Molecular Dynamics of phycocyanobilin binding bacteriophytochromes: A detailed study of structural and dynamic properties.

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Results from the force field parametrization of phycocyanobilin (PCB)

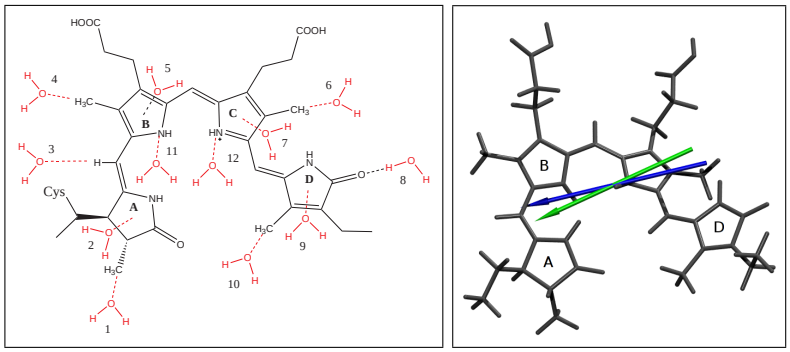


Figure S1: **Left picture**: Relative orientations of several water molecules with respect PCB, for the evaluation of atomic partial charges for the cofators. **Right picture:** Illustration of the molecular dipole moments of and PCB, calculated via HF/6-31G(d) (green) and reproduced (blue) with the set of derived atomic partial charges during the force field parametrization procedure.

Table S1: Minimum interaction energies (E_{min}) and distances (R_{min}) for PCB-water complexes, see left picture of Figure S1, evaluated at the ab initio (HF/6-31G(d)) and empirical levels. Distances are given in Å. Energies in kcal/mol and dipole moments (right picture of Figure S1) in Debye.

	HF/6-31G(d)		empirical/CHARMM		deviations	
	E_{min} (kcal/mol)	R_{min} (Å)	E_{min} (kcal/mol)	R_{min} (Å)	ΔE	ΔR
1	-2.647	3.38	-2.780	3.40	-0.134	-0.02
2	-4.229	4.07	-4.077	4.08	0.152	0.01
3	-6.282	2.63	-5.919	2.54	0.363	-0.09
4	-3.311	3.33	-3.325	3.32	-0.014	-0.01
5	-3.416	3.72	-4.696	3.34	-1.280	-0.38
6	-3.401	3.20	-3.283	3.30	0.118	0.1
7	-2.882	3.55	-2.867	3.48	0.015	-0.07
8	-2.586	2.15	-2.673	1.87	-0.087	-0.28
9	-2.673	3.39	-2.979	3.35	-0.306	-0.04
10	0.369	3.03	0.226	3.27	-0.143	-0.24
11	-7.073	2.25	-7.386	2.15	-0.313	-0.10
12	-5.346	3.09	-5.237	1.99	0.109	-1.10
average difference energy: 0.245 [kcal/mol]						
average difference distance: 0.212 (Å)						
Dipole Moment/HF-6-31G(d): tot = 7.438 x = 2.874 y = 6.465 z = -2.295						
Dipole Moment/empirical : tot = 8.771 x = 5.759 y = 6.152 z = -2.434						

Table S2: Atom types, labels and optimized partial charges of the PCB molecule. Values related to the propionate chains are written in italic letters. New atom types are written in bold letters. The position of atoms are illustrated in Figure S3.

