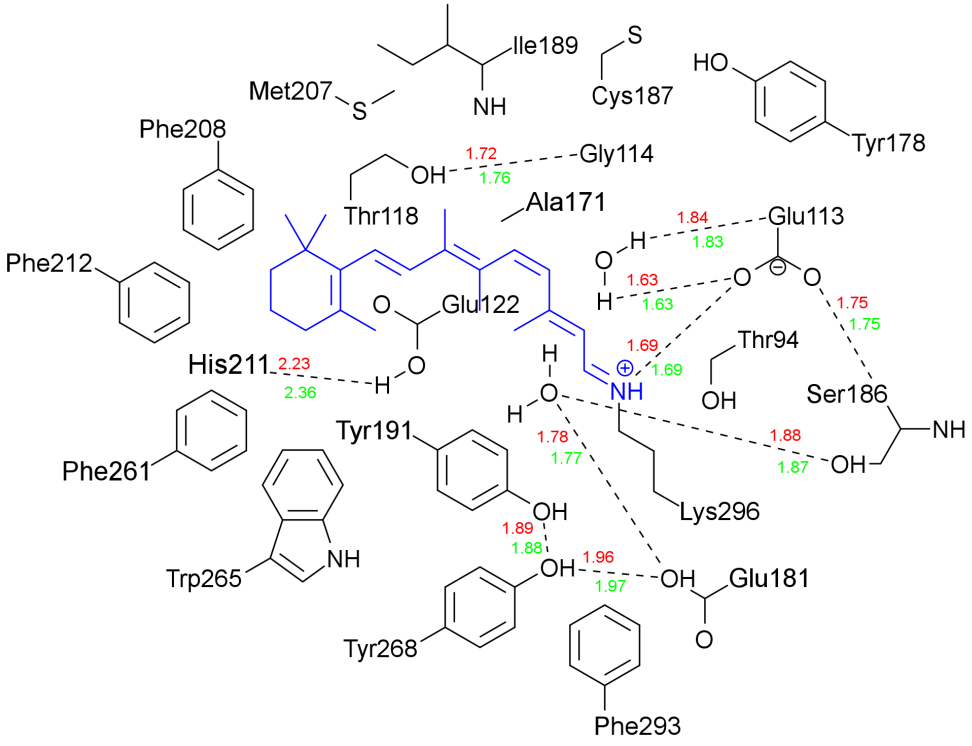

Figure S5: Hydrogen bond network surrounding 10-m-RPSB chromophore within opsin, resulting from CASSCF/MM relaxation. In red distances (in Å) for dark state 10-m-Rh, in green for 10-m-Batho.

Table S1: Bond lengths [Å] and BLAs calulated for various RPSB chromophores within rhodopsin at CASSCF/Amber94 level of theory. Positions of side chains of all residues within 4 Å of any QM atom were relaxed during geometry optimization.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
C5-C6	1.354	1.357	1.355	1.355	1.354
C6-C7	1.492	1.495	1.492	1.492	1.493
C7-C8	1.350	1.352	1.352	1.350	1.350
C8-C9	1.472	1.460	1.471	1.480	1.479
C9-C10	1.362	1.357	1.363	1.362	1.360
C10-C11	1.459	1.456	1.455	1.481	1.485
C11-C12	1.362	1.362	1.361	1.357	1.355
C12-C13	1.469	1.472	1.452	1.458	1.476
C13-C14	1.370	1.369	1.363	1.362	1.367
C14-C15	1.442	1.445	1.442	1.445	1.445
C15-N16	1.288	1.288	1.289	1.289	1.288
BLA	0.119	0.118	0.115	0.126	0.130
			S1 LE		
C5-C6		1.366	1.363	1.363	1.360
C6-C7		1.477	1.467	1.470	1.479
C7-C8		1.376	1.432	1.376	1.377
C8-C9		1.416	1.384	1.428	1.429
C9-C10		1.407	1.446	1.418	1.418
C10-C11		1.393	1.389	1.444	1.455
C11-C12		1.442	1.435	1.399	1.382
C12-C13		1.399	1.379	1.417	1.431
C13-C14		1.447	1.443	1.419	1.416
C14-C15		1.382	1.401	1.388	1.388
C15-N16		1.349	1.311	1.347	1.347
BLA		0.016	-0.001	0.042	0.055
			S1 CT		
C5-C6	1.361	1.369	1.367	1.370	1.365
C6-C7	1.465	1.454	1.455	1.454	1.462
C7-C8	1.389	1.394	1.395	1.398	1.384
C8-C9	1.401	1.386	1.397	1.397	1.410
C9-C10	1.459	1.441	1.465	1.482	1.461
C10-C11	1.356	1.354	1.355	1.358	1.364
C11-C12	1.487	1.488	1.486	1.488	1.484
C12-C13	1.362	1.359	1.358	1.359	1.364
C13-C14	1.476	1.481	1.462	1.449	1.456
C14-C15	1.375	1.373	1.377	1.382	1.378
C15-N16	1.346	1.349	1.347	1.337	1.338
BLA	-0.028	-0.035	-0.032	-0.031	-0.019
			CI		
C5-C6	1.364	1.371	1.366	1.363	1.363
C6-C7	1.461	1.451	1.458	1.467	1.466
C7-C8	1.380	1.388	1.383	1.377	1.377
C8-C9	1.413	1.397	1.408	1.419	1.418
C9-C10	1.437	1.421	1.441	1.447	1.447
C10-C11	1.362	1.363	1.361	1.371	1.370
C11-C12	1.475	1.474	1.474	1.478	1.477
C12-C13	1.365	1.367	1.360	1.358	1.369
C13-C14	1.447	1.446	1.437	1.440	1.443
C14-C15	1.377	1.378	1.373	1.368	1.380
C15-N16	1.337	1.336	1.339	1.343	1.333
BLA	-0.011	-0.015	-0.015	-0.011	-0.006
			batho		
C5-C6	1.355	1.357 (1.351)[a]	1.355	1.355	1.355
C6-C7	1.490	1.484 (1.489)	1.489	1.494	1.493
C7-C8	1.349	1.352 (1.351)	1.349	1.349	1.349
C8-C9	1.469	1.458 (1.457)	1.469	1.477	1.475
C9-C10	1.358	1.355 (1.357)	1.357	1.362	1.368
C10-C11	1.459	1.450 (1.456)	1.464	1.486	1.470
C11-C12	1.353	1.357 (1.356)	1.353	1.357	1.361
C12-C13	1.474	1.461 (1.466)	1.454	1.453	1.464
C13-C14	1.362	1.366 (1.369)	1.358	1.360	1.368
C14-C15	1.443	1.440 (1.439)	1.440	1.443	1.441
C15-N16	1.286	1.287 (1.287)	1.286	1.286	1.288
BLA	0.123	0.113 (0.116)	0.120	0.126	0.121

