Supporting Information for "Protonation of the Biliverdin IXα Chromophore in the Red and Far-red Photoactive States of Bacteriophytochrome"

Vaibhav Modi,[1] Serena Donnini,[2] Gerrit Groenhof,[1] Dmitry Morozov[1]*

[1]Department of Chemistry and NanoScience Center, University of Jyväskylä, P. O. Box 35, 40014 University of Jyväskylä, Jyväskylä, Finland

[2]Department of Biological and Environmental Science and NanoScience Center, University of Jyväskylä, P. O. Box 35, 40014 University of Jyväskylä, Jyväskylä, Finland.

Corresponding Author
*dmitry.morozov@jyu.fi

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Molecular modelling

The residues unresolved from X-ray crystallography in PDB models 4O0P and 4O01 were added using loop modelling, which are listed in Table S1. The longest range of missing residue (17 amino acids) lies at the N -terminal end, and the other 5 unresolved loop regions in the protein complex vary within length of 1-6 residues. A total of 100 new models were generated from loop optimization step of modelling using simulated annealing technique and sorted again based on DOPE score to retrieve an optimal phytochrome conformation.

Table S1. Missing residue regions in DrBphP crystal structures 4O0P (Pr) and 4O01 (Pfr). The amino acid residues are denoted by single letter abbreviations and the two chains of protein homo-dimer are named A and B.

PDB Model	Residues
4O0P (Pr-dark form)	A 1-17 (MASMTGGQQMGRGSMSR) A 121-123 (AAG) A 146-151 (WDSTGP) A 415 (A) A 445-447 (GEG) A 472-476 (QAKDD) A 521-523 (HHH) B 1-17 (MASMTGGQQMGRGSMSR) B 121-123 (AAG) B 145-151 (AWDSTGP) B 415 (A) B 472-476 (QAKDD) B 518-523 (HHHHHH)
4O01 (Pfr-illuminated form)	A 1-19 (MASMTGGQQMGRGSMSRDP) A 220-221 (PA) A 145-149 (AWDST) A 465-475 (WGGATPDQAKD) A 521-523 (HHH) B 1-19 (MASMTGGQQMGRGSMSRDP) B 220-221 (PA) B 145 (A) B 149 (T) B 465-475 (WGGATPDQAKD) B 521-523 (HHH)

A total of 81 and 84 water molecules were added in the Pr and Pfr phytochrome cavities. The buried water molecules placed by DOWSER were refined by energy optimization and short equilibration simulations. An environment comparable to the high resolution (1.45 Å) crystal structure of DrBphP CBD domain (PDB: 2O9C)[8] was obtained (Figure S1). After the energy minimization and short equilibration runs the water molecules added with DOWSER form a network of non-covalent interactions with the chromophore and adjacent residues analogous to those in the experimentally resolved CBD structure.

Table S2. DOWSER parameters for BV chromophore generated using the atomic charges, bond lengths, angles, and dihedrals parameters derived for AMBER03 force field.

Atom name	Type	Backward	Forward	Bond	Angle	Dihedral
CBA	CH2	NOT	CAA	0	0	0
CAA	CH1	CBA	C3A	1.510	0	0
C3A	CR	CAA	C2A	1.343	125.9	0
C4A	CR	C3A	NOT	1.39	120	180
C2A	CH1	C3A	C1A	1.510	120	0
CMA	CH3	C2A	NOT	1.541	114	60
C1A	CR	C2A	N_A	1.604	112.1	0
O_A	O	C1A	NOT	1.226	124	180
N_A	N	C1A	NOT	1.375	112.1	0
HNA	H	N_A	NOT	1.026	126.9	180
CHB	CH1	C4A	C1B	1.393	122	60
C1B	CR	CHB	C2B	1.406	125.1	180
N_B	N	C1B	NOT	1.381	125.3	0
HNB	H	N_B	NOT	1.026	122.3	0
C2B	CR	C1B	C3B	1.439	126.5	180
CMB	CH3	C2B	NOT	1.497	123.5	0
C3B	CR	C2B	C4B	1.402	106.9	180
CAB	CH2	C3B	CBB	1.497	129	180
CBB	CH2	CAB	CGB	1.526	114	60
CGB	CR	CBB	NOT	1.522	116.6	180
O1B	O	CGB	NOT	1.222	12.5	60
O2B	O	CGB	NOT	1.363	111.5	60
C4B	CR	C3B	NOT	1.456	107.1	0

CHC	CH1	C4B	C1C	1.404	125	180
C1C	CR	CHC	C2C	1.406	126	180
N_C	N	C1C	NOT	1.381	125.3	0
HNC	H	N_C	NOT	1.026	122.3	0
C2C	CR	C1C	C3C	1.439	126.5	180
CAC	CH2	C2C	CBC	1.497	123.5	0
CBC	CH2	CAC	CGC	1.526	114	60
CGC	CR	CBC	NOT	1.522	116.6	180
O1C	O	CGC	NOT	1.222	126.5	60
O2C	O	CGC	NOT	1.363	111.5	60
C3C	CR	C2C	C4C	1.402	106.9	180
CMC	CH3	C3C	NOT	1.497	129	180
C4C	CR	C3C	NOT	1.456	107.1	0
CHD	CH1	C4C	C1D	1.404	125	180
C1D	CR	CHD	C2D	1.406	126	60
N_D	N	C1D	NOT	1.381	125.3	0
HND	H	N_D	NOT	1.026	122.3	0
C2D	CR	C1D	C3D	1.439	126.5	180
CMD	CH3	C2D	NOT	1.497	123.5	0
C3D	CR	C2D	C4D	1.402	106.9	180
C3D	CAD	CBD	CH1	1.404	120	180
CBD	CH2	CAD	NOT	1.363	120	180
C4D	CR	C3D	O_D	1.455	120	0
O_D	O	C4D	NOT	1.226	130.7	180

* The atom names refer to BV representation in Figure 1a.

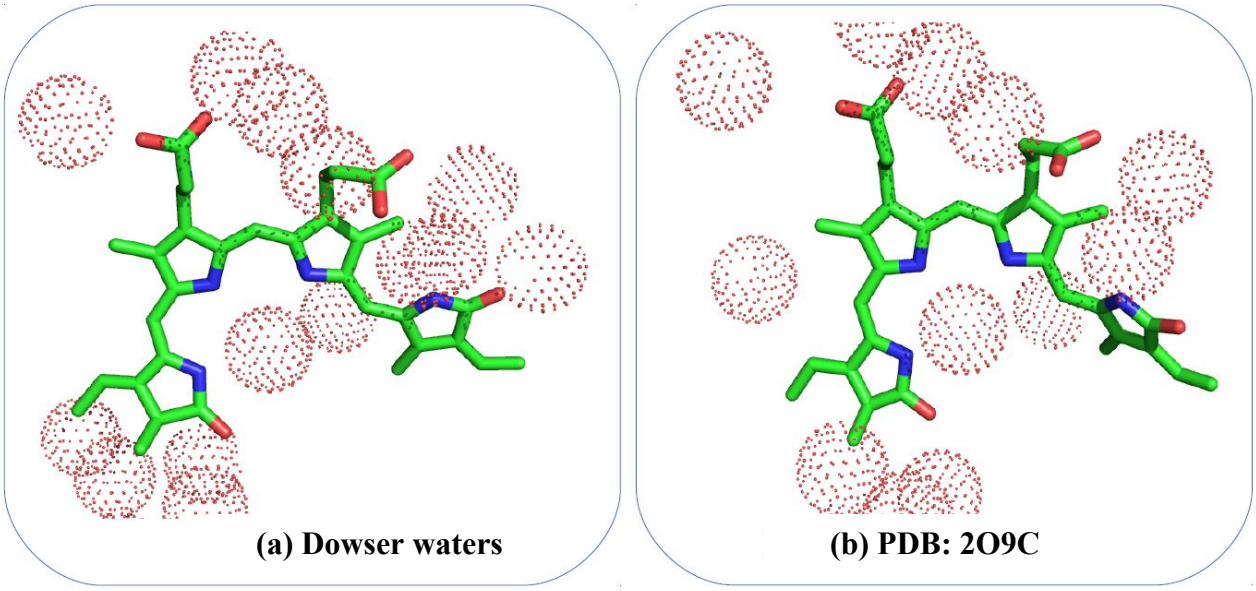

Figure S1. A dotted sphere representation of water molecules added in the vicinity of biliverdin using DOWSER program and equilibrated using MD simulations, labelled as DOWSER water. The arrangement of DOWSER water molecules is identical to the crystal water arrangement around chromophore in high resolution (1.45 Å) structure of CBD domain from DrBphP in dark form.

The tautomeric form of Histidine (His) residues and protonation state of amino-acids with ionizable side chains are summarized in Table S3. The protonation form for other residues of photo-sensory core were assigned based on their standard pK_a values in solution.

Table S3. Histidine residue tautomeric forms defined using pKa estimation for DrBphP MM simulations.

Residue	**Protonated nitrogen atom(s)**	
	Pr	**Pfr**
His44	ε2	ε2
His52	ε2	ε2
His61	ε2	ε2
His124	ε2 and δ1	ε2 and δ1
His130	ε2	ε2
His152	δ1	δ1
His210	ε2	ε2
His215	ε2 and δ1	δ1
His233	ε2	ε2
His274	ε2	δ1
His304	ε2	δ1

His305	ε2	ε2
His348	ε2	ε2
His349	ε2	ε2 and δ1
His358	ε2 and δ1	ε2
His363	δ1	ε2
His481	ε2	ε2 and δ1
His498	ε2	ε2
His518	ε2	ε2
His519	ε2	ε2 and δ1
His520	ε2	ε2 and δ1
His521	δ1	ε2
His522	ε2 and δ1	ε2
His523	ε2 and δ1	ε2 and δ1

Force field parameters for BV chromophore

Table S4. Atomic charges and atom types for Pr state of BV chromophore used in the AMBER03 force field for the MM calculations. The hydrogen atoms are denoted Ax, where the letter A is the index of heavy atom connected to the corresponding hydrogen atom and x = {a, b, c} denotes the equivalent hydrogen atoms.

