Supporting Information

CpeF is the bilin lyase that ligates the doubly linked phycoerythrobilin on β-phycoerythrin in the cyanobacterium *Fremyella diplosiphon*

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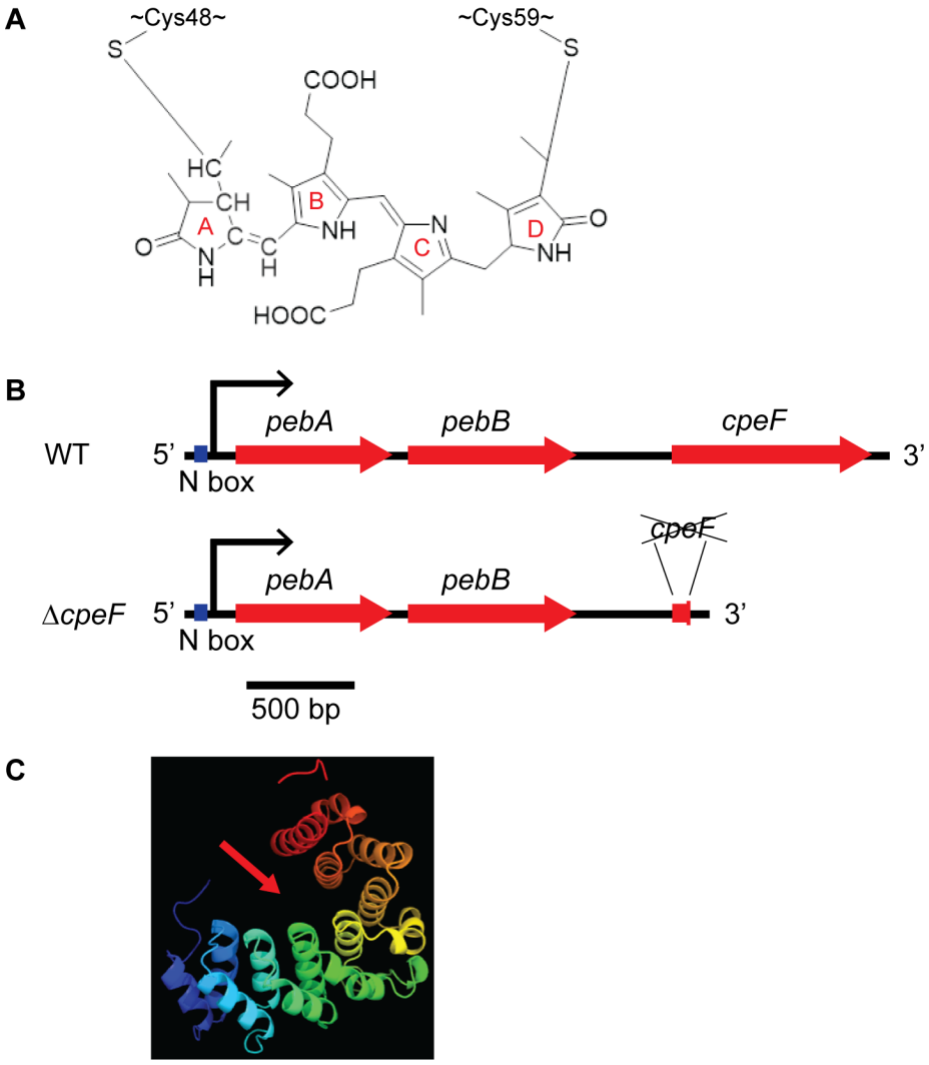

Fig. S1: Doubly bound PEB structure, the *peb* operon and modeled CpeF structure
(A) Structure of PEB doubly bound to specific Cys residues (Cys-48 and Cys-59 of CpeB from *F. diplosiphon*) of phycobiliproteins by thioether linkages to the A and D rings (1). **(B)** Diagrams of the *peb* operon containing the genes that encode the PEB synthesis proteins (PebA and PebB) and the bilin lyase CpeF from WT and Δ*cpeF* strains of *F. diplosiphon*. Possible transcription regulation site is labeled "N box" (2). **(C)** Predicted 3D structure of CpeF from *F. diplosiphon* using Phyre[2] prediction system (3,4). The red arrow indicates the possible PEB binding location in the groove of CpeF.