[a] In parantheses there are data for *9-dm-Batho* in which the twisting around the chromophore's C6–C7 bond is +79°

Table S2: Dihedral angles [in degrees] calulated for various RPSB chromophores within rhodopsin at CASSCF/Amber94 level of theory. Positions of side chains of all residues within 4 Å of any QM atom were relaxed during geometry optimization.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
5-6-7-8	-55.3	-52.0	-51.0	-51.4	-51.7
6-7-8-9	178.8	-176.5	177.7	179.6	179.2
7-8-9-10	-175.0	175.0	-174.2	-159.2	-158.5
8-9-10-11	173.9	-179.3	171.1	177.7	177.3
9-10-11-12	169.2	176.8	173.6	149.0	141.2
10-11-12-13	-5.1	-1.9	-5.8	-8.6	-9.8
11-12-13-14	168.0	161.8	175.2	168.8	158.1
12-13-14-15	-179.1	-176.0	179.7	178.9	-178.9
13-14-15-16	166.2	166.4	168.6	169.6	170.3
H-11-12-H	-1.9	-2.5	-0.9	-3.1	-3.7
			S1 LE		
5-6-7-8		-51.1	-49.8	-48.4	-50.2
6-7-8-9		-176.1	179.0	179.8	179.4
7-8-9-10		173.9	-176.2	-163.0	-160.8
8-9-10-11		-179.1	169.9	177.9	177.8
9-10-11-12		177.8	174.3	151.3	143.2
10-11-12-13		-5.4	-7.9	-12.8	-14.3
11-12-13-14		163.7	174.6	170.7	159.7
12-13-14-15		-172.6	-178.2	-177.8	-176.2
13-14-15-16		167.1	170.4	169.5	170.9
H-11-12-H		-3.5	-2.2	-6.1	-5.3
			S1 CT		
5-6-7-8	-51.1	-43.3	-45.3	-38.5	-38.9
6-7-8-9	179.6	-174.1	-179.0	-179.6	-178.3
7-8-9-10	179.2	169.0	176.9	-176.7	-177.3
8-9-10-11	176.7	-172.9	172.3	-169.1	-164.8
9-10-11-12	179.8	178.9	-179.0	168.1	167.9
10-11-12-13	-28.3	-25.8	-20.3	-40.8	-65.7
11-12-13-14	178.4	173.2	179.6	-175.9	179.6
12-13-14-15	-169.3	-166.8	-170.0	-174.1	-163.7
13-14-15-16	173.3	169.5	174.3	173.1	172.9
H-11-12-H	-21.5	-22.5	-13.3	-37.8	-66.7
			CI		
5-6-7-8	-38.4	-27.1	-36.0	-40.9	-39.6
6-7-8-9	-173.1	-168.0	-173.6	-176.2	-176.1
7-8-9-10	175.4	174.2	177.2	-175.3	-177.1
8-9-10-11	-156.8	-159.0	-153.3	-152.1	-150.4
9-10-11-12	-177.6	178.3	-175.7	-179.2	177.6
10-11-12-13	-86.8	-85.7	-87.1	-85.0	-82.2
11-12-13-14	-178.5	179.7	-179.4	179.5	177.3
12-13-14-15	-165.3	-163.2	-166.7	-167.4	-171.0
13-14-15-16	178.2	173.3	177.7	175.2	173.7
H-11-12-H	-83.7	-86.3	-86.6	-86.6	-85.4
			batho		
5-6-7-8	-45.7	-30.7 (79.3)[a]	-42.1	-46.9	-48.7
6-7-8-9	-166.4	-162.0 (-173.5)	-164.8	-166.4	-167.6
7-8-9-10	178.8	-175.8 (167.0)	177.8	-170.0	-168.0
8-9-10-11	-153.6	-150.7 (-166.6)	-152.6	-145.4	-147.1
9-10-11-12	-172.5	174.4 (163.7)	-160.1	-151.1	-172.4
10-11-12-13	-152.0	-151.5 (-163.7)	-150.7	-142.9	-145.0
11-12-13-14	-152.2	-171.4 (171.0)	-168.1	176.1	-171.8
12-13-14-15	-154.0	-156.1 (-166.0)	-157.6	-160.4	-155.3
13-14-15-16	175.8	174.6 (173.0)	169.2	165.3	175.4
H-11-12-H	-175.2	-174.3 (-178.0)	-174.5	-175.7	-172.9