Atom name**	**Atom**	**Atom Type**	**BV charges (e) – Pr Dark state**				
			Allprot	**Deprot-A**	**Deprot-B**	**Deprot-C**	**Deprot-D**
CBA	C	CT	-0.125784	-0.203762	-0.103050	-0.229543	-0.124648
HBA1	H	HC	0.141099	0.136623	0.090903	0.147943	0.114963
HBA2	H	HC	0.141099	0.136623	0.090903	0.147943	0.114963
CAA	C	CM	-0.128990	-0.114071	-0.138173	-0.214065	-0.212512
HAA	H	HC	0.197467	0.183367	0.197327	0.211276	0.200575
C3A	C	CM	-0.222884	-0.256168	-0.140716	-0.117059	-0.209827
C2A	C	CT	0.150926	0.197132	0.179136	0.323745	0.495323
H2A	H	HC	0.078532	0.029831	0.122425	0.003098	-0.046148
CMA	C	CT	-0.232203	-0.063887	-0.137302	-0.100033	-0.306863
HMA1	H	HC	0.071541	0.011709	0.040031	0.022898	0.070351
HMA2	H	HC	0.071541	0.011709	0.040031	0.022898	0.070351

HMA3	H	HC	0.071541	0.011709	0.040031	0.022898	0.070351
C1A	C	C	0.494423	0.653508	0.496733	0.424996	0.454889
OA	O	O	-0.566941	-0.712171	-0.627838	-0.583277	-0.578586
NA	N	NA	-0.371493	-0.581040	-0.176933	-0.209379	-0.406888
HNA	H	H	0.312913	-----	0.283875	0.271741	0.0353203
C4A	C	CC	0.293610	0.444239	0.048805	-0.024534	0.111921
CHB	C	C*	-0.508717	-0.620033	-0.326311	-0.170602	-0.236868
HHB	H	HC	0.180791	0.165923	0.136282	0.106955	0.121715
C1B	C	CC	0.299924	0.326981	0.321287	0.000552	-0.040423
C2B	C	C*	0.096990	0.075598	0.082417	0.137246	0.197569
CMB	C	CT	-0.407671	-0.218837	-0.241250	-0.324154	-0.368699
HMB1	H	HC	0.137772	0.068741	0.076523	0.096788	0.109631
HMB2	H	HC	0.137772	0.068741	0.076523	0.096788	0.109631
HMB3	H	HC	0.137772	0.068741	0.076523	0.096788	0.109631
C3B	C	C*	-0.223157	-0.161872	-0.236870	-0.359789	-0.385011
C4B	C	CC	0.204458	0.023029	0.200591	0.168143	0.192624
NB	N	NA	-0.461834	-0.226986	-0.548085	-0.239061	-0.277691
HNB	H	H	0.294567	0.183123	-----	0.237684	0.264059
CAB	C	CT	-0.049467	0.026304	-0.056134	0.256050	0.258543
HAB1	H	HC	0.059532	0.016007	0.028238	-0.023859	-0.019262
HAB2	H	HC	0.059532	0.016007	0.028238	-0.023859	-0.019262
CBB	C	CT	-0.147753	0.073027	0.009740	-0.097219	-0.123706
HBB1	H	HC	0.032167	-0.049630	-0.014053	-0.003895	0.000576
HBB2	H	HC	0.032167	-0.049630	-0.014053	-0.003895	0.000576
CGB	C	C	0.868953	0.837699	0.843738	0.837507	0.839885
O1B	O	O2	-0.799804	-0.815523	-0.824408	-0.833546	-0.830977
O2B	O	O2	-0.799804	-0.815523	-0.824408	-0.833546	-0.830977
CHC	C	CC	-0.300568	-0.186229	-0.217611	-0.238604	-0.345144
HHC	H	HC	0.260852	0.228768	0.229372	0.235788	0.247287
C1C	C	C*	0.338657	0.130317	0.126218	0.235670	0.240732
NC	N	NA	-0.715401	-0.369563	-0.363737	-0.563945	-0.520438
HNC	H	H	0.429492	0.343208	0.219782	-----	0.338459

C4C	C	CC	0.575162	0.122641	0.132139	0.374510	0.388743
C3C	C	CC	-0.059559	0.081143	0.053854	0.037294	0.148245
CMC	C	CT	-0.354912	-0.408173	-0.296450	-0.494114	-0.332064
HMC1	H	HC	0.120436	0.124811	0.097032	0.147399	0.118294
HMC2	H	HC	0.120436	0.124811	0.097032	0.147399	0.118294
HMC3	H	HC	0.120436	0.124811	0.097032	0.147399	0.118294
C2C	C	C*	-0.098782	-0.173870	-0.179305	-0.174327	-0.139621
CAC	C	CT	0.098471	-0.017298	-0.045656	-0.001461	-0.152533
HAC1	H	HC	0.018879	0.016670	0.020068	0.004290	0.065392
HAC2	H	HC	0.018879	0.016670	0.020068	0.004290	0.065392
CBC	C	CT	-0.201235	0.118531	-0.015955	0.145156	0.092259
HBC1	H	HC	0.032777	-0.076538	-0.014929	-0.084033	-0.052608
HBC2	H	HC	0.032777	-0.076538	-0.014929	-0.084033	-0.052608
CGC	C	C	0.928626	0.852904	0.852455	0.885512	0.877954
O1C	O	O2	-0.830879	-0.808738	-0.809695	-0.834242	-0.828726
O2C	O	O2	-0.830879	-0.808738	-0.809695	-0.834242	-0.828726
CHD	C	CC	-0.591351	-0.281211	-0.297969	-0.299437	-0.623872
HHD	H	HC	0.186294	0.194954	0.187372	0.125432	0.166525
C1D	C	C*	0.429679	0.189137	0.183425	0.210773	0.525233
ND	N	NA	-0.756385	-0.596745	-0.584351	-0.635954	-0.769332
HND	H	H	0.492973	0.416667	0.414284	0.408138	-----
C4D	C	C	0.802707	0.700539	0.695758	0.736142	0.849571
OD	O	O	-0.574791	-0.621307	-0.620724	-0.618986	-0.619391
C3D	C	CC	-0.088126	-0.039825	-0.033711	-0.059290	-0.153860
C2D	C	C*	0.009894	0.000393	-0.008482	0.015008	-0.019325
CMD	C	CT	-0.304626	-0.180547	-0.216321	-0.125136	-0.187728
HMD1	H	HC	0.107849	0.072109	0.066567	0.052565	0.065375
HMD2	H	HC	0.107849	0.072109	0.066567	0.052562	0.065375
HMD3	H	HC	0.107849	0.072109	0.066567	0.052565	0.065375
CAD	C	CM	-0.168161	-0.172837	-0.177418	-0.182681	-0.116367
HAD	H	HC	0.160099	0.155533	0.145617	0.164358	0.139860
CBD	C	CM	-0.373148	-0.462792	-0.399914	-0.429720	-0.415734

HBD1	H	HC	0.185031	0.194473	0.175472	0.187626	0.179261
HBD2	H	HC	0.185031	0.194473	0.175472	0.187626	0.179261

** The atom names refer to BV representation in Figure 1a.

Table S5. Atomic charges and atom types for Pr state of BV chromophore used in the AMBER03 force field for the MM calculations. The hydrogen atoms are denoted Ax, where A is the index of heavy atom connected to the corresponding hydrogen atom and x = {a, b, c} denotes the equivalent hydrogen atoms.

Atom name**	**Atom**	**Atom Type**	**BV charges (e) – Pfr Illuminated state**				
			Allprot	**Deprot-A**	**Deprot-B**	**Deprot-C**	**Deprot-D**
CBA	C	CT	-0.160213	-0.189742	-0.149204	-0.134423	-0.136000
HBA1	H	HC	0.142406	0.139983	0.127506	0.124373	0.125055
HBA2	H	HC	0.142406	0.139983	0.127506	0.124373	0.125055
CAA	C	CM	-0.150339	-0.116493	-0.143944	-0.183544	-0.128359
HAA	H	HC	0.206208	0.168066	0.169436	0.177800	0.161937
C3A	C	CM	-0.190843	-0.284840	-0.184399	-0.158934	-0.246548
C2A	C	CT	0.288527	0.325334	0.338244	0.301547	0.379720
H2A	H	HC	0.035952	-0.022687	-0.007789	0.010866	-0.008850
CMA	C	CT	-0.257769	-0.269885	-0.283010	-0.391525	-0.337182
HMA1	H	HC	0.073841	0.061502	0.072656	0.112922	0.083724
HMA2	H	HC	0.073841	0.061502	0.072656	0.112922	0.083724
HMA3	H	HC	0.073841	0.061502	0.072656	0.112922	0.083724
C1A	C	C	0.431185	0.626008	0.491165	0.500974	0.514265
OA	O	O	-0.575833	-0.689026	-0.606700	-0.628127	-0.587148
NA	N	NA	-0.213914	-0.622246	-0.314624	-0.288515	-0.441656
HNA	H	H	0.212987	-----	0.282468	0.257195	0.341992
C4A	C	CC	0.095123	0.502234	0.146009	0.065336	0.174924
CHB	C	C*	-0.334989	-0.666589	-0.412996	-0.247561	-0.321444
HHB	H	HC	0.166613	0.191091	0.175175	0.154362	0.170634
C1B	C	CC	0.152106	0.349842	0.345132	0.036036	0.038104
C2B	C	C*	0.090712	0.072554	0.040966	0.091563	0.125958
CMB	C	CT	-0.258848	-0.207441	-0.319834	-0.306687	-0.316116
HMB1	H	HC	0.085754	0.065913	0.084576	0.085749	0.091067
HMB2	H	HC	0.085754	0.065913	0.084576	0.085749	0.091067