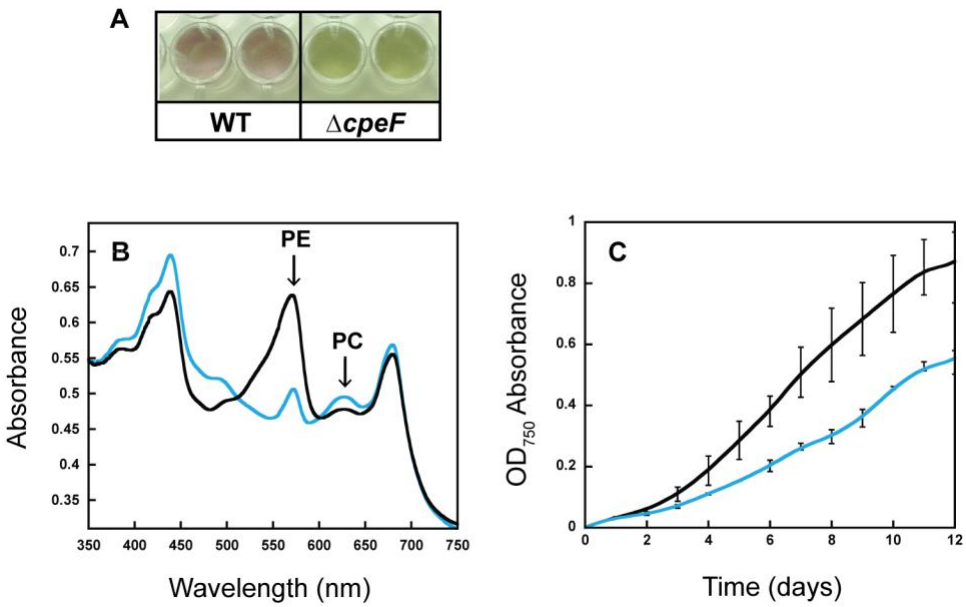

Fig. S2: Phenotypes of *F. diplosiphon* strains grown in ~15 µmol m^{-2} s^{-1} of green light
(A) Liquid cell cultures of WT and Δ*cpeF F. diplosiphon* grown in approximately ~15 µmol m^{-2} s^{-1} of green light. **(B)** Whole cell absorbance spectra of WT (black line) and Δ*cpeF* (cyan line) cells. PE and PC peaks are indicated with arrows. **(C)** Average growth curves of two independent replicates each for WT (black line) and Δ*cpeF* (cyan line) cells with OD readings taken at 750 nm every 24 hours for 12 days. Error bars represent the ranges.