[a] In parantheses there are data for *9-dm-Batho* in which the twisting around the chromophore's C6–C7 bond is +79°

Table S3: Bond lengths [Å] and BLAs calulated for various RPSB chromophores within rhodopsin at B3LYP/Amber94 level of theory. Positions of side chains of all residues within 4 Å of any QM atom were relaxed during geometry optimization.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
C5-C6	1.367	1.366	1.369	1.371	1.369
C6-C7	1.459	1.465	1.455	1.455	1.461
C7-C8	1.367	1.366	1.371	1.373	1.369
C8-C9	1.435	1.430	1.433	1.438	1.437
C9-C10	1.389	1.376	1.393	1.404	1.398
C10-C11	1.412	1.419	1.408	1.423	1.427
C11-C12	1.391	1.388	1.393	1.395	1.390
C12-C13	1.421	1.426	1.405	1.406	1.425
C13-C14	1.405	1.406	1.396	1.399	1.406
C14-C15	1.400	1.405	1.402	1.401	1.401
C15-N16	1.326	1.323	1.326	1.329	1.326
BLA	0.051	0.058	0.046	0.046	0.054
			batho		
C5-C6	1.374	1.365	1.374	1.373	1.372
C6-C7	1.446	1.451	1.447	1.451	1.448
C7-C8	1.377	1.370	1.379	1.377	1.376
C8-C9	1.428	1.421	1.426	1.427	1.427
C9-C10	1.399	1.379	1.401	1.413	1.413
C10-C11	1.403	1.413	1.403	1.410	1.408
C11-C12	1.393	1.383	1.397	1.404	1.405
C12-C13	1.407	1.416	1.398	1.396	1.404
C13-C14	1.408	1.403	1.398	1.403	1.411
C14-C15	1.393	1.397	1.394	1.393	1.392
C15-N16	1.330	1.324	1.329	1.330	1.330
BLA	0.035	0.049	0.034	0.032	0.031

Table S4: Dihedral angles [in degrees] calulated for various RPSB chromophores within rhodopsin at B3LYP/Amber94 level of theory. Positions of side chains of all residues within 4 Å of any QM atom were relaxed during geometry optimization.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
5-6-7-8	-41.9	-44.0	-39.1	-34.4	-34.7
6-7-8-9	169.5	-176.3	167.0	178.5	172.9
7-8-9-10	176.7	168.4	177.0	-178.9	-176.3
8-9-10-11	168.9	-179.4	166.5	175.4	178.6
9-10-11-12	176.3	175.9	178.2	165.0	158.6
10-11-12-13	-15.2	-9.1	-14.2	-23.5	-28.1
11-12-13-14	174.6	169.7	-177.9	177.9	171.6
12-13-14-15	179.4	-172.1	-179.0	179.3	180.0
13-14-15-16	170.0	164.3	169.1	169.4	169.5
H-11-12-H	-9.3	-5.6	-7.0	-14.2	-18.2
			batho		
5-6-7-8	-12.3	43.6	-16.9	-28.9	-29.5
6-7-8-9	-165.2	-168.6	-165.0	-173.3	-172.7
7-8-9-10	164.9	173.9	167.9	-178.9	-178.0
8-9-10-11	-143.8	-158.3	-141.7	-143.9	-143.2
9-10-11-12	-179.4	165.6	-173.8	-171.6	-175.1
10-11-12-13	-152.3	-158.2	-146.4	-141.0	-137.8
11-12-13-14	-170.9	174.3	-178.4	-178.5	-175.1
12-13-14-15	-159.7	-159.4	-164.4	-158.8	-158.1
13-14-15-16	-176.8	175.9	176.5	176.5	179.3
H-11-12-H	-162.4	-173.0	-162.9	-157.4	-154.7