atom	atom type	partial charge	atom	atom type	partial charge
CAC	CPM	-0.159	**C3B**	**CPY6**	0.320
HAC	HA	0.080	O_B	O	-0.337
C1C	C	0.289	**CHB**	**CPY2**	-0.448
H2C	HA	0.057	HHB	HA	0.219
N_C	NR1	-0.500	CAB	CE1	-0.180
H_C	H	0.302	HAB	HE1	0.090
H3C	H	0.144	HBB	HE1	0.090
C4C	CA	0.237	CBB	CE2	-0.270
C3C	CT1	0.017	HV1	HE1	0.090
C2C	CT1	0.365	HV2	HE2	0.090
O_C	O	-0.495	CBC	CT2	-0.160
CHD	**CPY3**	-0.501	HL1	HA	0.090
HHD	HA	0.214	HL2	HA	0.090
HV3	HA	0.090	HL3	HA	0.090
C1D	CPA	0.375	CMC	CT3	-0.179
N_D	NR1	-0.543	HE1	HA	0.038
H_D	H	0.306	HE2	HA	0.038
C4D	CPA	0.364	HE3	HA	0.038
C3D	CPB	-0.041	CMD	CT3	0.056
C2D	CPB	-0.161	HD1	HA	0.022
CHA	CPM	-0.111	HD2	HA	0.022
C1A	CPA	0.169	HHA	HA	0.319
N_A	NR1	-0.654	HD3	HA	0.022
H_A	H	0.355	CMA	CT3	-0.103
C2A	**CPY4**	-0.038	HA1	HA	0.066
C3A	CPB	-0.175	HA2	HA	0.066
C4A	CPA	0.437	HA3	HA	0.066
C4B	C	0.306	CMB	CT3	-0.018
N_B	NR1	-0.560	HB1	HA	0.049
H_B	H	0.375	HB2	HA	0.049
C1B	CA	0.452	HB3	HA	0.049
C2B	**CPY5**	-0.370	*CBD*	*CT2*	*-0.28*
HO3	*HA*	*0.09*	*O2A*	*OC*	*-0.76*
HO4	*HA*	*0.09*	*O1A*	*OC*	*-0.76*
CGD	*CC*	*0.62*	*CAA*	*CT2*	*-0.18*
O2D	*OC*	*-0.76*	*HO5*	*HA*	*0.09*
O1D	*OC*	*-0.76*	*HO6*	*HA*	*0.09*
CBA	*CT2*	*-0.28*	*CAD*	*CT2*	*-0.18*
HO1	*HA*	*0.09*	*HO7*	*HA*	*0.09*
HO2	*HA*	*0.09*	*HO8*	*HA*	*0.09*
CGA	*CC*	*0.62*			

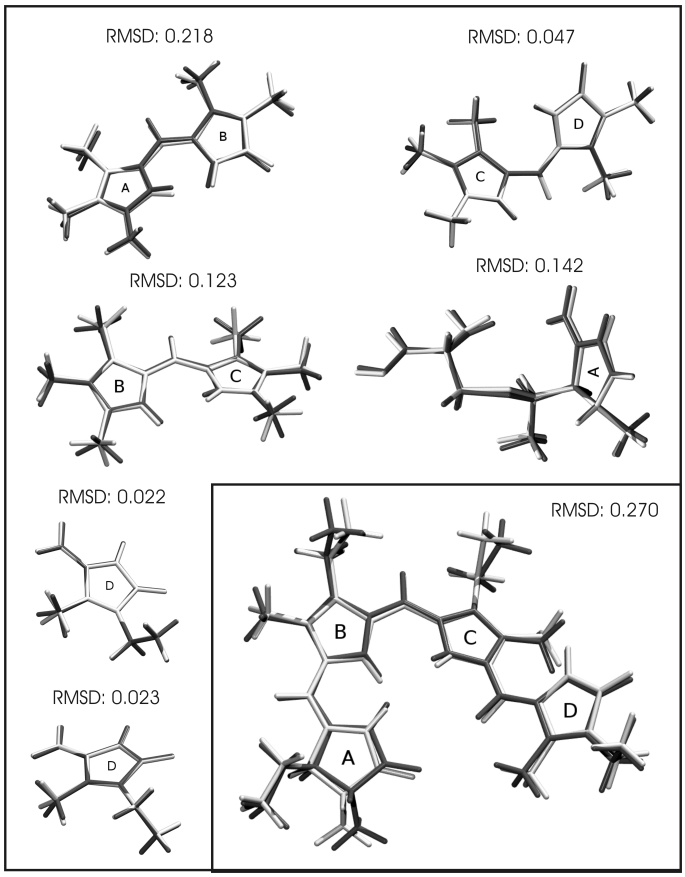


Figure S2: Optimized ab initio (DFT/B3LYP/6-31g(d)) (dark grey) and empirical (light grey) structures of the PCB chromophore without propionate chains and the small model fragments used in the parametrization procedure. RMS deviations, in Å, are given for non hydrogen atoms after optimal superimposition.

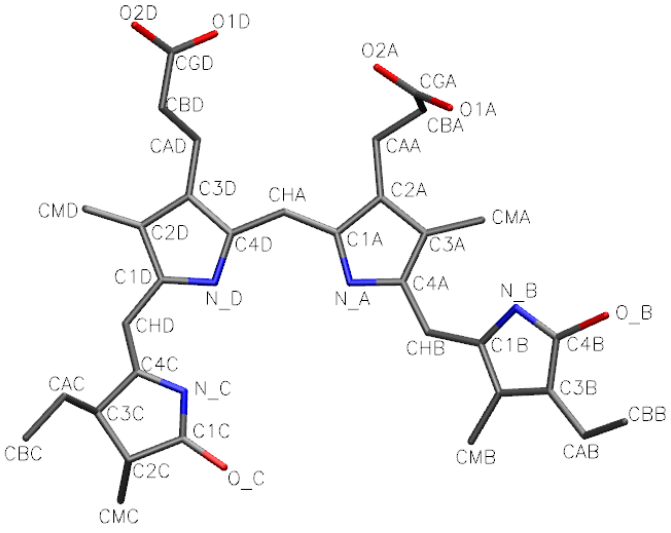


Figure S3: Labeling of all non hydrogen atoms of PCB.