HMB3	H	HC	0.085754	0.065913	0.084576	0.085749	0.091067
C3B	C	C*	-0.107975	-0.153443	-0.254932	-0.274516	-0.322487
C4B	C	CC	0.019292	0.036231	0.185116	0.093966	0.244521
NB	N	NA	-0.330574	-0.250914	-0.545432	-0.261497	-0.378494
HNB	H	H	0.281417	0.206126	-----	0.295026	0.305018
CAB	C	CT	0.011177	0.017465	0.168369	0.236018	0.228751
HAB1	H	HC	0.037059	0.016123	0.014037	-0.005678	0.004231
HAB2	H	HC	0.037059	0.016123	0.014037	-0.005678	0.004231
CBB	C	CT	0.005539	0.081418	-0.192695	-0.201297	-0.235382
HBB1	H	HC	-0.024865	-0.051371	0.024860	0.020123	0.034541
HBB2	H	HC	-0.024865	-0.051371	0.024860	0.020123	0.034541
CGB	C	C	0.836315	0.838509	0.869845	0.869237	0.869446
O1B	O	O2	-0.804384	-0.816067	-0.838478	-0.839682	-0.836738
O2B	O	O2	-0.804384	-0.816067	-0.838478	-0.839682	-0.836738
CHC	C	CC	-0.131675	-0.209971	-0.167397	-0.183526	-0.462604
HHC	H	HC	0.208094	0.235189	0.225111	0.222066	0.255630
C1C	C	C*	0.083961	0.132181	0.081733	0.200414	0.378707
NC	N	NA	-0.519627	-0.316647	-0.266306	-0.522486	-0.670893
HNC	H	H	0.392200	0.326444	0.291588	-----	0.382728
C4C	C	CC	0.258241	0.014106	-0.001728	0.287223	0.446492
C3C	C	CC	0.012488	0.128103	0.087547	0.097066	0.095018
CMC	C	CT	-0.425555	-0.388585	-0.232960	-0.304866	-0.489485
HMC1	H	HC	0.133515	0.115665	0.070119	0.083725	0.152595
HMC2	H	HC	0.133515	0.115665	0.070119	0.083725	0.152595
HMC3	H	HC	0.133515	0.115665	0.070119	0.083725	0.152595
C2C	C	C*	0.125036	-0.168090	-0.170476	-0.219102	-0.147368
CAC	C	CT	-0.064168	-0.015186	0.079050	0.042756	-0.056536
HAC1	H	HC	0.041743	0.019364	-0.012050	0.003489	0.038338
HAC2	H	HC	0.041743	0.019364	-0.012050	0.003489	0.038338
CBC	C	CT	-0.030445	0.059306	0.07455	0.104223	0.040694
HBC1	H	HC	-0.007975	-0.062378	-0.091380	-0.067907	-0.037704
HBC2	H	HC	-0.007975	-0.062378	-0.091380	-0.067907	-0.037704

CGC	C	C	0.850120	0.868532	0.854408	0.866815	0.866541
O1C	O	O2	-0.749769	-0.821857	-0.821538	-0.827200	-0.817653
O2C	O	O2	-0.749769	-0.821857	-0.821538	-0.827200	-0.817653
CHD	C	CC	-0.399765	-0.272894	-0.228179	-0.335205	-0.722267
HHD	H	HC	0.187392	0.231610	0.182002	0.204315	0.224194
C1D	C	C*	0.346411	0.261188	0.205031	0.294617	0.719153
ND	N	NA	-0.790471	-0.818128	-0.787264	-0.838345	-0.847725
HND	H	H	0.441957	0.44451	0.413430	0.455579	----
C4D	C	C	0.814382	0.841195	0.840706	0.834491	0.875858
OD	O	O	-0.621731	-0.676482	-0.668571	-0.656471	-0.615953
C3D	C	CC	-0.068393	-0.133935	-0.139937	-0.067046	-0.131867
C2D	C	C*	0.010889	0.052690	0.130575	0.013815	-0.117855
CMD	C	CT	-0.244646	-0.262942	-0.312858	-0.272259	-0.262124
HMD1	H	HC	0.110513	0.118988	0.123464	0.116000	0.112664
HMD2	H	HC	0.110513	0.118988	0.123464	0.116000	0.112664
HMD3	H	HC	0.110513	0.118988	0.123464	0.116000	0.112664
CAD	C	CM	-0.173485	-0.120615	-0.135393	-0.163882	-0.097059
HAD	H	HC	0.173013	0.153962	0.166629	0.173063	0.152614
CBD	C	CM	-0.418324	-0.483019	-0.472113	-0.475402	-0.460241
HBD1	H	HC	0.191871	0.190620	0.189349	0.189567	0.182800
HBD2	H	HC	0.191871	0.190620	0.189349	0.189567	0.182800

** The atom names refer to BV representation in Figure 1a.

Table S6. Atomic charges used for Pr state Cys24 residue in the AMBER03 force field for the MD simulations.

Atom	Charges (e) – Pr Dark state				
	Pr	**Deprot-A**	**Deprot-B**	**Deprot-C**	**Deprot-D**
N	-0.406250	-0.406250	-0.406250	-0.406250	-0.406250
H	0.276625	0.276625	0.276625	0.276625	0.276625
CA	0.000000	0.000000	0.000000	0.000000	0.000000
HA	0.081325	0.081325	0.081325	0.081325	0.081325
CB	-0.209278	-0.199800	-0.180751	-0.201389	-0.221453
HB1	0.163720	0.176122	0.176122	0.159492	0.166868
HB2	0.163720	0.176122	0.176122	0.159492	0.166868
SG	-0.251045	-0.299206	-0.287515	-0.257517	-0.253368
C	-0.606750	-0.606750	-0.606750	-0.606750	-0.606750
O	-0.558450	-0.558450	-0.558450	-0.558450	-0.558450

Table S7. Atomic charges used for Pfr state Cys24 residue in the AMBER03 force field for the MD simulations.

Atom	Charges (e) – Pfr Illuminated state				
	Pr	**Deprot-A**	**Deprot-B**	**Deprot-C**	**Deprot-D**
N	-0.406250	-0.406250	-0.406250	-0.406250	-0.406250
H	0.276625	0.276625	0.276625	0.276625	0.276625
CA	0.000000	0.000000	0.000000	0.000000	0.000000
HA	0.081325	0.081325	0.081325	0.081325	0.081325
CB	-0.199281	-0.213535	-0.210651	-0.202524	-0.199082
HB1	0.155258	0.166013	0.162773	0.159005	0.155258
HB2	0.155258	0.166013	0.162773	0.159005	0.155258
SG	-0.268582	-0.249284	-0.270392	-0.275985	-0.268582
C	-0.606750	-0.606750	-0.606750	-0.606750	-0.606750
O	-0.558450	-0.558450	-0.558450	-0.558450	-0.558450

Additional results from MD simulations

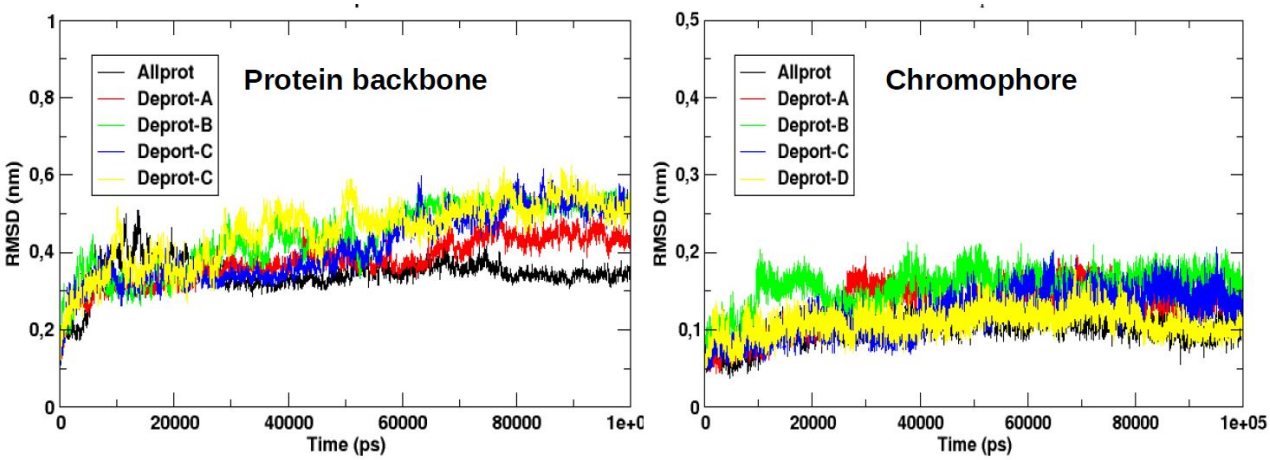

Figure S2. Root mean-square deviation (rmsd) for the protein backbone and the chromophore as a function of time over 100ns of MD trajectories for all 5 protonation models of DrBphP in dark state.