Table S5: Bond lengths [Å] and BLAs calulated for various RPSB chromophores within rhodopsin at CASSCF/Amber94 level of theory. Protein cavity was frozen during geometry optimization procedure.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
C5-C6	1.355	1.354	1.355	1.356	1.356
C6-C7	1.494	1.494	1.493	1.495	1.496
C7-C8	1.351	1.351	1.352	1.351	1.351
C8-C9	1.474	1.460	1.473	1.483	1.481
C9-C10	1.362	1.357	1.364	1.363	1.362
C10-C11	1.461	1.455	1.456	1.481	1.486
C11-C12	1.363	1.363	1.362	1.358	1.357
C12-C13	1.469	1.469	1.449	1.457	1.476
C13-C14	1.370	1.370	1.365	1.363	1.369
C14-C15	1.440	1.442	1.442	1.444	1.444
C15-N16	1.290	1.289	1.290	1.289	1.289
BLA	0.119	0.117	0.115	0.125	0.129
			S1 CT		
C5-C6	1.363	1.370	1.367	1.370	1.366
C6-C7	1.466	1.453	1.458	1.455	1.466
C7-C8	1.388	1.398	1.393	1.394	1.383
C8-C9	1.402	1.385	1.399	1.399	1.413
C9-C10	1.458	1.445	1.463	1.478	1.460
C10-C11	1.356	1.355	1.355	1.360	1.364
C11-C12	1.488	1.488	1.486	1.487	1.484
C12-C13	1.361	1.362	1.357	1.357	1.364
C13-C14	1.477	1.475	1.462	1.456	1.456
C14-C15	1.375	1.376	1.376	1.379	1.377
C15-N16	1.347	1.348	1.348	1.342	1.338
BLA	-0.028	-0.034	-0.031	-0.031	-0.017
			CI		
C5-C6	1.365	1.371	1.368	1.364	1.364
C6-C7	1.465	1.453	1.460	1.471	1.470
C7-C8	1.379	1.387	1.383	1.378	1.376
C8-C9	1.415	1.398	1.412	1.422	1.420
C9-C10	1.437	1.421	1.443	1.449	1.447
C10-C11	1.363	1.365	1.363	1.370	1.371
C11-C12	1.475	1.475	1.477	1.480	1.480
C12-C13	1.366	1.363	1.359	1.361	1.365
C13-C14	1.448	1.452	1.442	1.439	1.451
C14-C15	1.374	1.372	1.372	1.379	1.374
C15-N16	1.340	1.344	1.343	1.334	1.342
BLA	-0.011	-0.018	-0.016	-0.006	-0.010
			batho		
C5-C6	1.357	1.358	1.357	1.357	1.357
C6-C7	1.493	1.489	1.493	1.498	1.496
C7-C8	1.349	1.351	1.350	1.350	1.351
C8-C9	1.469	1.459	1.471	1.480	1.475
C9-C10	1.358	1.355	1.357	1.361	1.370
C10-C11	1.457	1.452	1.470	1.489	1.470
C11-C12	1.351	1.356	1.354	1.359	1.362
C12-C13	1.477	1.466	1.453	1.452	1.463
C13-C14	1.361	1.366	1.361	1.362	1.369
C14-C15	1.443	1.439	1.440	1.442	1.440
C15-N16	1.287	1.288	1.287	1.287	1.290
BLA	0.124	0.116	0.121	0.126	0.119

Table S6: Dihedral angles [in degrees] calulated for various RPSB chromophores within rhodopsin at CASSCF/Amber94 level of theory. Protein cavity was frozen during geometry optimization procedure.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
5-6-7-8	-54.3	-56.4	-53.7	-51.0	-48.6
6-7-8-9	179.4	-178.3	178.9	-179.8	-177.9
7-8-9-10	-174.1	175.9	-173.8	-159.5	-162.5
8-9-10-11	173.3	-179.8	171.7	176.9	177.8
9-10-11-12	168.8	176.8	175.2	151.0	143.8
10-11-12-13	-5.9	-2.5	-7.7	-11.0	-11.0
11-12-13-14	167.6	164.1	176.8	170.8	158.5
12-13-14-15	179.8	-175.9	-178.6	179.9	-179.7
13-14-15-16	169.7	166.6	170.2	168.9	172.6
H-11-12-H	-2.5	-2.2	-0.2	-3.4	-4.8
			S1 CT		
5-6-7-8	-50.1	-43.0	-46.0	-37.4	-38.5
6-7-8-9	179.5	-174.2	-179.1	179.8	-178.8
7-8-9-10	178.9	169.7	177.4	-176.4	-176.4
8-9-10-11	177.1	-171.1	172.1	-171.3	-164.4
9-10-11-12	179.9	178.5	-178.6	164.8	167.7
10-11-12-13	-29.4	-29.7	-19.6	-32.2	-66.0
11-12-13-14	178.2	172.6	179.3	-176.2	179.4
12-13-14-15	-169.4	-166.3	-170.7	-175.4	-163.2
13-14-15-16	174.1	170.9	175.0	171.7	173.5
H-11-12-H	-22.7	-27.2	-12.5	-29.0	-67.0
			CI		
5-6-7-8	-37.7	-26.5	-33.5	-40.2	-40.1
6-7-8-9	-172.6	-168.5	-172.9	-176.3	-176.5
7-8-9-10	175.9	175.6	178.1	-175.3	-176.9
8-9-10-11	-157.8	-159.2	-153.7	-154.4	-152.4
9-10-11-12	-175.7	179.0	-175.8	-179.4	179.1
10-11-12-13	-89.4	-86.3	-88.4	-81.3	-83.5
11-12-13-14	-176.9	178.2	-177.3	179.7	178.4
12-13-14-15	-165.6	-162.9	-165.8	-168.0	-171.0
13-14-15-16	179.6	176.3	177.4	175.0	175.0
H-11-12-H	-84.5	-86.5	-87.0	-80.7	-85.8
			batho		
5-6-7-8	-44.0	-36.1	-40.2	-46.1	-45.6
6-7-8-9	-165.5	-160.7	-162.1	-165.1	-165.3
7-8-9-10	-179.9	-173.9	177.6	-168.5	-170.5
8-9-10-11	-155.3	-150.7	-152.4	-145.4	-145.7
9-10-11-12	-176.0	178.4	-154.8	-150.8	-172.4
10-11-12-13	-154.9	-152.1	-149.2	-139.7	-143.9
11-12-13-14	-143.2	-164.3	-172.8	168.3	-171.8
12-13-14-15	-154.0	-155.0	-156.4	-157.9	-155.9
13-14-15-16	177.8	176.3	174.2	171.0	175.7
H-11-12-H	-176.5	-173.7	-174.1	-176.7	-171.9

