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Supporting information for article:

Structure of allophycocyanin B from *Synechocystis* PCC 6803 reveals the structural basis for the extreme red-shift of the terminal emitter in phycobilisomes

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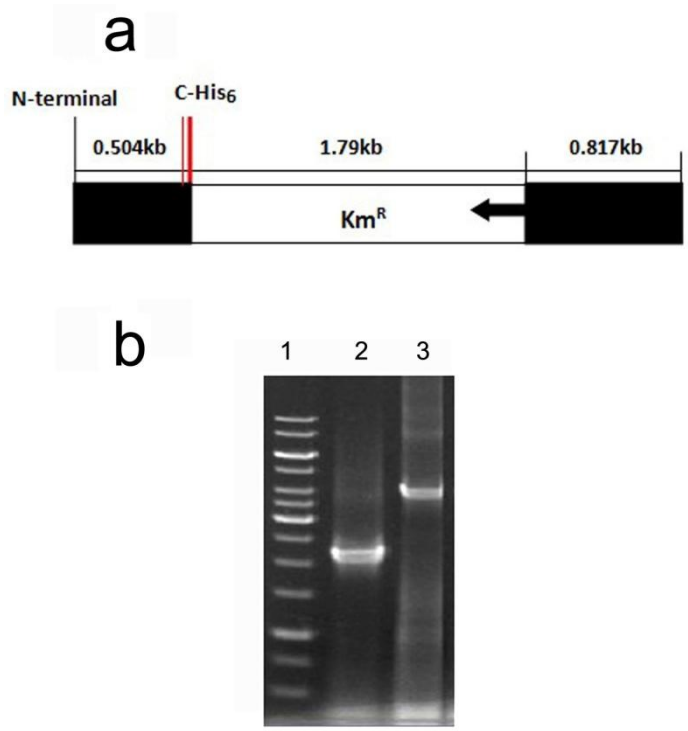

Figure S1 Construction of *Synechocystis* mutant containing His-tagged ApcD. **a)** Construction scheme for mutation plasmid pBlue-*apcD-histag*-Km^R-downstream. **b)** Complete segregation checked via PCR. **1**. DNA ladder (from top to bottom): 10,000, 8,000, 6,000, 5,000, 4,000, 3,500, 3,000, 2,500, 2,000, 1,500, 1,000, 750, 500 bp; **2**. Wild type (2171 bp); **3**. Mutant (3933 bp).