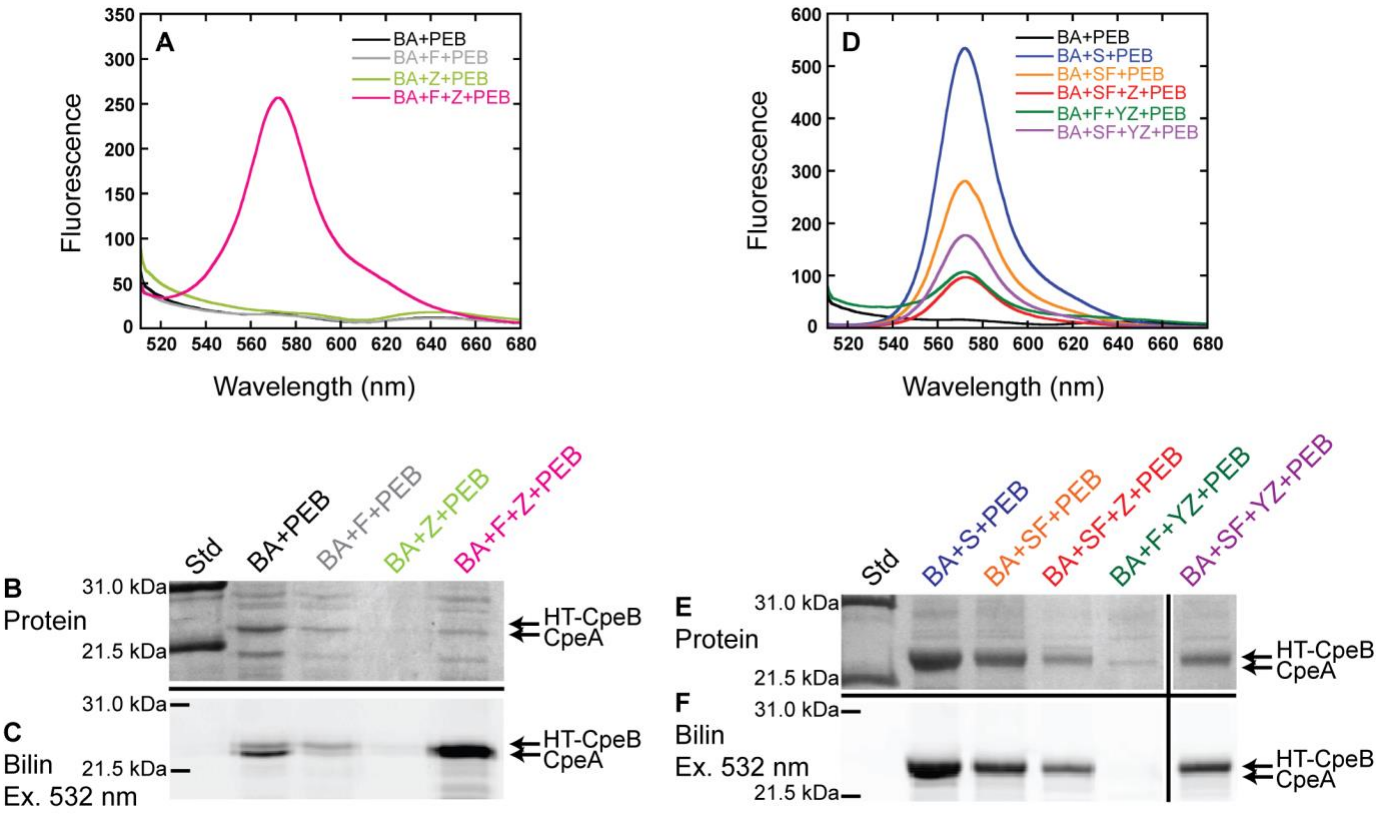

Fig. S3: Recombinant CpeF coexpressions with HT-CpeBA
(A) Fluorescence emission (excitation set at 490 nm) spectra of purified HT-CpeBA obtained from *E. coli* cells expressing pCpeBA and pNT-PebS (BA+PEB; black) in addition to pCpeF2 (BA+F+PEB; grey); pNT-CpeZ3 (BA+Z+PEB; lime green); or pCpeF2 and pNT-CpeZ3 (BA+F+Z+PEB; magenta). All spectra were obtained using undiluted samples. **(B)** The Coomassie-stained SDS-polyacrylamide gel for purified HT-CpeBA samples from panel A. Lane "Std" indicates the molecular weight standard. **(C)** The Zn-enhanced fluorescence of the gel in panel B excited at 532 nm. **(D)** Fluorescence emission (excitation set at 490 nm) spectra of purified HT-CpeBA obtained from *E. coli* cells expressing pCpeBA and pNT-PebS (BA+PEB; black) in addition to pCpeS (BA+S+PEB; blue); pCpeSF2 (BA+SF+PEB; orange); pCpeSF2 and pNT-CpeZ3 (BA+SF+Z+PEB; red); pCpeF2 and pNT-CpeYZ (BA+F+YZ+PEB; green); or pCpeSF2 and pNT-CpeYZ (BA+SF+YZ+PEB; purple). All spectra were obtained by diluting the samples 151-fold except for the two samples BA+PEB and BA+F+YZ+PEB which were undiluted. **(E)** The Coomassie-stained SDS-polyacrylamide gel for purified HT-CpeBA samples from panel D. Lane "Std" indicates the molecular weight standard. **(F)** The Zn-enhanced fluorescence of the gel in panel E excited at 532 nm. These results are representative of three independent replicates.

Table S1: Spectral properties of PBS purified from WT and Δ*cpeF*

Strain and fraction	PE Abs λ_{max} (nm)	PC Abs λ_{max} (nm)	AP Abs λ_{max} (nm)	Fluorescence emission λ_{max} (nm) excited at 490 nm
WT bottom	568	615	649	582 (sh), 669
Δ*cpeF* middle	577	617	649	576 (sh), 673
Δ*cpeF* bottom	577	617	649	576 (sh), 673

"Abs" is an abbreviation for absorbance.
"sh" is an abbreviation for shoulder in the spectrum.

Table S2: Observed MALDI-MS peaks of tryptic digested recombinant HT-CpeB peptides

Sample*	CpeB Cys-80 (*m/z*)	CpeB Cys-165 (*m/z*)	CpeB Cys-48/Cys-59 (*m/z*)
B+F+PEB	ND	ND	ND
B+S+PEB	1250.7	ND	ND
B+S+F+PEB	1250.7	ND	ND
BZ+F+PEB	ND	ND	ND
BZ+S+PEB	1250.7	ND	ND
BZ+S+F+PEB	1250.7	ND	4627.4

CpeB peptides contain a PEB chromophore (587 *m/z*) at the specified Cys residues when indicated by an *m/z* value which are represented with a +1 charge state.
"ND" represents PEB modified peptides that were not detected in MS results.
* See Fig. 4 for sample descriptions.