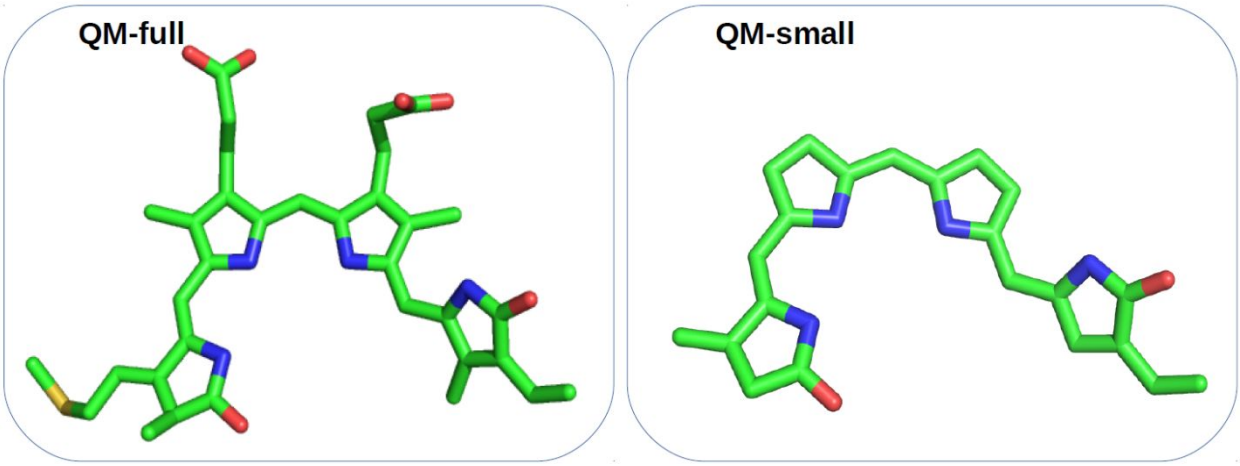

Figure S3. BV chromophore atoms included in the QM-full and QM-small subsystem for the single state excited state energy calculations using TD-DFT and XMCQDPT2 level of theory. In the QM-small subsystem the propionate side chains and methyl groups were replaced by hydrogen atoms.

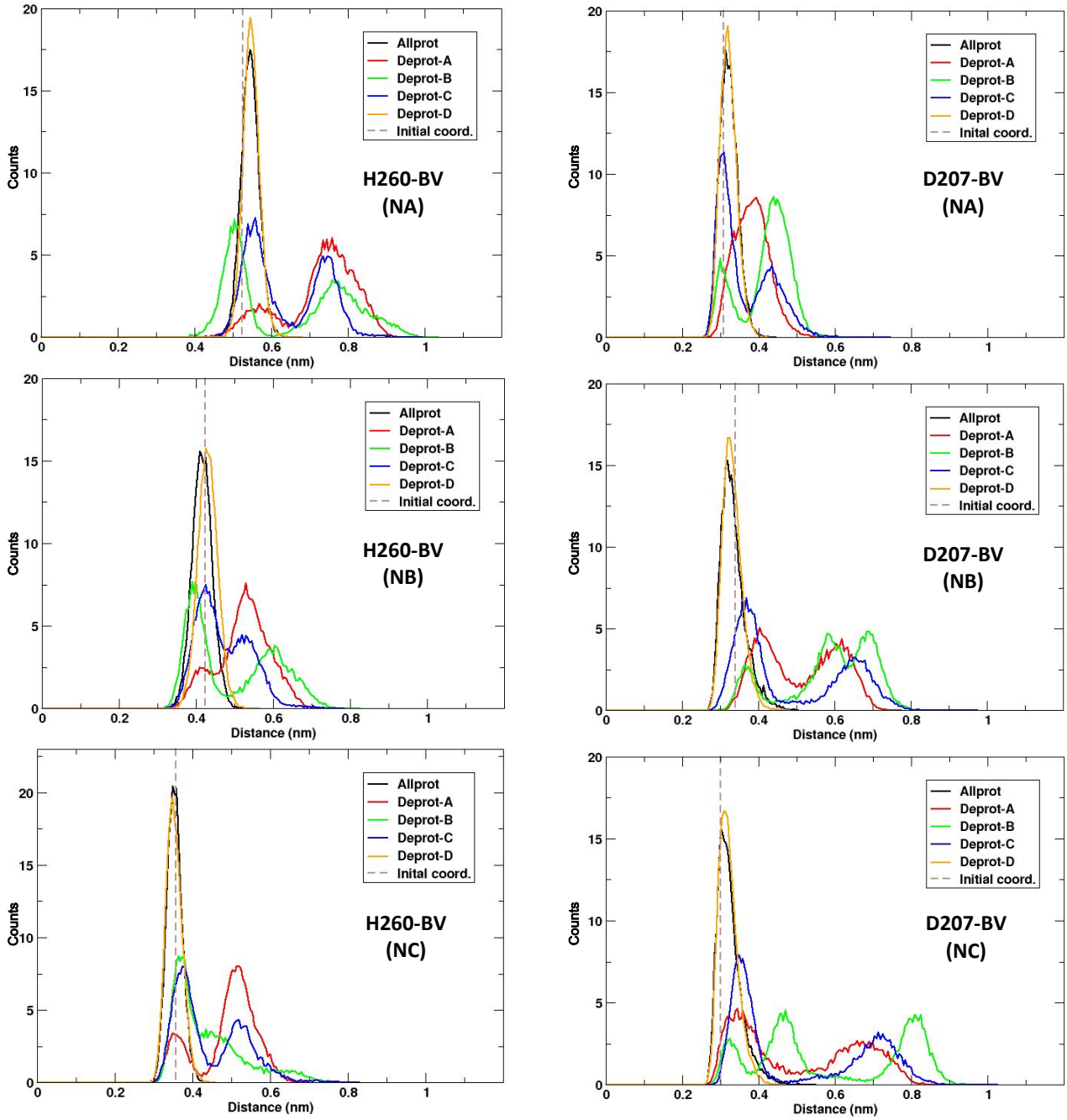

Figure S4. Distance distribution plots of the polar contacts between the BV A, B, and C pyrrole ring N-atoms, His260, and Asp207 in the chromophore binding pocket (shown earlier in Figure 3) for the 5 Pr state models with different protonation form of BV.

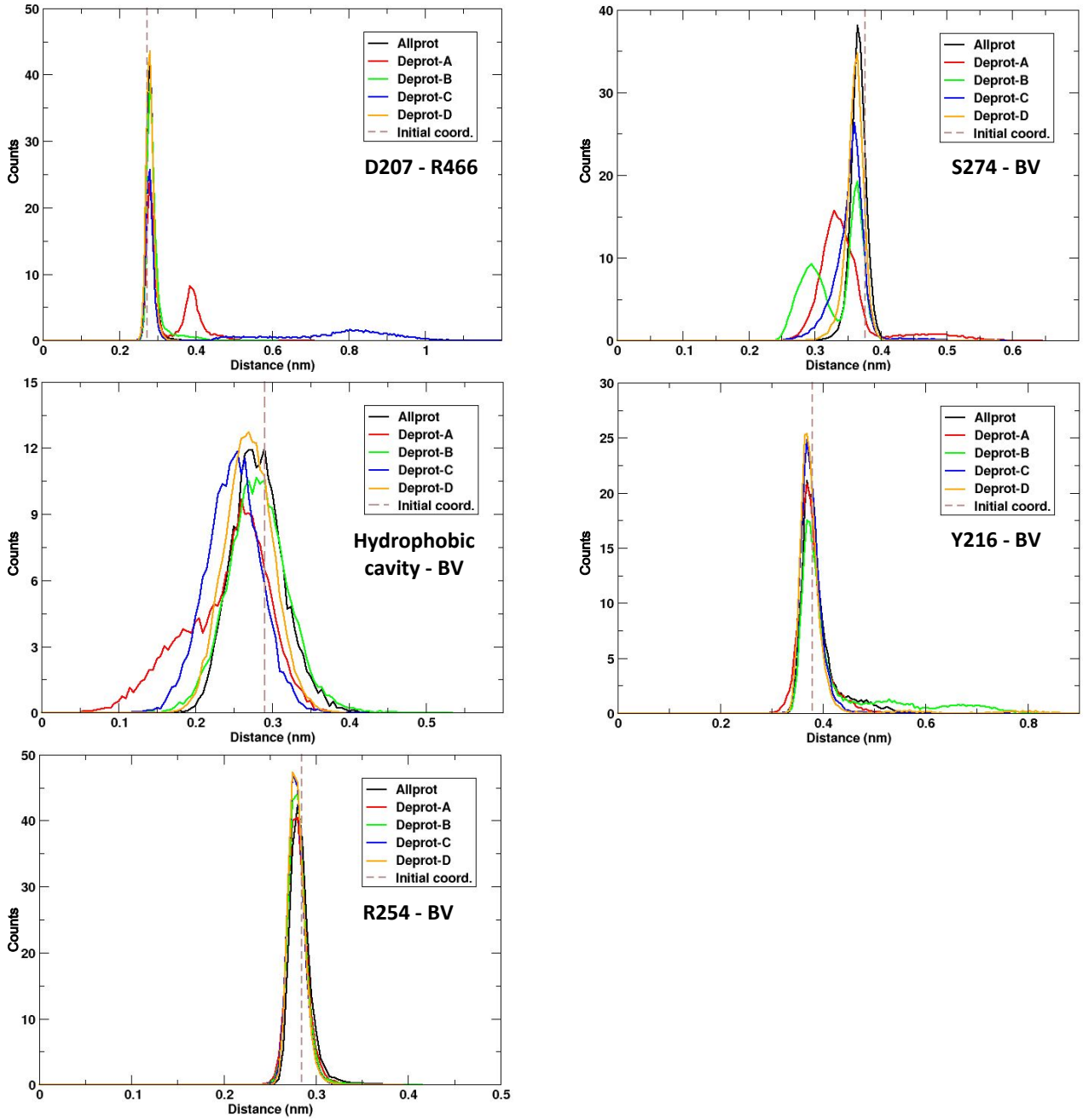

Figure S5: Distance distribution plots for the 5 Pr state models with different protonation form of BV. The electrostatic interactions formed by salt-bridge Asp207-Arg466, hydrophobic cavity residues (Tyr 176, Phe203. Tyr263) and D-ring of chromophore, chromophore B/C-ring side-chain carboxylate group and Tyr 216, Arg254, and Ser274 which stabilize the chromophore in the CBD domain in Pr state.