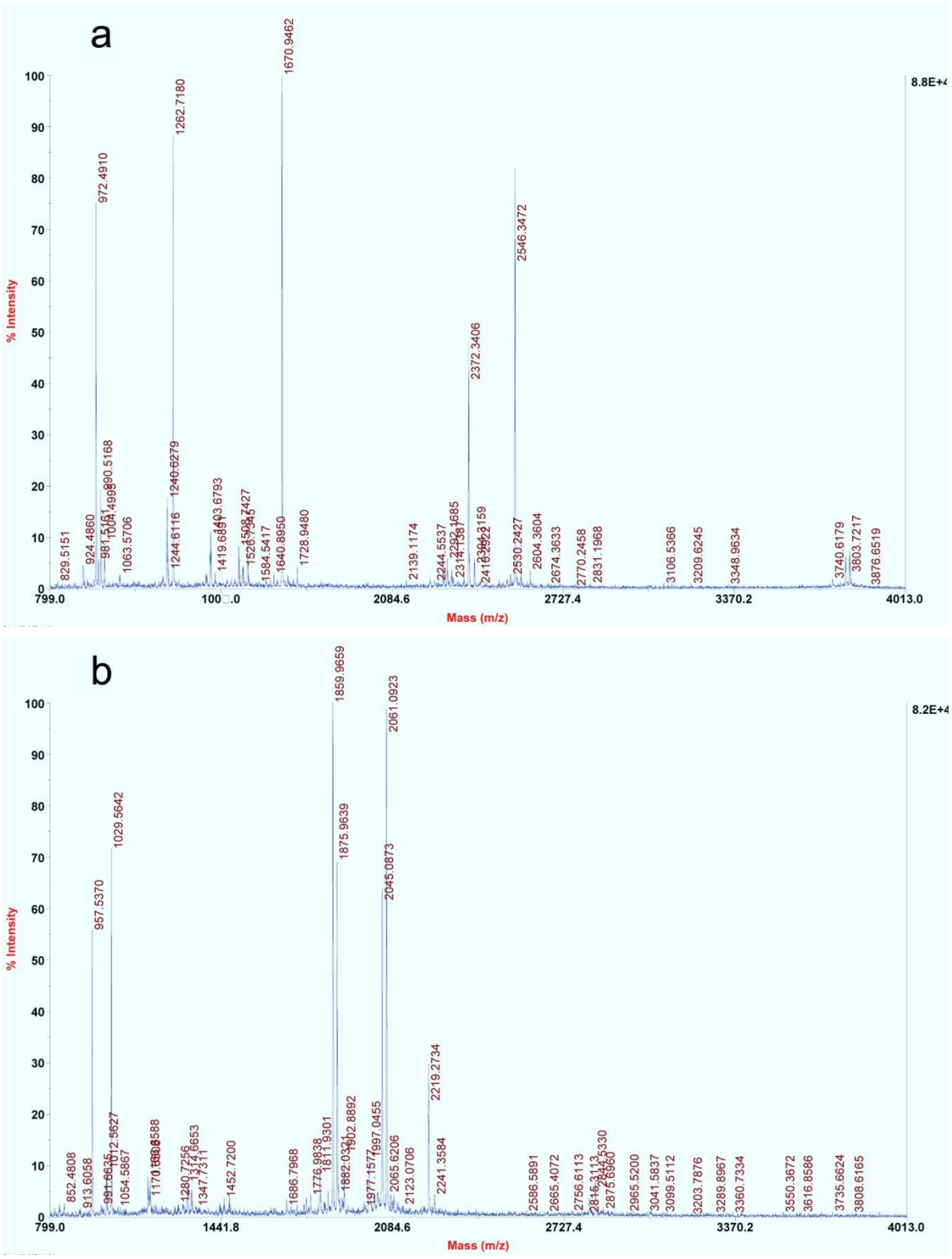

Figure S2 MALDI-TOF mass spectra of AP-B subunits extracted from the 2D electrophoresis (Supplementary Fig. S1). **a)** ApcD, **b)** ApcB.

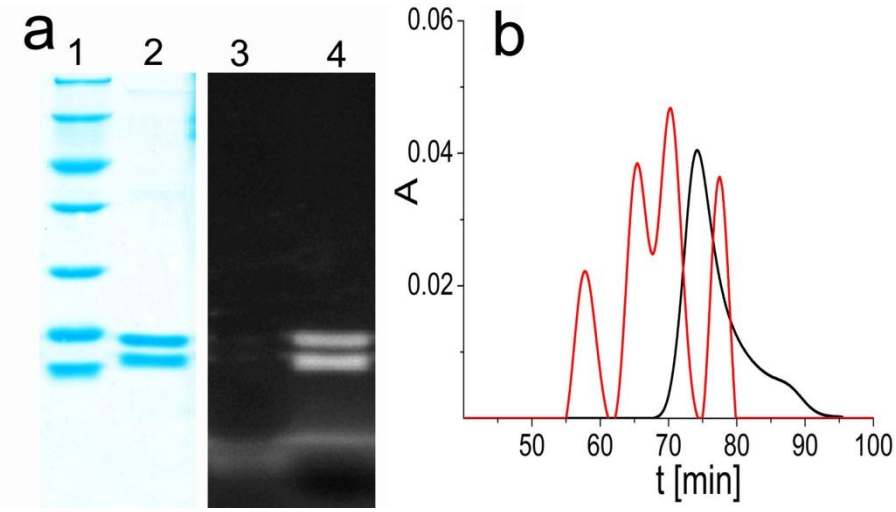

Figure S3 Characterization of affinity-purified AP-B used for crystallization. a) SDS-PAGE of the sample (lanes 2,4) and standard proteins (lanes 1,3). Lanes 1,2 were stained with Coomassie brilliant blue, lanes 3,4 show the zinc induced fluorescence of the same gel. No protein was found with mw <14 kDa, showing the absence of L_C. Molecular markers (lane 1, from top to bottom): 116, 66.2, 45, 35, 25, 18.4, 14.4 kDa. **b)** Gel filtration of the native sample (black) and a protein standard (red) on Superdex 200 in KPB (20 mM, pH 7.2) containing NaCl (0.1 M). The main peak of AP-B eluting at 74.1 min corresponds to a mw of 104 kDa, corresponding to a trimer $(ApcD/ApcB)_3$ (calculated 108 kDa). The molecular markers (peaks from left to right of the red-labeled chromatogram) have masses of 443, 200, 150 and 66 kDa.