Table S7: Bond lengths [Å] and BLAs calulated for various RPSB chromophores within rhodopsin at B3LYP/Amber94 level of theory. Protein cavity was frozen during geometry optimization procedure.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
C5-C6	1.366	1.365	1.368	1.368	1.368
C6-C7	1.465	1.465	1.462	1.464	1.465
C7-C8	1.366	1.368	1.369	1.369	1.369
C8-C9	1.441	1.430	1.442	1.442	1.441
C9-C10	1.390	1.378	1.392	1.403	1.400
C10-C11	1.416	1.419	1.413	1.425	1.428
C11-C12	1.392	1.390	1.394	1.393	1.392
C12-C13	1.422	1.425	1.406	1.410	1.423
C13-C14	1.408	1.408	1.396	1.396	1.408
C14-C15	1.401	1.402	1.402	1.403	1.400
C15-N16	1.327	1.327	1.327	1.327	1.329
BLA	0.054	0.056	0.050	0.053	0.053
			batho		
C5-C6	1.374	1.367	1.371	1.369	1.370
C6-C7	1.454	1.451	1.455	1.459	1.457
C7-C8	1.376	1.373	1.373	1.370	1.372
C8-C9	1.431	1.423	1.432	1.436	1.432
C9-C10	1.401	1.379	1.398	1.405	1.409
C10-C11	1.404	1.416	1.404	1.417	1.410
C11-C12	1.395	1.380	1.394	1.398	1.402
C12-C13	1.409	1.419	1.398	1.399	1.405
C13-C14	1.409	1.402	1.399	1.399	1.411
C14-C15	1.393	1.396	1.395	1.396	1.392
C15-N16	1.331	1.323	1.329	1.329	1.332
BLA	0.037	0.050	0.039	0.043	0.036

Table S8: Dihedral angles [in degrees] calulated for various RPSB chromophores within rhodopsin at B3LYP/Amber94 level of theory. Protein cavity was frozen during geometry optimization procedure.

	Rh	*9-dm-Rh*	*13-dm-Rh*	*10-m-13-dm-Rh*	*10-m-Rh*
			FC		
5-6-7-8	-42.7	-44.8	-41.2	-37.9	-37.1
6-7-8-9	174.5	-176.0	173.1	177.6	-178.2
7-8-9-10	176.3	169.1	175.5	-179.6	-178.9
8-9-10-11	171.8	-179.5	170.4	179.2	-177.8
9-10-11-12	175.5	175.9	176.9	167.6	160.8
10-11-12-13	-12.7	-10.0	-11.0	-20.3	-27.9
11-12-13-14	174.0	168.4	178.8	176.4	169.1
12-13-14-15	-177.5	-173.7	-178.5	-179.8	-178.6
13-14-15-16	168.1	166.5	170.6	170.1	170.4
H-11-12-H	-6.8	-6.6	-4.4	-12.1	-18.1
			batho		
5-6-7-8	-13.3	45.8	-25.2	-35.7	-31.3
6-7-8-9	-156.8	-155.8	-161.1	-169.1	-165.0
7-8-9-10	163.8	173.1	170.5	-176.4	-179.5
8-9-10-11	-138.4	-159.0	-142.6	-146.7	-140.6
9-10-11-12	-177.2	171.4	-174.0	-171.6	-174.8
10-11-12-13	-149.8	-161.7	-150.6	-138.2	-137.9
11-12-13-14	-167.1	-175.5	-173.5	176.8	-174.4
12-13-14-15	-161.2	-172.0	-159.0	-155.7	-158.9
13-14-15-16	-177.8	173.5	178.8	172.8	-178.8
H-11-12-H	-159.5	-171.5	-162.8	-160.4	-155.2

Table S9: MS-CASPT/MM absorption λ_{max} and oscillator strengths for $S_0 \rightarrow S_1$ vertical excitations, as well as photon energy storage, calculated at CASSCF/MM and B3LYP/MM ground-state equilibrium geometries optimized within protein environment in which side chains of all residues within 4 Å of any QM atom were relaxed. In paranethesis there are MS-CASPT2/MM values for geometries obtained from geometry optimization within frozen protein matrix. CASSCF wave function was obtained from state-average calculations over two roots with equal weights.

Model	λ_{max} [nm]		f[a]		storage [kcal/mol]	
	CASSCF/MM geometry					
Rh	469	(467)	0.608	(0.643)	-	-
Batho	480	(491)	0.521	(0.454)	20.7	(20.3)
9-dm-Rh	437	(458)	0.402	(0.656)	-	-
9-dm-Batho[b]	462	(485)	0.318	(0.543)	21.2	(20.3)
9-dm-Batho[c]	429	-	0.843	-	11.4	-
13-dm-Rh	456	(472)	0.452	(0.663)	-	-
13-dm-Batho	480	(487)	0.471	(0.569)	23.9	(21.1)
10-m-13-dm-Rh	480	(482)	0.423	(0.447)	-	-
10-m-13-dm-Batho	493	(491)	0.366	(0.369)	18.2	(17.5)
10-m-Rh	485	(492)	0.332	(0.345)	-	-
10-m-Batho	490	(503)	0.591	(0.636)	11.6	(9.2)
	B3LYP/MM geometry					
Rh	485	(481)	1.391	(1.417)	-	-
Batho	521	(522)	1.760	(1.720)	16.8	(20.3)
9-dm-Rh	456	(469)	1.284	(1.376)	-	-
9-dm-Batho	466	(463)	1.647	(1.634)	10.1	(20.0)
13-dm-Rh	491	(483)	1.467	(1.403)	-	-
13-dm-Batho	519	(513)	1.718	(1.729)	15.6	(20.2)
10-m-13-dm-Rh	509	(488)	1.443	(1.370)	-	-
10-m-13-dm-Batho	525	(505)	1.668	(1.656)	12.7	(16.3)
10-m-Rh	508	(513)	1.211	(1.285)	-	-
10-m-Batho	534	(522)	1.654	(1.623)	11.4	(14.3)