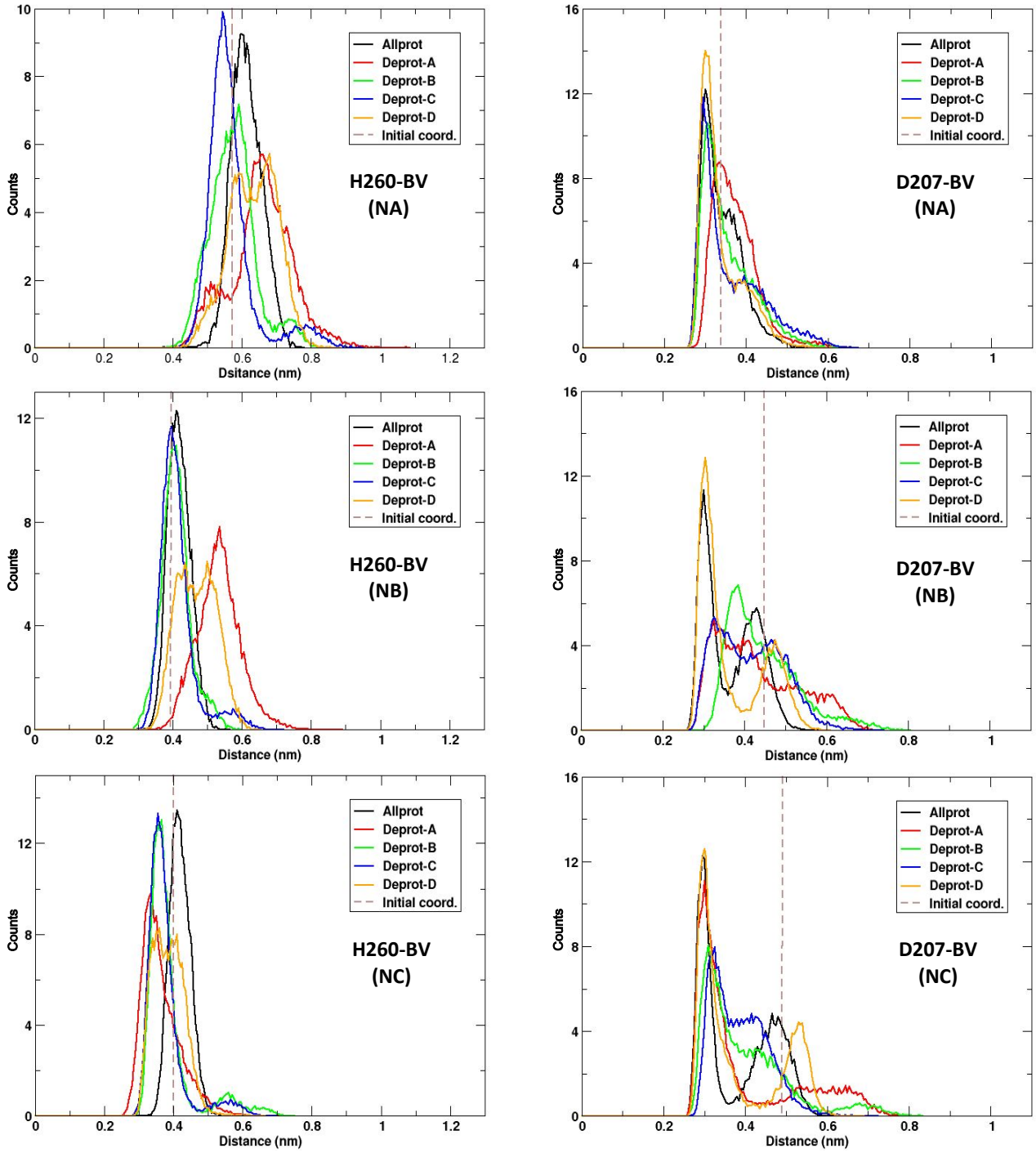

Figure S6. Distance distribution plots of the polar contacts between the BV A, B, and C pyrrole ring N-atoms, His260, and Asp207 in the chromophore binding pocket (shown earlier in Figure 3) for the 5 Pfr state models with different protonation form of BV.

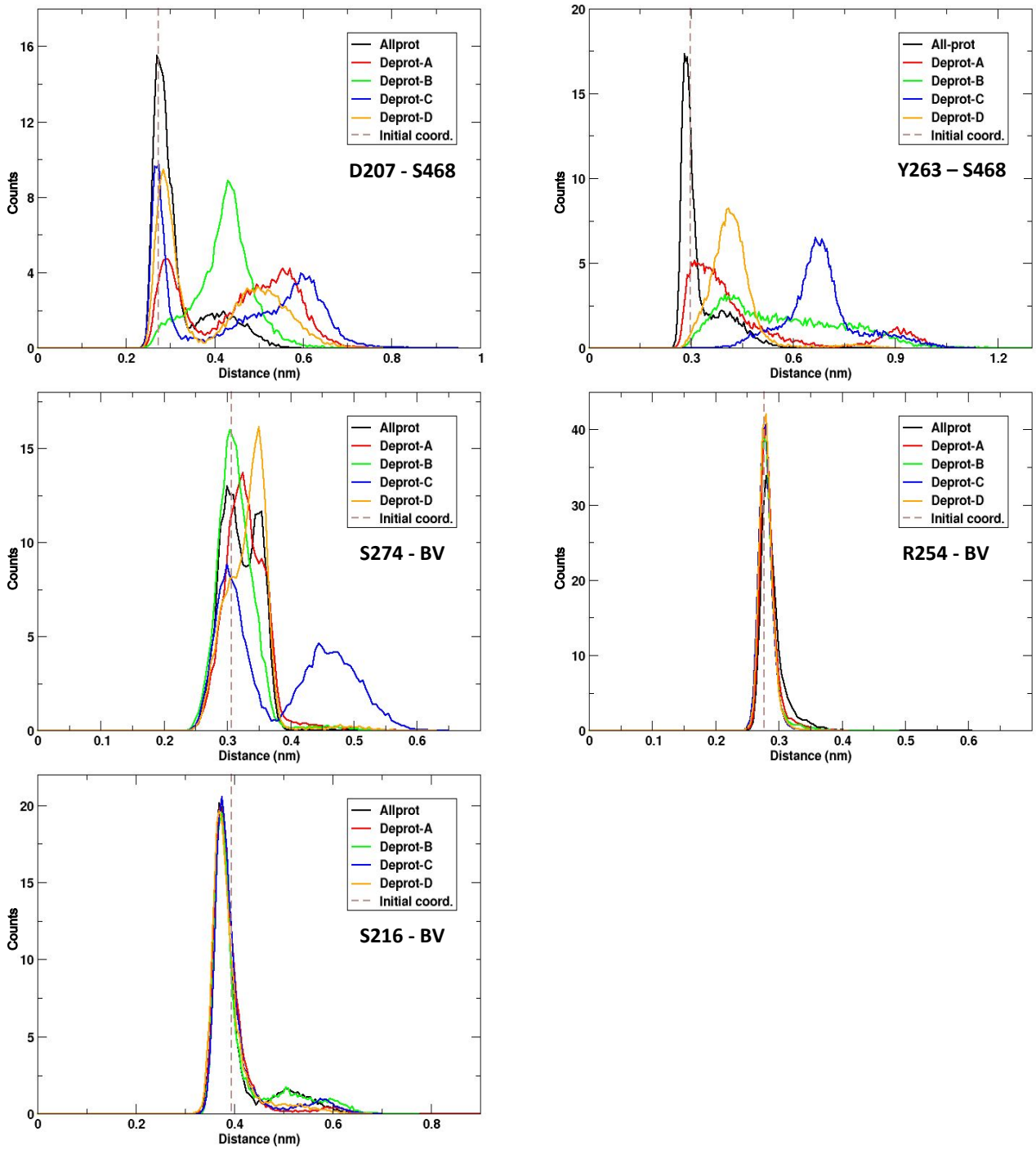

Figure S7: Distance distribution plots for salt-bridge Asp207-Arg466, Tyr263-Ser468, chromophore B/C-ring side-chain carboxylate group and Tyr 216, Arg254, and Ser274 which are the conserved non-covalent interaction in active site of Pfr state.