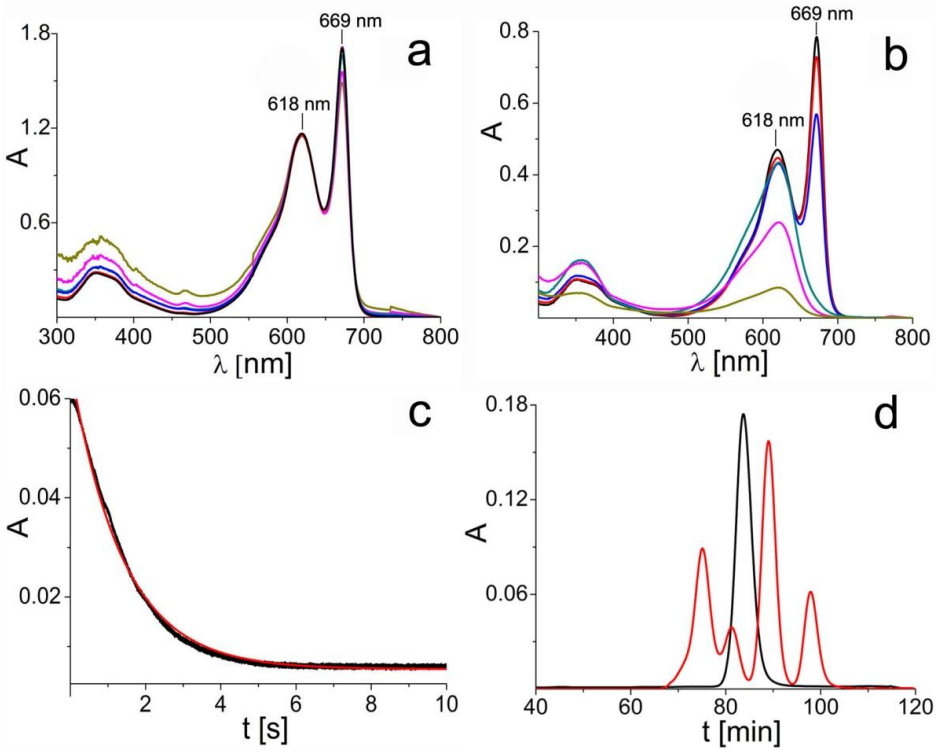

Figure S4 Absorption spectral changes of AP-B induced by dilution or addition of urea. **a)** Absorption changes (normalized at 618 nm) induced by dilution of AP-B with KPB (20 mM, pH 7.2) containing NaCl (0.1 M). The red-most absorptions at 669 nm began to decrease at AP-B concentrations below 0.1 μM. Sample concentrations (based on trimer) are 1.5 (black), 0.75 (red), 0.38 (blue), 0.19 (dark cyan), 0.095 (magenta), 0.048 μM (dark yellow). **b)** Absorption changes induced by addition of urea: 0 M (black), 0.5 M (red), 1 M (blue), 2 M (dark cyan), 4 M (magenta) and 8 M (dark yellow). The red-shifted trimer absorption nearly disappeared already at 2 M urea. **c)** Dissociation kinetics (dark line) with urea fitted with a one-exponential model (red line), giving a decay half-time of 1.38 s with R-square of 0.9962. AP-B in KPB (20 mM, pH 7.2) containing NaCl (0.1M) was mixed with 4 M urea in the same buffer (1:1, v/v) in a stopped-flow apparatus and the absorption monitored at 669 nm. **d)** Gel filtration of the AP-B sample (black) on Superdex 200 in KPB (20 mM, pH 7.2) containing NaCl (0.1 M) and urea (2 M). The main peak of AP-B eluting at 83.7 min corresponds to a mw of 40 kDa, corresponding to a monomer $(ApcD/ApcB)_1$ (calculated 36 kDa). The molecular markers (peaks from left to right of the red-labeled chromatogram) have masses of 66, 45, 29 and 12 kDa.