[a] Oscillator strengths calculated using SA-CASSCF wavefunction and MS-CASPT2 vertical excitation energies

[b] The structure in which the twisting around the chromophore's C6–C7 bond is -31° in relaxed and -36° in frozen protein environment

[c] The structure in which the twisting around the chromophore's C6–C7 bond is +79°

Table S10: CASPT2/MM absorption λ_{max} (in paranthesis MS-CASPT2/MM), oscillator strengths, and changes in dipole moment for $S_0 \rightarrow S_1$ vertical excitations, as well as photon energy storage, calculated at CASSCF/MM and B3LYP/MM ground-state equilibrium geometries obtained from calculations in which positions of side chains of all residues within 4 Å of any QM atom were relaxed. CASSCF wave function was obtained from state-average calculations over three roots with equal weights.

Model	λ_{max} [nm]		f	storage [kcal/mol]	$\Delta\mu$ [Debye]
	CASSCF/MM geometry				
Rh	501	(480)	0.537	-	11.1
Batho	506	(486)	0.462	20.6	13.2
9-dm-Rh	465	(473)	0.457	-	8.5
9-dm-Batho[a]	519	(515)	0.471	21.1	10.1
9-dm-Batho[b]	457	(442)	0.666	12.2	9.7
13-dm-Rh	487	(487)	0.472	-	9.4
13-dm-Batho	508	(500)	0.443	24.2	11.7
10-m-13-dm-Rh	500	(480)	0.413	-	13.2
10-m-13-dm-Batho	512	(510)	0.357	18.1	12.0
10-m-Rh	493	(474)	0.364	-	13.5
10-m-Batho	528	(507)	0.524	12.6	10.9
	B3LYP/MM geometry				
Rh	537	(489)	1.037	-	13.7
Batho	609	(500)	1.187	18.2	14.5
9-dm-Rh	490	(470)	0.966	-	12.1
9-dm-Batho	520	(450)	1.278	10.9	14.4
13-dm-Rh	543	(477)	1.128	-	15.0
13-dm-Batho	618	(515)	1.094	17.4	13.8
10-m-13-dm-Rh	562	(493)	1.138	-	15.0
10-m-13-dm-Batho	638	(530)	1.040	15.1	13.1
10-m-Rh	558	(525)	0.879	-	13.0
10-m-Batho	654	(536)	1.027	13.7	12.4

[a] The structure in which the twisting of the chromophore's C6–C7 bond is -31°

[b] The structure in which the twisting of the chromophore's C6–C7 bond is +79°

Table S11: CASPT2/MM absorption λ_{max} (in paranthesis MS-CASPT2/MM), oscillator strengths, and changes in dipole moment for $S_0 \rightarrow S_1$ vertical excitations, as well as photon energy storage, calculated at CASSCF/MM and B3LYP/MM ground-state equilibrium geometries obtained from calculations in which protein matrix was frozen. CASSCF wave function was obtained from state-average calculations over three roots with equal weights.

Model	λ_{max} [nm]		f	storage [kcal/mol]	$\Delta\mu$ [Debye]
	CASSCF/MM geometry				
Rh	499	(477)	0.562	-	11.7
Batho	509	(486)	0.422	19.6	14.8
9-dm-Rh	488	(470)	0.569	-	11.0
9-dm-Batho[a]	519	(505)	0.484	20.4	11.6
13-dm-Rh	505	(480)	0.572	-	11.7
13-dm-Batho	516	(484)	0.510	21.0	14.5
10-m-13-dm-Rh	499	(482)	0.429	-	12.8
10-m-13-dm-Batho	507	(495)	0.357	17.6	13.8
10-m-Rh	499	(480)	0.384	-	14.0
10-m-Batho	545	(514)	0.546	10.3	11.4
	B3LYP/MM geometry				
Rh	523	(475)	1.115	-	13.7
Batho	607	(508)	1.170	22.0	13.1
9-dm-Rh	508	(469)	1.093	-	13.4
9-dm-Batho	511	(447)	1.299	20.6	14.5
13-dm-Rh	526	(474)	1.094	-	14.6
13-dm-Batho	588	(496)	1.201	21.5	13.8
10-m-13-dm-Rh	536	(487)	1.065	-	13.9
10-m-13-dm-Batho	575	(485)	1.178	17.4	14.4
10-m-Rh	567	(506)	0.991	-	13.3
10-m-Batho	622	(543)	1.040	16.2	11.1

[a] The structure in which the twisting of the chromophore's C6–C7 bond is -31°

Table S12: CASPT2//CASSCF/MM relative energies [kcal/mol] of the structures discussed in the text with respect to the S0 state energy of the dark state structure of each rhodopsin analogue. CASSCF wave function was obtained from state-average calculations over three roots with equal weights. Geometries were obtained from calculations in which positions of side chains of all residues within 4 Å of any QM atom were relaxed.