Additional results from QM/MM calculations

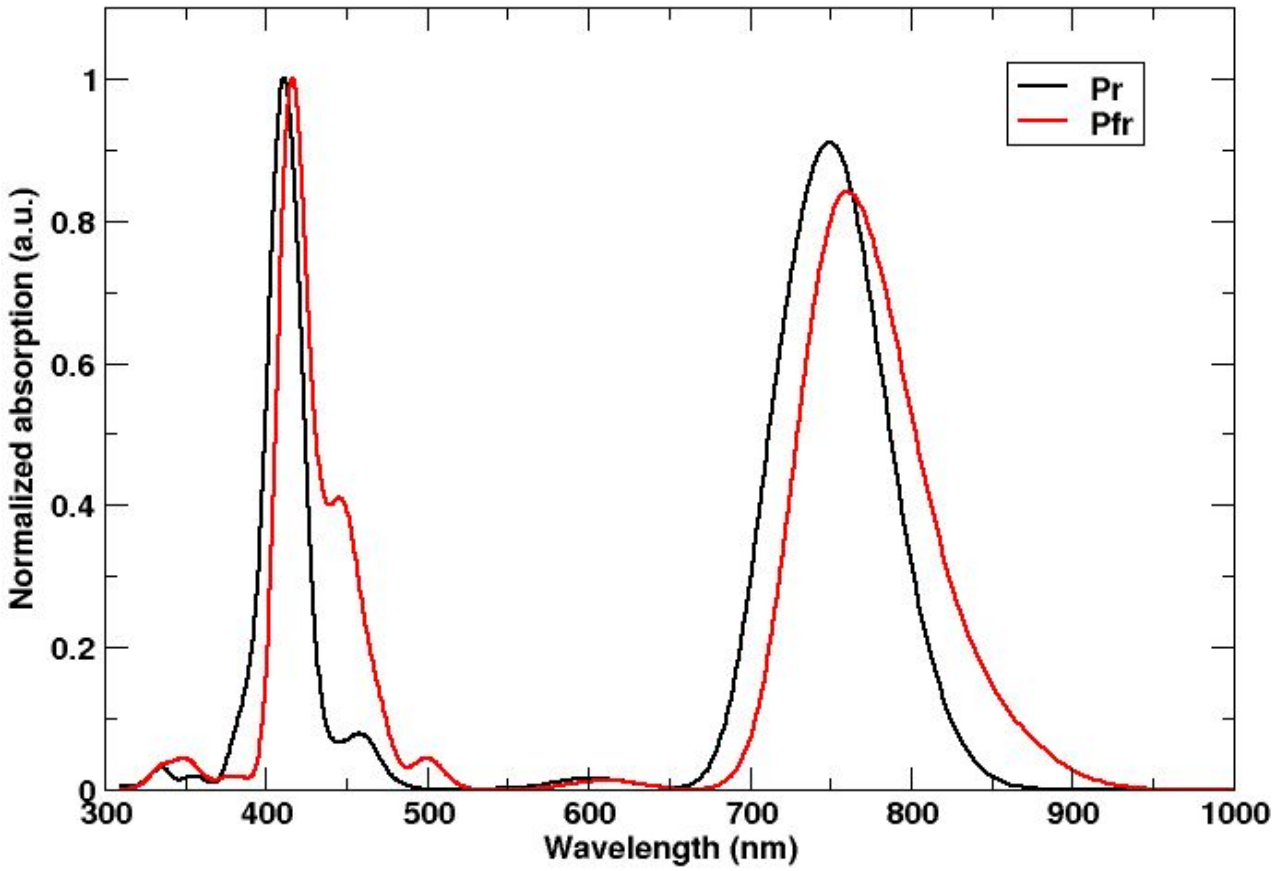

Figure S8: Inclusion of more states (nstates=10) in the xMCQDPT2 calculations reproduces the Soret-band for the Pr and Pfr fully protonated BV models.

Effect of QM subsystem size and protein environment: Figure S9 compares the UV/Vis spectra for the different QM subsystem sizes for the Pr state with a fully protonated BV. The inclusion of pyrrole water or protein environment (using point charges) in the QM subsystem does not show significant change in the Q band maxima, with the corresponding peak observed at ~690 nm. This suggests that the point charge representation of the pyrrole water is sufficiently accurate. Excluding the protein environment and the propionate side chains of the chromophore do not have any effect on the Q-band maxima, which indicates that future computations could be carried out with the QM-small subsystem to save computational effort without any qualitative loss.

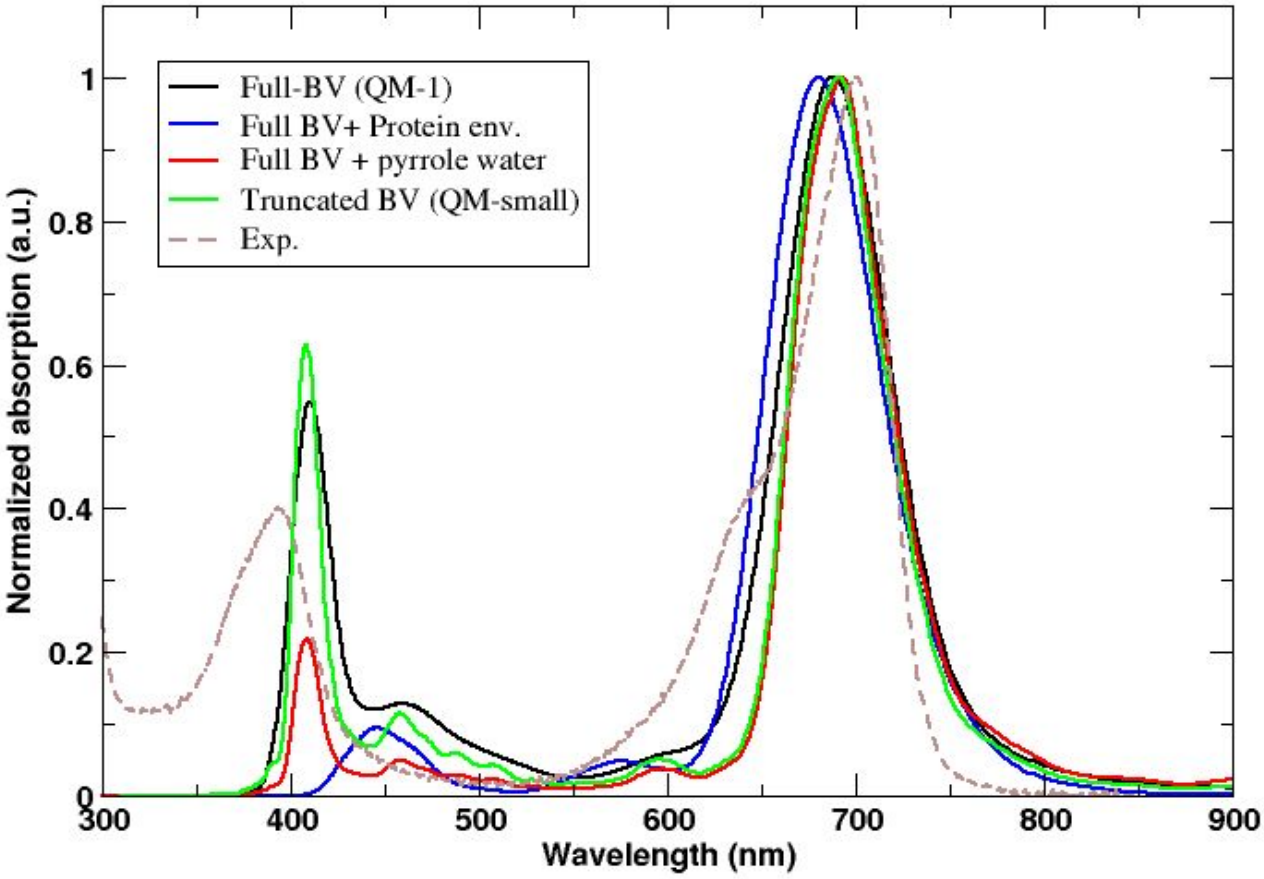

Figure S9. UV/Vis absorption spectra for the Pr state with fully protonated chromophore calculated using BLYP/6-31G(d,p) level of theory for the QM-full sub-system (see Figure S3) with the pyrrole water (blue) and without the pyrrole water (black). The experimental spectrum is shown as brown dotted line.

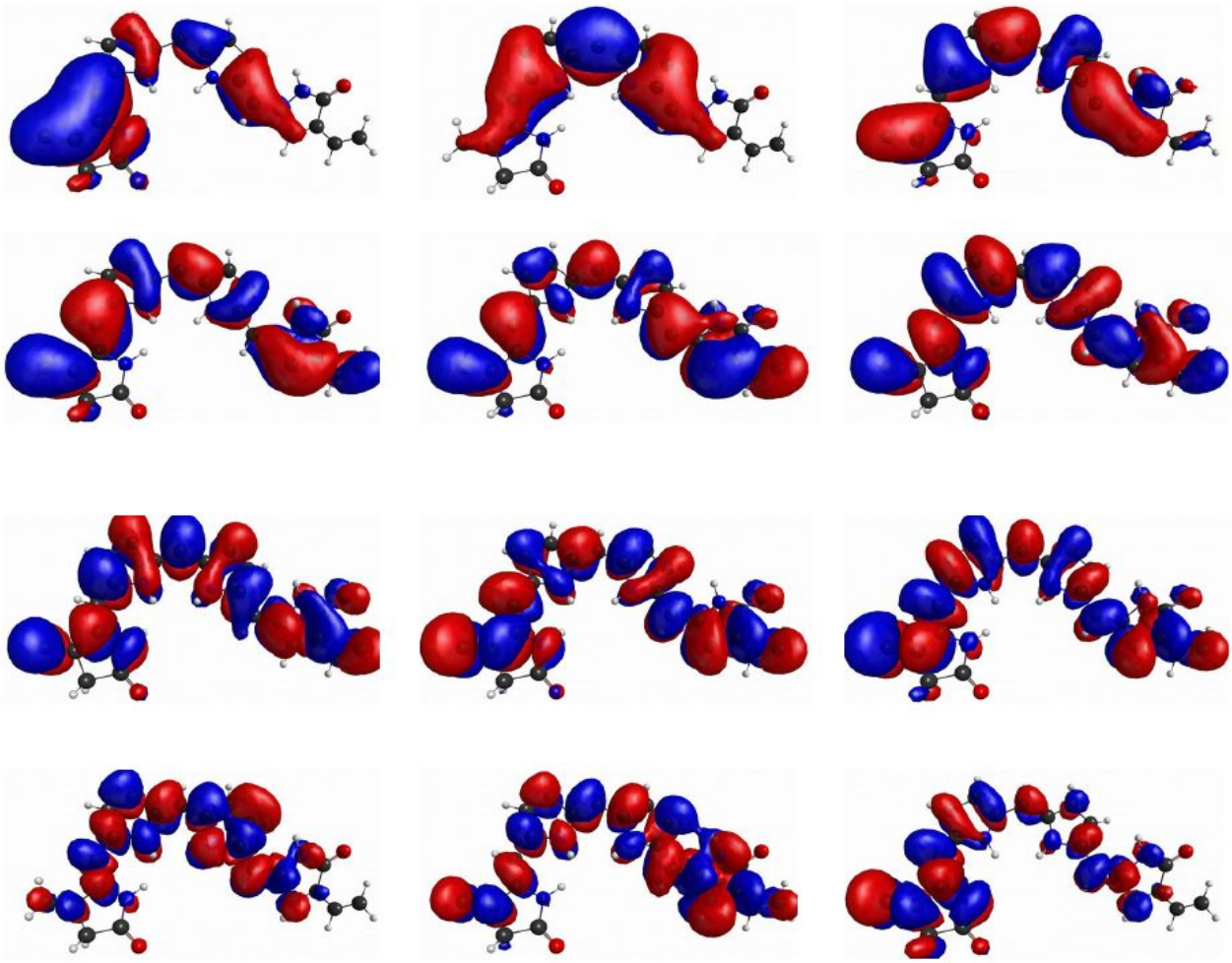

Figure S10: CASSCF (12,12) active space orbitals used in the XMCQDPT2 excited state energy calculations.