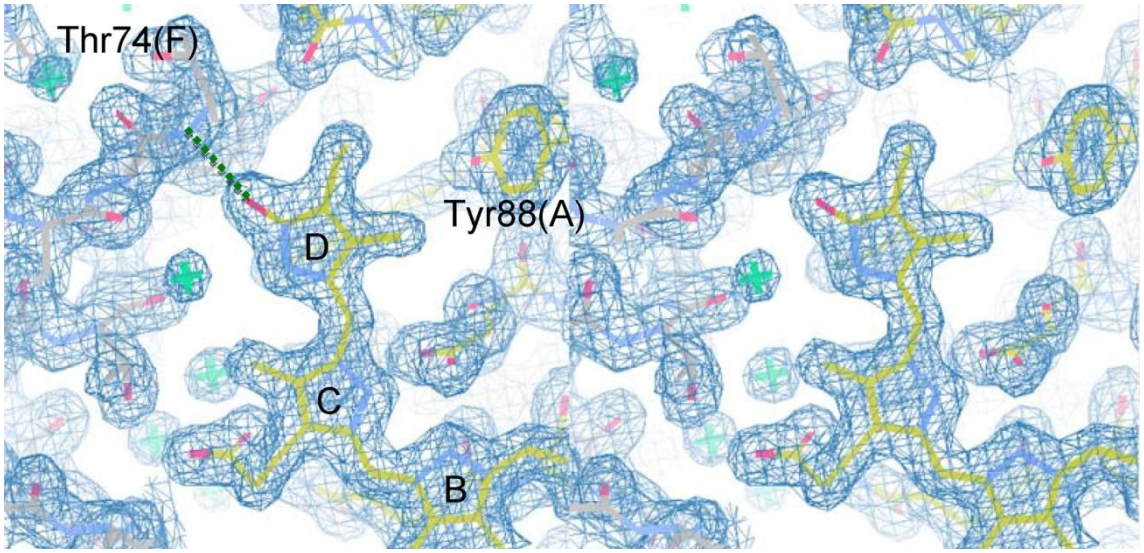

Figure S5 Side-by-side stereo view of the 2Fo-Fc map (contoured at 2σ) at the interface between two monomers (chain A of ApcD in yellow; chain F of ApcB in grey). The rings B/C/D of the PCB chromophore are nearly co-planar. The green dashed line marks the hydrogen bond between the main chain nitrogen of Thr74(F) and the carbonyl group of ring D.

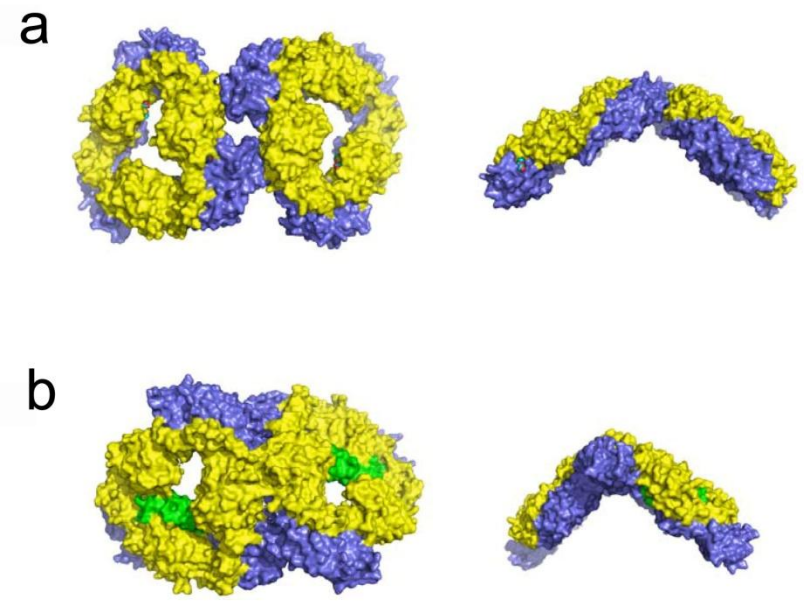

Figure S6 Edge-to-edge interaction between two trimers in AP-B (**a**) and APC (**b**; PDB ID 1B33). α subunits are colored in blue and β subunits in yellow, and the small core linker, L_C, in green**.**

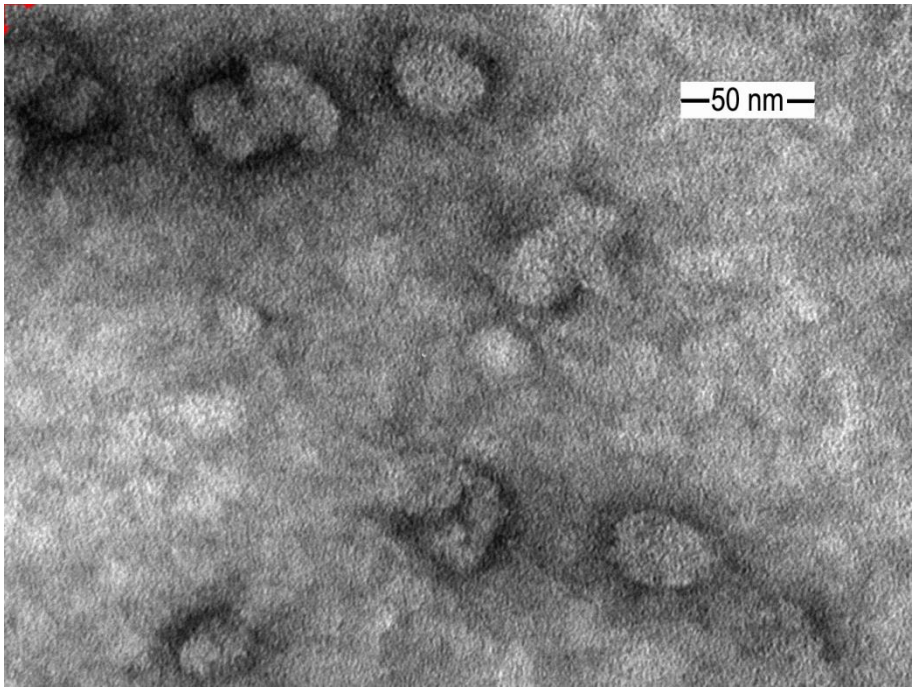

Figure S7 AP-B aggregates on the surface of a copper grid as seen by negative staining electron microscopy. Magnification 300,000 x.