Model	state	GS	S1 (LE)	S1 (CT)	CI	batho
		[kcal/mol]				
		CASPT2				
Rh	S0	0.0		12.7	36.9	20.6
	S1	57.1		45.5	40.5	77.2
	S2	82.8		61.0	88.7	106.6
9-dm-Rh	S0	0.0	4.0	15.1	40.3	21.1 (12.2)[a]
	S1	61.6	53.4	48.4	45.4	76.3 (74.7)
	S2	81.8	59.9	64.1	92.2	94.0 (97.4)
13-dm-Rh	S0	0.0	3.8	13.6	40.3	24.2
	S1	58.7	50.1	47.0	43.7	80.5
	S2	80.6	55.3	65.1	89.9	107.0
10-m-13-dm-Rh	S0	0.0	1.3	14.5	31.8	18.1
	S1	57.2	44.9	40.8	36.8	74.0
	S2	86.4	66.8	70.3	84.4	104.5
10-m-Rh	S0	0.0	2.1	23.3	23.8	12.6
	S1	58.0	45.4	31.6	29.0	67.2
	S2	82.8	70.4	75.1	72.0	90.9
		MS-CASPT2				
Rh	S0	0.0		5.6	39.2	20.7
	S1	59.6		52.8	44.6	79.6
	S2	91.6		72.2	91.9	114.5
9-dm-Rh	S0	0.0	0.5	7.8	42.5	19.3 (11.6)[a]
	S1	60.5	53.7	54.9	49.5	74.8 (76.3)
	S2	94.0	73.4	75.9	95.4	108.3 (107.4)
13-dm-Rh	S0	0.0	-0.5	7.2	43.8	23.8
	S1	58.7	51.5	55.3	47.4	81.1
	S2	92.1	68.9	74.7	93.5	118.4
10-m-13-dm-Rh	S0	0.0	-0.7	6.5	34.0	17.1
	S1	59.6	50.5	50.2	39.0	73.2
	S2	91.2	70.4	75.7	86.7	113.5
10-m-Rh	S0	0.0	0.1	13.1	24.8	9.7
	S1	60.3	48.7	43.2	31.1	66.4
	S2	85.3	73.9	78.1	73.7	99.5

[a] In parantheses there are data for *9-dm-Batho* in which the twisting around the chromophore's C6–C7 bond is +79°

Table S13: CASPT2//CASSCF/MM relative energies [kcal/mol] of the structures discussed in the text with respect to the S0 state energy of the dark state structure of each rhodopsin analogue. CASSCF wave function was obtained from state-average calculations over three roots with equal weights. Geometries were obtained from calculations in which protein cavity was frozen.

Model	state	GS	S1 (CT)	CI	batho
		[kcal/mol]			
Rh	S0	0.0	11.8	36.3	19.6
	S1	57.3	44.8	39.1	75.9
	S2	82.6	68.0	87.6	108.2
9-dm-Rh	S0	0.0	13.5	37.6	20.4
	S1	58.6	45.2	41.1	75.5
	S2	82.8	68.3	87.8	98.0
13-dm-Rh	S0	0.0	10.4	38.0	21.0
	S1	56.7	44.5	42.3	76.5
	S2	82.0	60.7	90.0	105.5
10-m-13-dm-Rh	S0	0.0	11.3	30.6	17.6
	S1	57.3	41.7	31.1	74.1
	S2	84.3	66.3	74.8	106.3
10-m-Rh	S0	0.0	20.8	25.4	10.3
	S1	57.4	29.5	28.5	63.2
	S2	78.1	68.6	78.4	86.8

Table S14: Change in dipole moment [Debye] and oscillator strength for the $S_0 \rightarrow S_2$ vertical excitations in RPSB chromophores within rhodopsin calculated using 3-root state-average CASSCF/MM (roots with equal weights) level of theory for CASSCF/MM and B3LYP/MM geometries. Optimized structures were obtained from geometry optimization in which side chains of all residues within 4 Å of any QM atom were relaxed. In paranthesis there are values for geometries obtained from calculations using frozen protein matrix.

Model	CASSCF/MM geometry		B3LYP/MM geometry	
	$\Delta\mu$ [Debye]	f	$\Delta\mu$ [Debye]	f
Rh	2.8 (3.1)	0.377 (0.362)	0.2 (0.0)	0.113 (0.080)
Batho	2.9 (3.1)	0.302 (0.220)	0.6 (0.8)	0.104 (0.135)
9-dm-Rh	3.1 (2.8)	0.478 (0.382)	0.7 (0.6)	0.235 (0.124)
9-dm-Batho[a]	4.9[a](4.1)[b]	0.473[a](0.416)[b]	1.4 (1.5)	0.006 (0.006)
9-dm-Batho[b]	1.6[c]	0.354[c]		
13-dm-Rh	3.5 (3.1)	0.446 (0.355)	0.1 (0.2)	0.037 (0.062)
13-dm-Batho	3.6 (3.1)	0.392 (0.331)	0.0 (0.7)	0.193 (0.121)
10-m-13-dm-Rh	3.6 (3.7)	0.338 (0.333)	0.3 (0.0)	0.030 (0.071)
10-m-13-dm-Batho	3.3 (3.4)	0.385 (0.372)	0.1 (1.0)	0.236 (0.050)
10-m-Rh	4.4 (5.8)	0.335 (0.325)	0.9 (0.2)	0.162 (0.052)
10-m-Batho	3.5 (3.5)	0.389 (0.365)	0.0 (0.1)	0.262 (0.265)

[a] The structure in which the twisting of the chromophore's C6–C7 bond is -31°
[b] The structure in which the twisting of the chromophore's C6–C7 bond is -36°
[c] The structure in which the twisting of the chromophore's C6–C7 bond is +79°

Table S15: CASPT2/MM absorption λ_{max}, oscillator strengths, and changes in dipole moment for $S_0 \rightarrow S_1$ vertical excitations, as well as photon energy storage, calculated **in vacuo** for CASSCF/MM and B3LYP/MM ground-state equilibrium geometries optimized within protein environment in which side chains of all residues within 4 Å of any QM atom were relaxed. In paranthesis there are values for geometries obtained from calculations using frozen protein matrix. CASSCF wave function was obtained from state-average calculations over two roots with equal weights.