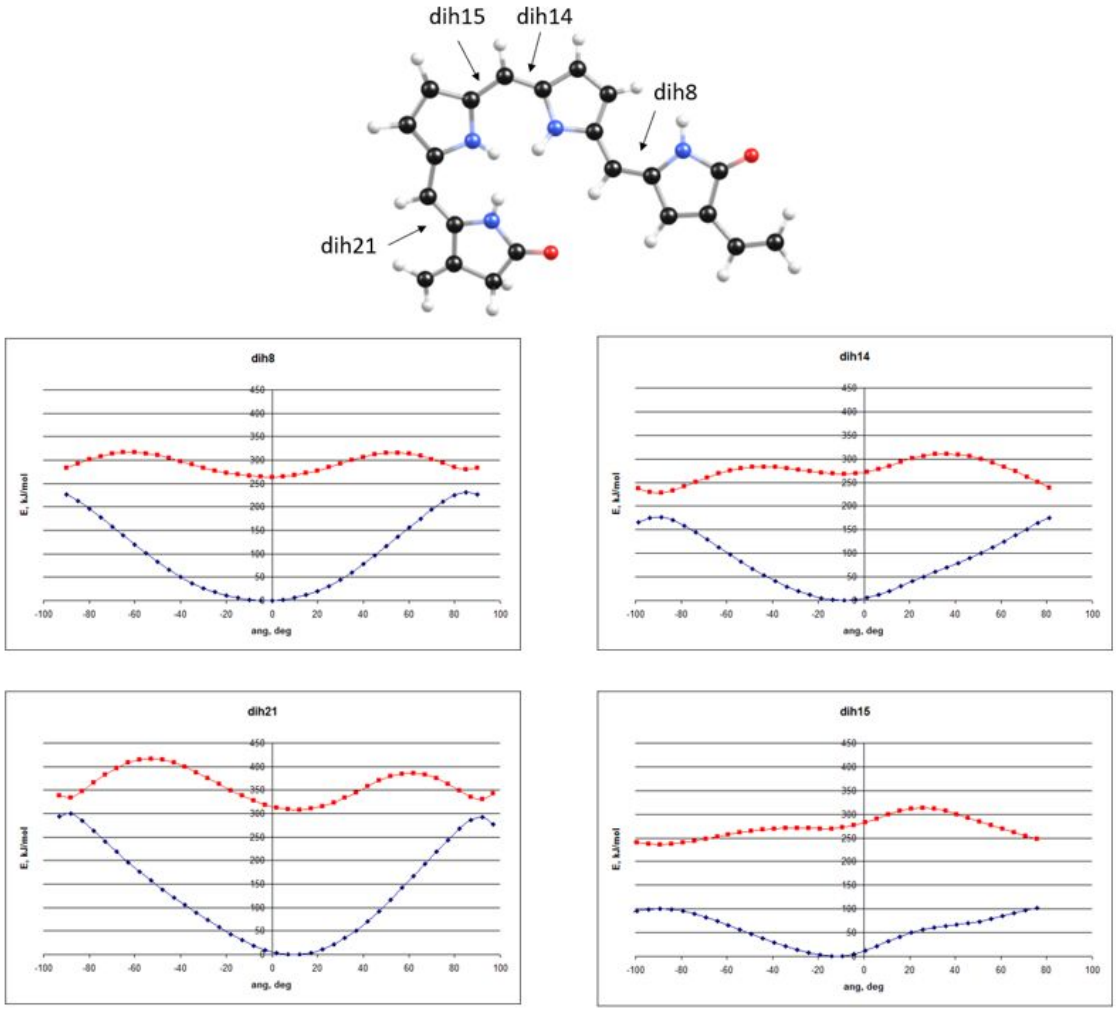

Figure S11: Excited-state relaxed energy scans of the most important biliverdin torsions. Excited S_1 state is shown with red line while ground S_0 state are blue line. The scans has been done at the SA(5)-CASSCF(12,12)/cc-pVDZ level of theory on the model chromophore *in vacuo*. The results show that (12,12) active space used in the work is stable with respect to the chromophore distortions.

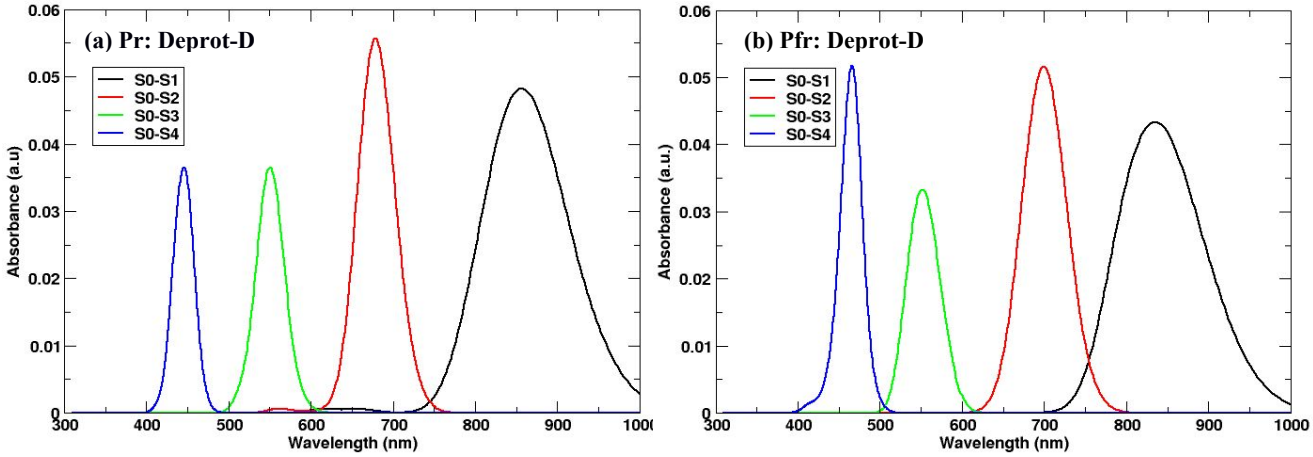

Figure S12. The contribution of different excited state transitions ($S_0 \rightarrow S_n$) to the absorption spectra computes with xMCQDPT2 method for the Pr and Pfr states with chromophore deprotonated at D-ring.

Proton release/uptake pathway

In our simulations we observe occasionally an exchange of the pyrrole water with bulk solvent molecules in both Pr anf Pfr. This exchange was observed by monitoring the hydrogen bond distance between BV and nearest water molecule. The pyrrole water exchange involving the residue His260 might provide a pathway for the proton from the chromophore D-ring.

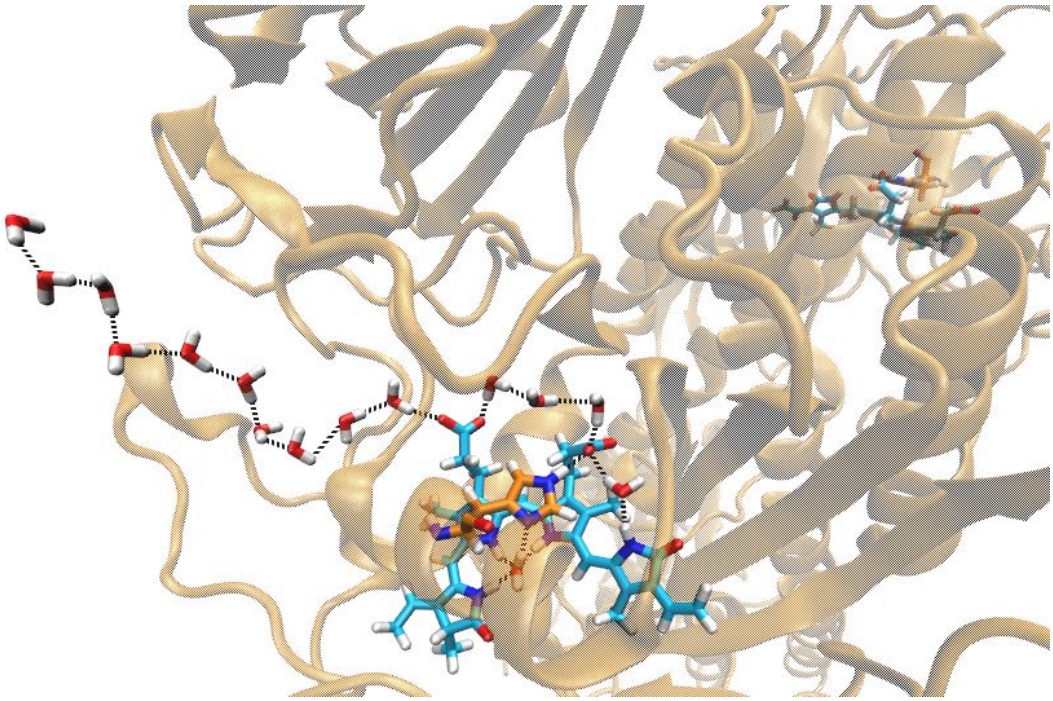

Figure S13: Snapshot from MD simulation of Pr state with a fully protonated chromophore illustrating a potential proton wire involving the chromophore, residue His260, pyrrole water and several solvent molecules. Colour code: carbon atoms of BV in turquoise and of the amino acid side chains involved in the proton wires in orange, oxygen atoms in red, nitrogen atoms in blue. Aliphatic hydrogen atoms are not shown.