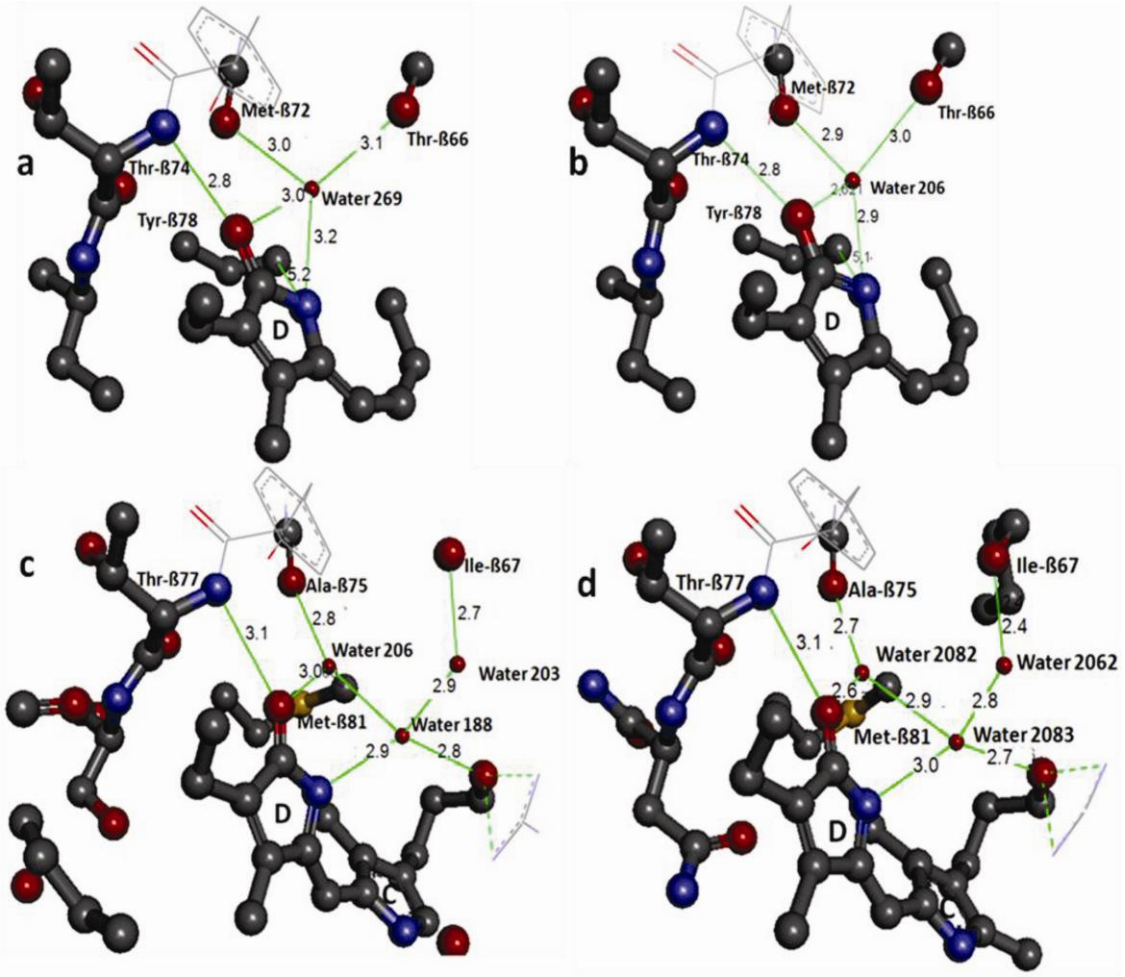

Figure S8 Characteristic inter-monomer interactions in trimers of allophycocyanins (top) and C-phycocyanins (bottom). H-bonding network of ring D amide group of PCB bound to the α-subunit with amino acid residues of ApcB. **a)** AP-B (this work), **b)** APC_3L_C (pdb 1B33) (Reuter *et al.*, 1999), **c)** CPC from *Fremyella diplosiphon* (pdb 1CPC) (Duerring *et al.*, 1991) and **d)** CPC from *Gracilaria chilensis* (pdb 2BV8) (Contreras-Martel *et al.*, 2007). The chromophore and residues <5.5 Å from ring D and/or interacting via H-bonds are shown in ball-and-stick representation. The Tyr-residue shown on top in stick representation is common to β-subunits of CPC (numbered as β78), AP-B and APC (numbered as β73). Distances indicated by green lines are given in grey. Thr66, Met-72, Thr74,75, Tyr78 and the single water are characteristic of allophycocyanin β-subunits. Ile67, Ala75, Met81 are characteristic of C-phycocyanin β-subunits. Thr74 and the corresponding Thr77 are common to both allophycocyanins and C-phycocyanins, respectively. Plots generated with Discovery Studio V3.5 (Accelrys).