Table S3: Oligonucleotide primers used in this study

Primer name[a]	Sequence (5' to 3')[b]	Plasmid(s) created
cpeF-f2	CCAACAGAGGAAGATCCCGCGCAAATTGAACGTCAATCCGAAGAAGATGATTGGTAAC	*cpeF* deletion construct
cpeF-r	CGGATTGACGTTCAATTTGCGCGGGATCTTCCTCTGTTGGAGCTTCTG	*cpeF* deletion construct
Nco-cpeF-r2	TCACCATGGATTACATCAACGTGCAGGTAGC	*cpeF* deletion construct
Nco-cpeF-f	TCACCATGGCCATTCCCCCAGGACTAGACTATCAGGTGAGCGATCGCGG	*cpeF* deletion construct
FdcpeA.R.EcoRI	AAGAATTCCTAGGAGAGAGAGTTAATAGCGTA	pCpeBA
HTcpeB.F.BamHI	AAGGATCCGATGCTTGATGCTTTTTCTAGAGC	pCpeB2; pCpeBA; pCpeBZ
HTcpeB.R.EcoRI	CCGAATTCTTAGCTCAAAGCAGAGATTACGCG	pCpeB2; pCpeBA; pCpeBZ
FdCpeB(C48S)	[phos]CAACGCTAGCTCCATGGTTTCTG	pCpeB(C48S)CpeA
FdCpeB(C59S)	[phos]CTGGAATGATCTCCGAAAACCAAGG	pCpeB(C59S)CpeA
pETDuet(XhoI del)	[phos]ACGTCGGTACCCTCCAGTCTGGTAAAGAAACCGCTG	pCpeB(C48S)CpeA; pCpeB(C59S)CpeA
HTcpeF2.F.XhoI	GCTCGAGAAGTCAATCACTCAACTCAG	pHT-CpeF2
HTcpeF2.R.HindIII	CCAAGCTTCCAATCATCTTCTTCGG	pHT-CpeF2
FdcpeF.F.NdeI	AATTTGTGCATATGAGTCAATCACTCAACTCAGAA	pCpeF2; pCpeSF2
FdcpeF.R.XhoI	AACTCGAGTTACCAATCATCTTCTTCGGATTG	pCpeF2; pCpeSF2
FdcpeS.F.NcoI	AACCATGGAAACCAAAGTGTTGATGAATATTACA	pCpeSF2
FdcpeS.R.PstI	AACTGCAGCTAGGCACCAGTGTTTATGAAAAAATG	pCpeSF2
FdcpeY.F.BglII	GATCAGATCTATGGATAAGCGCTTTTTTAATTTC	pNT-CpeYZ
FdcpeZ.R.XhoI	GATCCTCGAGTTATTTTTCTCCCCGCTG	pNT-CpeYZ
cpeZ.F.EcoRV	TGGATATCGATGCCGACAACAGAAG	pCpeBZ; pNT-CpeZ3
cpeZ.R.XhoI	CACTCGAGTTTATTTTTCTCCCCGCT	pCpeBZ; pNT-CpeZ3

[a] "F" and "R" are the forward and reverse primers, respectively.

[b] Engineered restriction enzyme sites are underlined.

Table S4: Plasmids used in this study

Plasmid name[a]	Recombinant proteins produced[b]	Parent vector	Antibiotic[c]	Reference
pPebS	Myovirus HO1 and HT-PebS	pACYCDuet-1	Cm	(5)
pNT-PebS	Myovirus HO1 and PebS	pACYCDuet-1	Cm	(6)
pCpeB2	*F. diplosiphon* HT-CpeB	pCDFDuet-1	Sp	This study
pCpeBA	*F. diplosiphon* HT-CpeB and CpeA co-expressed on 1 mRNA	pETDuet-1	Ap	This study
pCpeB(C48S)CpeA	*F. diplosiphon* HT-CpeB(C48S) and CpeA	pCpeBA	Ap	This study
pCpeB(C59S)CpeA	*F. diplosiphon* HT-CpeB(C59S) and CpeA	pCpeBA	Ap	This study
pCpeBZ	*F. diplosiphon* HT-CpeB and CpeZ	pCpeB2	Sp	This study
pCpeF2	*F. diplosiphon* CpeF	pCDFDuet-1	Sp	This study
pHT-CpeF2	*F. diplosiphon* HT-CpeF	pBAD-*Myc*-His A	Ap	This study
pCpeS	*F. diplosiphon* CpeS	pCOLADuet-1	Km	(7)
pCpeSF2	*F. diplosiphon* CpeS and CpeF	pCDFDuet-1	Sp	This study
pNT-CpeYZ	*F. diplosiphon* CpeY and CpeZ	pCOLADuet-1	Km	This study
pNT-CpeZ3	*F. diplosiphon* CpeZ	pCOLADuet-1	Km	This study

[a] "NT-" is an abbreviation for "Non-Tagged" referring specifically to hexahistidine-tags.
[b] Proteins produced as hexahistidine-tagged fusions are indicated as "HT-".
[c] Antibiotic resistance used to select for presence of the plasmid (Ap: ampicillin; Cm: chloramphenicol; Km: kanamycin; Sp: spectinomycin).

References

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