Table S3: Average deviations for several internal coordinates (39 bond length, 40 bond angles, 8 torsion angles and 15 improper torsion angle) between in vacuo minimized structures of PCB without propionate chains (see picture on the right bottom of Figure S2), using optimized MM parameters compared to DFT/B3LYP/6-31g(d) optimized geometries.

	bond length [Å]	bond angle [deg]	torsion angle [deg]	improper torsion angle [deg]
PCB	0.001	0.905	2.321	0.688

Table 4: Optimized parameters for newly defined dihedral angles of the PCB chromophore. The related model systems (numbering I-O) are given in Figure S4.

dihedral parameter	K_χ (kcal/mol)	n	χ_0 (deg)
linking fragment 1 (O)			
CT2 SE CT1 CT3	4.0	1	180.0
CT2 SE CT1 CT3	1.1	2	150.0
CT2 SE CT1 CT3	1.0	3	0.0
CT2 SE CT1 CT3	1.1	8	0.0
linking fragment 2 (N)			
SE CT1 CT1 CA	0.50	3	0.00
SE CT1 CT1 CA	2.00	2	265.0
double bond pyrrolinone fragment ring C-D (K)			
CPA-CPY2-CA-CPY5	9.5	1	0.0
CPA-CPY2-CA-CPY5	3.5	2	185.0
single bond pyrrolinone fragment ring C-D (J)			
CPB-CPA-CPY2-CA	1.9	3	0.0
CPB-CPA-CPY2-CA	4.2	2	140.0
CPB-CPA-CPY2-CA	2.1	1	243.0
pyrrol fragment 1 (M)			
CPY4-CPA-CPM-CPA	9.0	2	183.0
CPY4-CPA-CPM-CPA	3.0	1	3.50
CPY4-CPA-CPM-CPA	2.0	3	189.0
pyrrol fragment 2 (M)			
CPB-CPA-CPM-CPA	8.5	2	184.0
CPB-CPA-CPM-CPA	2.0	1	0.0
CPB-CPA-CPM-CPA	1.9	3	196.0
double bond pyrrolinone fragment ring A-B (L)			
CT1-CA-CPY3-CPA	5.1	1	0.0
CT1-CA-CPY3-CPA	2.1	2	180.0
single bond pyrrolinone fragment ring A-B (J)			
CA-CPY3-CPA-CPB	0.30	3	60.0
CA-CPY3-CPA-CPB	2.70	2	170.0
ethyl fragment (I)			
CPY5 CPY6 CT2 CT3	0.30	1	0.0
CPY5 CPY6 CT2 CT3	0.12	2	180.0
CPY5 CPY6 CT2 CT3	0.25	4	0.0