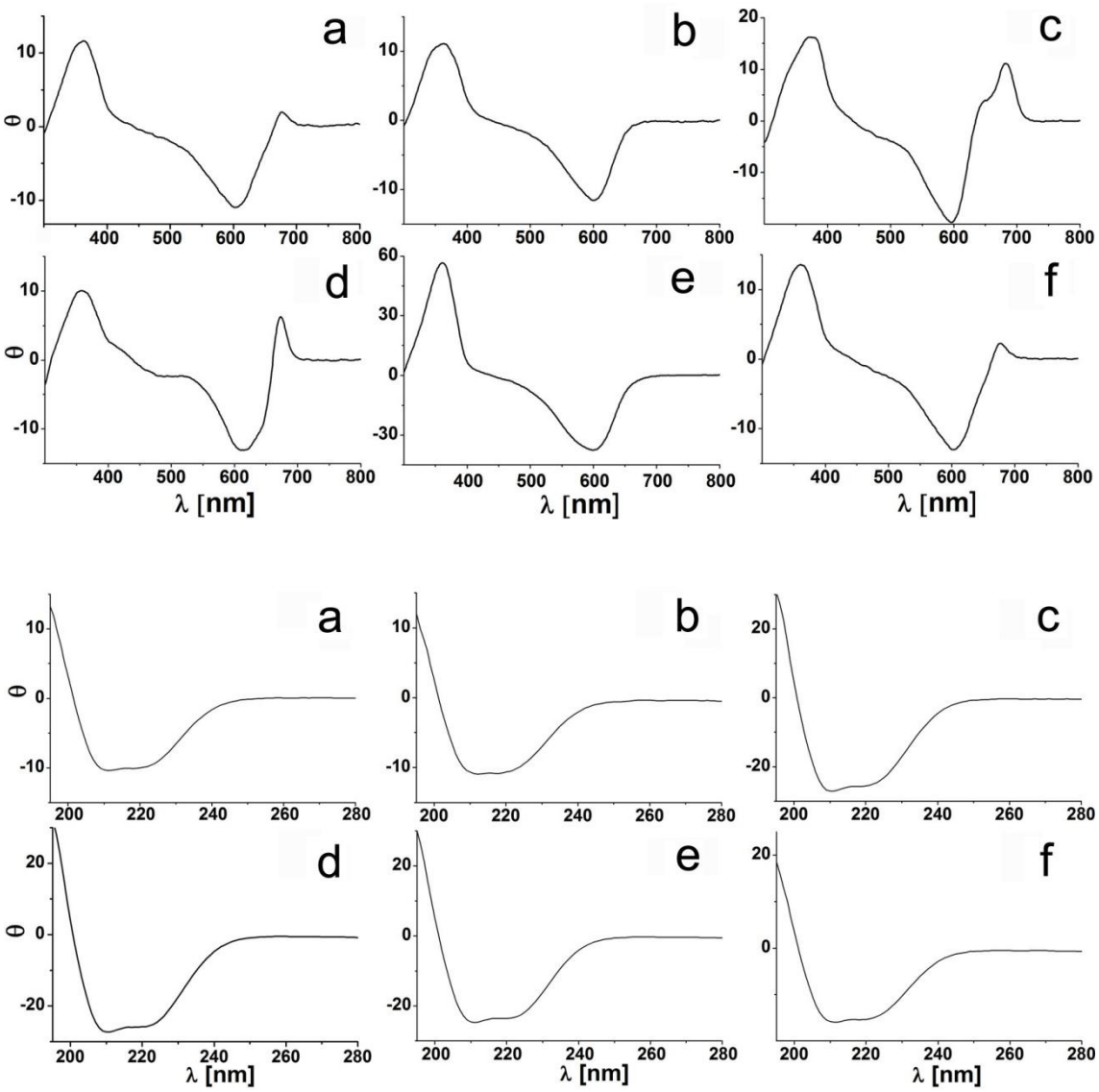

Figure S9 Visible (top) and UV (bottom) circular dichroism (CD) spectra of chromophorylated ApcD and its mutants. His-tagged wild type ApcD (**a**), and mutated apoproteins Y65V (**b**), Q80T (**c**), Y85L (**d**), W87Y (**e**), and M126V (**f**) were generated and chromophorylated with PCB in *E. coli* and then purified via Ni^{2+} affinity column. All spectra were measured in KPB (50 mM, pH 7.2) containing NaCl (0.5 M). The quantitative absorption and fluorescence spectra are shown in Supplementary Table S3.