The effect of the including Range-Separation on the chromophore force field

While the CAM-B3LYP functional has shown good performance for charge transfer excitations and excited-state dynamics in conjugated systems, it may not be the optimal choice for describing the ground state properties in such systems (M. A. Rohrdanz, J. Herbert: Simultaneous benchmarking of ground- and excited-state properties with long-range-corrected density functional theory, *J. Chem. Phys.* **129** (2008) 034107), we show below (Fig. S14 and Table S8) that the most critical force field parameters, namely bond lengths, angles and charges are essentially the same, irrespective of whether the geometry has been optimized with B3LYP or CAM-B3LYP."

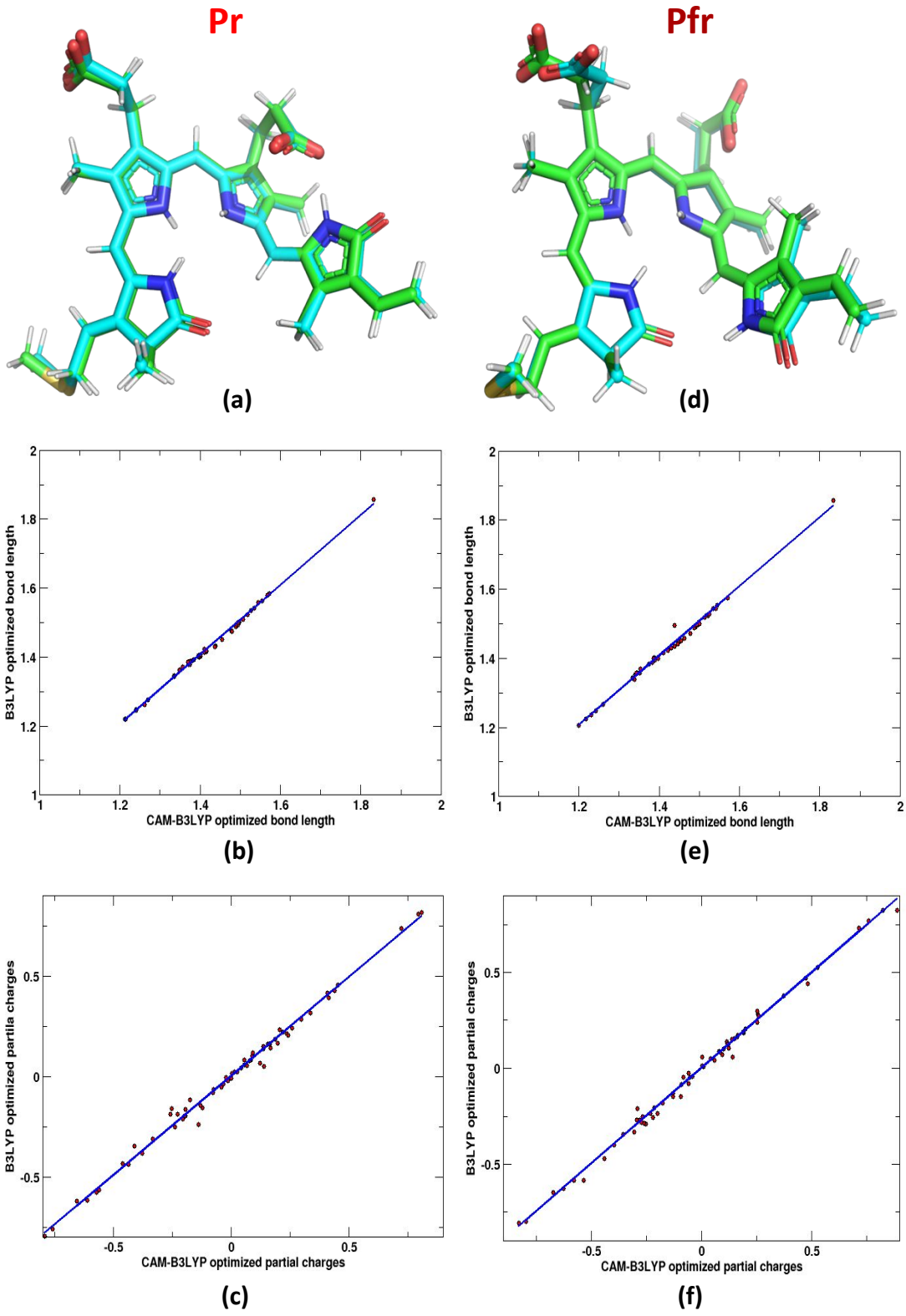

Figure S14: A superimposed stick representation of the optimized BV chromophore geometry using B3LYP (cyan) and CAM-B3LYP (green) functionals and basis set 6-31G* for (a) Pr and (d) Pfr state with fully protonated chromophore. A linear regression fit of the optimized geometry bond lengths (b)-Pr, (e)-Pfr and partial charges (c)-Pr, (f)-Pfr to compare the effect of using B3LYP instead of CAM-B3LYP for geometry optimization step in the RESP procedure.

Table S8: Comparison of DFT functionals based on the bond lengths for optimized BV chromophore for Pr and Pfr state full protonated chromophore.

Bonds	Pr (fully protonated BV)			Pfr (fully protonated BV)		
	CAM-B3LYP	B3LYP	Std. dev.	CAM-B3LYP	B3LYP	Std. dev.
CBA-S (CYS24)	1.832	1.858	-0.026	1.834	1.857	-0.023
CBA-CAA	1.494	1.494	0.000	1.439	1.495	-0.056
CAA-C3A	1.335	1.346	-0.011	1.341	1.352	-0.011
C3A-C2A	1.518	1.524	-0.006	1.491	1.491	0.000
C2A-CMA	1.535	1.543	-0.008	1.535	1.544	-0.009
C2A-C1A	1.526	1.534	-0.008	1.497	1.497	0.000
C1A-OA	1.213	1.219	-0.006	1.230	1.236	-0.006
C1A-NA	1.372	1.378	-0.006	1.339	1.339	0.000
NA-C4A	1.400	1.403	-0.003	1.449	1.449	0.000
C4A-C3A	1.478	1.476	0.002	1.488	1.488	0.000
C4A-CHB	1.350	1.363	-0.013	1.344	1.357	-0.013
CHB-C1B	1.438	1.432	0.006	1.439	1.434	0.005
C1B-NB	1.375	1.388	-0.013	1.382	1.389	-0.007
C1B-C2B	1.396	1.405	-0.009	1.388	1.403	-0.015
C2B-CMB	1.497	1.502	-0.005	1.497	1.499	-0.002
C2B-C3B	1.415	1.417	-0.002	1.430	1.429	0.001
C3B-CAB	1.489	1.489	0.000	1.499	1.505	-0.006
CAB-CBB	1.544	1.559	-0.015	1.541	1.545	-0.004
CBB-CGB	1.572	1.583	-0.011	1.570	1.575	-0.005
CGB-O1B	1.240	1.247	-0.007	1.242	1.247	-0.005
CGB-O2B	1.261	1.261	0.000	1.260	1.267	-0.007
C3B-C4B	1.411	1.422	-0.011	1.386	1.400	-0.014
C4B-NB	1.401	1.406	-0.005	1.389	1.394	-0.005

C4B-CHC	1.398	1.400	-0.002	1.447	1.441	0.006
CHC-C1C	1.381	1.391	-0.010	1.345	1.357	-0.012
C1C-NC	1.410	1.414	-0.004	1.422	1.423	-0.001
C1C-C2C	1.415	1.419	-0.004	1.521	1.523	-0.002
C2C-CAC	1.491	1.495	-0.003	1.545	1.554	-0.009
CAC-CBC	1.568	1.579	-0.011	1.525	1.529	-0.004
CBC-CGC	1.554	1.562	-0.008	1.517	1.524	-0.007
CGC-O1C	1.240	1.245	-0.005	1.200	1.206	-0.006
CGC-O2C	1.270	1.277	-0.007	1.351	1.357	-0.006
C2C-C3C	1.398	1.405	-0.007	1.514	1.518	-0.004
C3C-CMC	1.497	1.500	-0.003	1.491	1.495	-0.004
C3C-C4C	1.400	1.407	-0.007	1.343	1.356	-0.013
C4C-NC	1.369	1.385	-0.016	1.410	1.415	-0.005
C4C-CHD	1.437	1.431	0.006	1.463	1.458	0.005
CHD-C1D	1.357	1.372	-0.015	1.344	1.357	-0.013
C1D-ND	1.375	1.379	-0.004	1.397	1.399	-0.002
C1D-C2D	1.479	1.475	0.004	1.477	1.473	0.004
C2D-CMD	1.494	1.499	-0.005	1.494	1.498	-0.004
C2D-C3D	1.349	1.362	-0.013	1.354	1.369	-0.015
C3D-CAD	1.454	1.451	0.003	1.455	1.452	0.003
CAD-CBD	1.336	1.343	-0.007	1.334	1.343	-0.009
C3D-C4D	1.507	1.509	-0.002	1.500	1.501	0.001
C4D-OD	1.214	1.221	-0.007	1.217	1.224	-0.007
C4D-ND	1.385	1.393	-0.008	1.375	1.383	-0.008