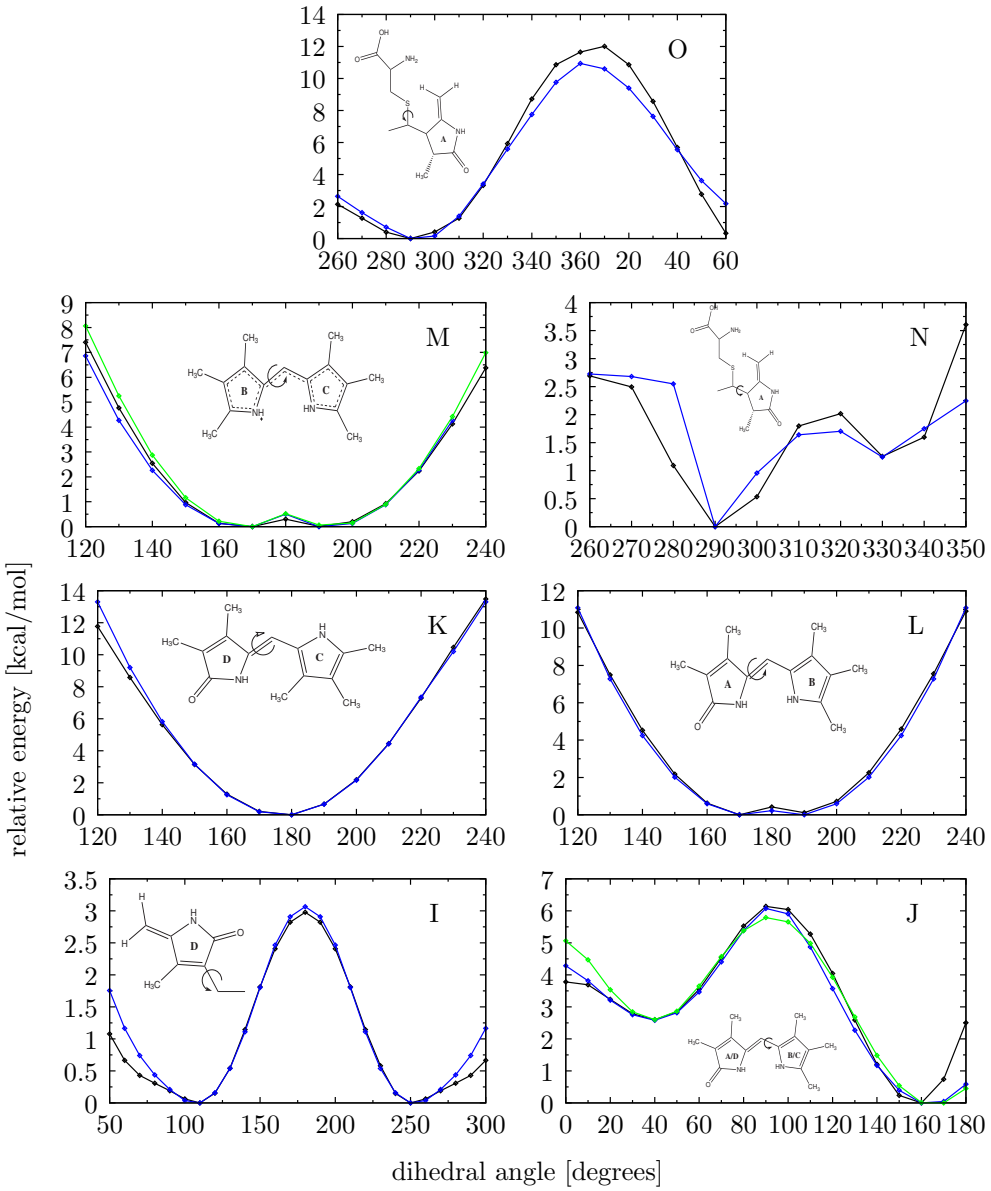


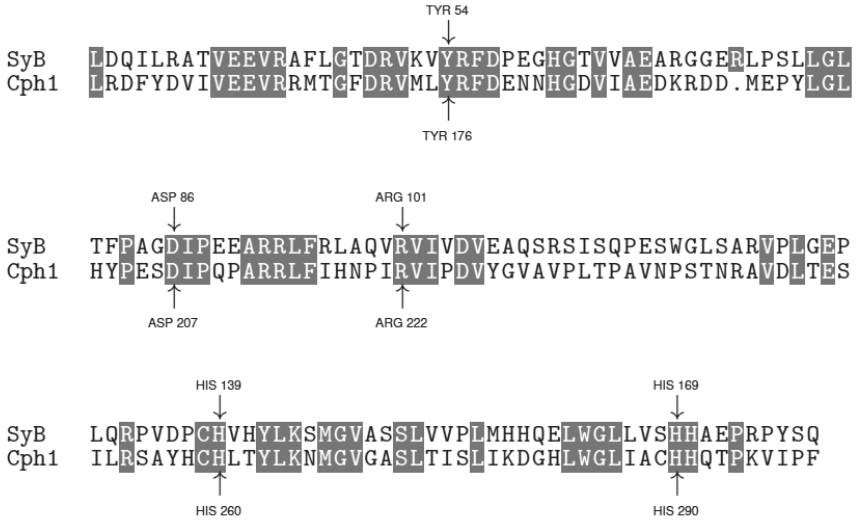
Figure S4: Potential energy surfaces for partially constrainted model compounds of PCB. The surfaces were scanned in steps of 10 degrees. The black curves denote the target data derived using ab initio methods (DFT/B3LYP/6-31G(d)), blue and green curves result from optimized MM torsion parameters. Potential curves were shifted by setting the lowest energy conformations for each curve equal zero. Curves in green color present either the same torsion in different fragments (picture J) or different torsions in the same fragment (picture M). Especially for picture M, ab initio results for both torsions in the central methine bridge were nearly identical, therefore only one set of target data was employed.