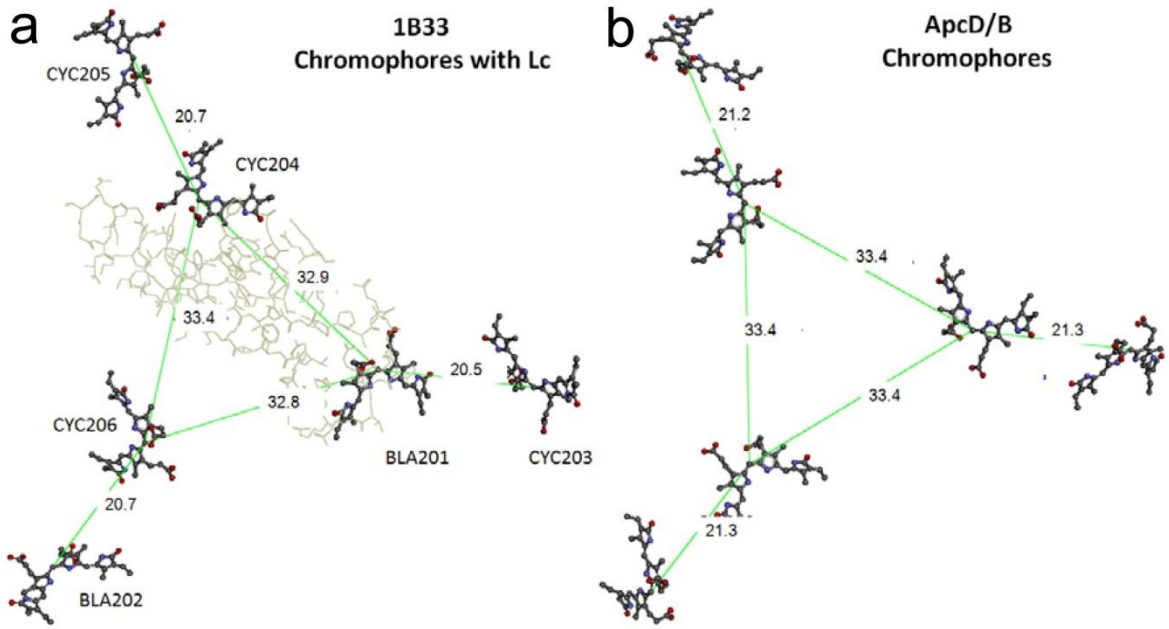

Figure S10 Distances between chromophores in APC_3L_C **(a)** (Reuter *et al.*, 1999) and AP-B **(b).** The center-to-center (C-10 to C-10) distances in AP-B are slightly longer than those in APC_3L_C. The trimers are seen from top. In APC_3L_C, the core linker, L_C, is in shown in light grey, and the numbering is from Reuter et al. (Reuter *et al.*, 1999) . Plots generated with Discovery Studio V3.5 (Accelrys).

Table S1 Primers for *Synechocystis* mutants containing ApcD-Histag (P1-P4) and primers for ApcD mutants (P5-P14).

Primer	Sequence	DNA
P1	5′-GGCGCCCTCGAGATGAGTGTAGTTAGTCAAGTTATTTTGCA-3′	*apcD-histag*
P2	5′-ATAGAATTCTCAATGATGATGATGATGATGGGACATAAACTGAATGATG TAA-3′	
P3	5′-CTGGAATTCTTTGTTTTGGCACGAAGATTAA-3′	*apcD downstream sequence*
P4	5′-CAGTCTAGAGGAAGCCATAGAAAAGGGGAAA-3′	
P5	5′-GTTTAAGAAGCACCCTGAAGTTCGTGCTCCCGGAG-3′	*apcD(Y65V)*
P6	5′-CTCCGGGAGCACGAACTTCAGGGTGCTTCTTAAAC-3′	
P7	5′-AGCGGCAATATAATACCTGTTTGCGCGATTACGGTTG-3′	*apcD(Q80T)*
P8	5′-CAACCGTAATCGCGCAAACAGGTATTATATTGCCGCT-3′	
P9	5′-GTGTTTGCGCGATTTAGGTTGGTATTTGCGCCT-3′	*apcD(Y85L)*
P10	5′-AGGCGCAAATACCAACCTAAATCGCGCAAACAC-3′	
P11	5′-CGCGATTACGGTTACTATTTGCGCCTAGTTAC-3′	*apcD(W87Y)*
P12	5′-GTAACTAGGCGCAAATAGTAACCGTAATCGCG-3′	
P13	5′-GTGCCAGGGATGGTTGACGCGGTAACTGTA-3′	*apcD(M126V)*
P14	5′-TACAGTTACCGCGTCAACCATCCCTGGCAC-3′	

Table S2 Plasmids used. The pACYCDuet, pCDFDuet and pET30, from Novagen, are T7 promoter expression vectors. pACYCDuet and pCDFDuet are designed to co-express two target proteins in *E. coli*.