Model	λ_{max} [nm]	f	storage [kcal/mol]	$\Delta\mu$ [Debye]
	CASSCF/MM geometry			
Rh	534 (535)	0.753 (0.759)	-	16.3 (16.2)
Batho	559 (563)	0.613 (0.499)	10.3 (12.1)	20.4 (21.2)
9-dm-Rh	529 (523)	0.745 (0.779)	-	17.1 (16.7)
9-dm-Batho[a]	574 (573)	0.854 (0.747)	8.1 (11.1)	19.8 (20.2)
9-dm-Batho[b]	482	1.042	4.2	15.1
13-dm-Rh	555 (549)	0.755 (0.789)	-	16.3 (15.9)
13-dm-Batho	570 (572)	0.678 (0.627)	12.2 (13.8)	20.6 (21.2)
10-m-13-dm-Rh	556 (552)	0.483 (0.507)	-	18.1 (17.6)
10-m-13-dm-Batho	570 (560)	0.524 (0.470)	9.9 (11.9)	20.6 (20.5)
10-m-Rh	554 (560)	0.372 (0.407)	-	19.0 (18.7)
10-m-Batho	563 (572)	0.841 (0.844)	3.9 (5.2)	17.0 (16.8)
	B3LYP/MM geometry			
Rh	535 (531)	1.500 (1.476)	-	12.4 (12.5)
Batho	576 (584)	1.880 (1.839)	8.3 (13.4)	9.4 (8.8)
9-dm-Rh	517 (519)	1.400 (1.431)	-	14.9 (14.5)
9-dm-Batho	508 (515)	1.761 (1.738)	2.0 (9.3)	12.4 (12.9)
13-dm-Rh	545 (534)	1.569 (1.497)	-	11.8 (12.4)
13-dm-Batho	586 (579)	1.889 (1.861)	9.3 (12.3)	8.7 (9.3)
10-m-13-dm-Rh	556 (545)	1.496 (1.454)	-	11.8 (12.1)
10-m-13-dm-Batho	601 (566)	1.825 (1.768)	9.2 (8.6)	7.4 (9.4)
10-m-Rh	566 (569)	1.358 (1.366)	-	12.4 (11.9)
10-m-Batho	604 (595)	1.765 (1.747)	8.0 (9.3)	6.7 (7.1)

[a] The structure in which the twisting of the chromophore's C6–C7 bond is -31°

[b] The structure in which the twisting of the chromophore's C6–C7 bond is +79°

Table S16: CASPT2/MM absorption λ_{max}, oscillator strengths, and changes in dipole moment for $S_0 \rightarrow S_1$ vertical excitations, as well as photon energy storage, calculated **in vacuo** for CASSCF/MM and B3LYP/MM ground-state equilibrium geometries optimized within protein environment in which side chains of all residues within 4 Å of any QM atom were relaxed. In paranthesis there are values for geometries obtained from calculations using frozen protein matrix. CASSCF wave function was obtained from state-average calculations over three roots with equal weights.

Model	λ_{max} [nm]	f	storage [kcal/mol]	$\Delta\mu$ [Debye]
	CASSCF/MM geometry			
Rh	533 (534)	0.735 (0.740)	-	15.9 (15.8)
Batho	552 (556)	0.674 (0.570)	9.7 (10.8)	20.3 (22.0)
9-dm-Rh	528 (530)	0.720 (0.737)	-	16.6 (16.2)
9-dm-Batho[a]	572 (571)	0.813 (0.726)	7.8 (10.9)	19.4 (19.6)
9-dm-Batho[b]	481	0.978	3.8	15.1
13-dm-Rh	553 (540)	0.732 (0.766)	-	16.1 (15.6)
13-dm-Batho	560 (559)	0.707 (0.682)	12.2 (13.8)	20.1 (20.8)
10-m-13-dm-Rh	549 (540)	0.542 (0.573)	-	18.4 (17.6)
10-m-13-dm-Batho	555 (565)	0.580 (0.506)	10.4 (8.8)	20.1 (22.4)
10-m-Rh	552 (555)	0.408 (0.447)	-	19.9 (19.6)
10-m-Batho	560 (569)	0.808 (0.804)	4.6 (6.2)	16.8 (16.6)
	B3LYP/MM geometry			
Rh	544 (538)	1.355 (1.338)	-	14.0 (14.0)
Batho	594 (606)	1.649 (1.594)	8.6 (14.0)	12.8 (12.5)
9-dm-Rh	528 (529)	1.258 (1.292)	-	15.9 (15.6)
9-dm-Batho	524 (526)	1.561 (1.570)	2.2 (9.4)	14.6 (14.8)
13-dm-Rh	552 (547)	1.419 (1.331)	-	13.9 (14.3)
13-dm-Batho	610 (600)	1.593 (1.594)	9.8 (12.6)	12.9 (13.1)
10-m-13-dm-Rh	564 (554)	1.348 (1.312)	-	13.8 (13.8)
10-m-13-dm-Batho	627 (585)	1.533 (1.522)	9.8 (9.1)	11.9 (12.8)
10-m-Rh	581 (583)	1.218 (1.219)	-	13.9 (13.5)
10-m-Batho	629 (619)	1.499 (1.490)	8.3 (9.6)	10.9 (11.1)

[a] The structure in which the twisting of the chromophore's C6–C7 bond is -31°

[b] The structure in which the twisting of the chromophore's C6–C7 bond is +79°