The protocol for molecular dynamics simulations

All calculations concerning heating, equilibration and production were performed with the NAMD program (version 2.6) using the CHARMM27 force field. At the beginning, the solvent water was heated up to 300 K and equilibrated for 80 ps while keeping the remaining parts of the system fixed. Subsequently, several energy minimization runs (1000 steps of conjugate gradient) were performed in which the initially harmonic constraints (15 kcal/mol$\mathrm{\AA}^2$) applied to all protein backbone atoms were gradually released until the entire system was free to move. Afterwards, the complete system was heated up to 300 K during 60 ps of Langevin dynamics with restraint protein backbone atoms. These steps were followed by 100 ps of molecular dynamics simulations under constant pressure, constant temperature (NPT) conditions using a combination of the Langevin Piston Nose-Hoover method, as implemented in NAMD. Here again, restraints were released stepwise until the system was totally unrestrained. The simulation of the equilibrated system was further proceeded for a 25 ns production run, using a reduced Langevin damping factor (from 5.0 to 1.0) in order to more closely approximate free dynamics (NPT conditions at 300K).

All simulations were done under periodic boundary conditions with the Particle-Mesh-Ewald method in use for the calculation of electrostatic interactions. For the van der Waals interactions, a cutoff (12 Å) was used in combination with a switching function. No scaling was applied to electrostatic interactions between 1-4 atom pairs. In order to use a 2 fs time step, all bond lengths between heavy atoms and hydrogen have been constrained to their minimum energy values by applying the SHAKE algorithm.

Sequence alignment between SyB(GAF) and Cph1



FigureS5: Sequence alignment of the two phytochrome species of Cph1 and SyB(GAF). A cutout is shown, containing the GAF fragment of both proteins. Equivalent residues in the cofactor binding pocket of both phytochromes which having different numbers in both systems are highlighted. Residues in gray colored boxes indicate a sequence match.

Some results of molecular dynamics simulations

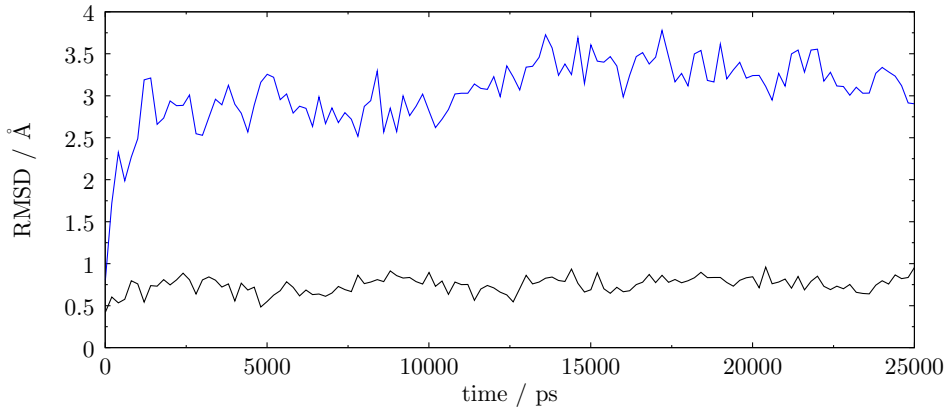


Figure S6: Root-mean-square deviations (RMSD) for all non-hydrogen protein (blue) and chromophore (black) atoms as a function of time.

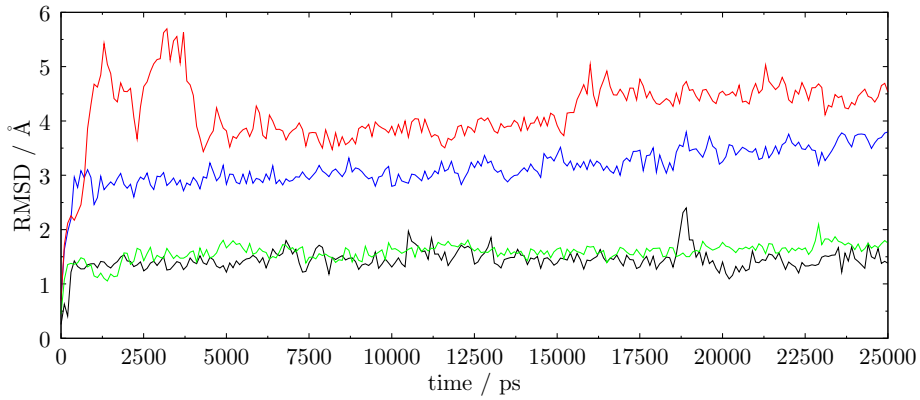


Figure S 7: Root-mean-square deviations (RMSD) for all non-hydrogen atoms of the protein (Pr:blue, Pfr:red) and chromophore (Pr:black, Pfr:green) of SyB(GAF) atoms as a function of the simulation time.