Using the three vector-derivatives together with compatible replicons and antibiotic resistance, 5 proteins could be co-expressed in the same cell, thereby generating the respective designed phycobiliproteins in *E. coli*. Subscripts indicate the strain of the parent organisms.

Antibiotic Resistance	Plasmids with P15A replicon pACYCDuet derivatives	Plasmids with CloDF13 replicon pCDFDuet derivatives	Plasmids with ColE1 replicon pET30 derivatives
Kanamycin			pET-apcD$_{PCC6803}$ pET-apcD(Y65V)$_{PCC6803}$ pET-apcD(Q80T)$_{PCC6803}$ pET-apcD(Y85L)$_{PCC6803}$ pET-apcD(W87Y)$_{PCC6803}$ pET-apcD(M126V)$_{PCC6803}$
Chloramphenicol	pACYC-ho1$_{PCC7120}$-pcyA$_{PCC7120}$		
Streptomycin		pCDF-cpcS$_{PCC7120}$	

Table S3 Quantitative absorption and fluorescence data of ApcD and its mutants from *Synechocystis* PCC 6803.

Proteins were generated and chromophorylated with PCB in *E. coli*, and purified by Ni-affinity column chromatography. Spectra were obtained in potassium phosphate buffer (20 mM, pH 7.0) containing NaCl (0.5 M). Extinction coefficients (Glazer & Fang, 1973) and fluorescence yields (Cai *et al.*, 2001) were determined by standard methods and averaged from two independent experiments. The last four rows give for comparison are from *Nostoc* PCC 7120 (Wang *et al.*, 2010).

Phycobiliproteins (after purification)	Absorption		Fluorescence (excitation at 580 nm)	
	λ_{max} [nm]	ε_{Vis} [$M^{-1}\bullet cm^{-1}$]	λ_{max} [nm]	Φ_F
Synechocystis				
PCB-ApcD	625	4.9×10^4	642	0.104
PCB-ApcD(Y65V)	606	5.4×10^4	633	0.130
PCB-ApcD(Q80T)	648	5.2×10^4	640	0.096
PCB-ApcD(Y85L)	648	6.7×10^4	655	0.091
PCB-ApcD(W87Y)	616	3.0×10^4	652	0.095
PCB-ApcD(M126V)	630	4.3×10^4	642	0.102
Nostoc				
PCB-ApcD	650	6.2×10^4	663	0.074
PCB-ApcD(W87E)	602	10.1×10^4	635	0.22
PCB-ApcD(Y116S)	601	5.6×10^4	640	0.10
PCB-ApcD(M126S)	600	7.2×10^4	638	0.07

Table S4 The red shift in absorption and the angle of the ring plane of PCB in the crystal structure of AP-B, APC and CPC.

The absorption maximum of α-81 of AP-B is taken from this work, and absorption maxima of α-81 of APC, APC-Lc and β-81 of AP-B, APC and APC-Lc (MacColl, 2004), and those of α-84 (Fairchild *et al.*, 1992), β-82 (Zhao *et al.*, 2006) and β-153 (Zhao *et al.*, 2007) of CPC are from the respective references.

Phycobiliproteins		Ring A/B N-C_4-C_6-N	Ring B/C N-C_9-C_{11}-N	Ring C/D N-C_{14}-C_{16}-N	∑	A_{max} (nm)
AP-B	α-81	16.6°	10.8°	2.6°	30.0°	669
(4PO5)	β-81	21.9°	16.8°	16.0°	54.7°	615
APC	α-81	20.0°	13.3°	0.6°	33.9°	650
(1ALL)	β-81	18.2°	17.8°	40.8°	76.8°	615
APC-Lc	α-81	27.8°	6.4°	3.5°	37.7°	652
(1B33)	β-81	20.7°	10.5°	36.3°	67.5°	615
C-PC	α-84	13.3°	18.8°	37.4°	69.5°	614
(1KTP)	β-82	21.4°	15.0°	42.2°	78.6°	618
	β-153	33.3°	20.9°	40.2°	94.4°	596

Supplementary References

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