Supporting Information

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SI Materials and Methods

Cloning and Expression. All genetic manipulations are carried out according to standard protocols (1). Plasmids pACYC-ho1-pcyA for PCB and pET-cph1(1-514) have been reported (2–4). To facilitate purification via Ni^{2+}-affinity chromatography as well as subsequent crystallization, *cpcE*, *cpcF*, and *nblB* from *Nostoc* are amplified by PCR (Table S4) and ligated into the expression vector pET30 (Novagen) via restriction sites NdeI and XhoI (Fermentas) for C-terminal fusion to a His_6 tag. Then *cpcE*(*Δhis-tag*) and *cpcF*(*Δhis-tag*), in which the His_6 tag is deleted, are generated from *cpcE* and *cpcF* via PCR with primers P5–P8. *cpcA* and *pecA* from *Nostoc* are amplified via PCR with primers P9, P10, P11, and P12 and ligated into the expression vector pET30 (Novagen) via restriction sites NcoI and XhoI (Fermentas) for fusing an N-terminal His_6 tag. *cpcE* and *cpcF*, *pecE* and *pecF*, *nblB*, and *cpcF* are amplified by PCR (for primers see Table S4) and then cloned in the two cloning sites of pCDFDuet (Novagen) via restriction sites NcoI, PstI, NdeI, and XhoI (Fermentas), respectively. To analyze the structural effects on lyase activities of CpcE, CpcF, PecE, and PecF, site-directed variants are constructed by using the MutanBEST kit (TaKaRa) with primers listed in Table S4.

For overexpression of the lyases and their variants, the respective plasmids (Table S5) are transformed into *Escherichia coli* BL21 (DE3) (Novagen). Cells are grown at 16 °C in Luria–Bertani (LB) medium supplemented with kanamycin (20 μg/mL) and induced with isopropyl β-D-thiogalactoside (1 mM), and then growth is continued for an additional 12 h after induction. For chromophorylation, pET-derived expression vectors for CpcA or PecA are transformed into *E. coli* BL21 (DE3) (Novagen) containing the PCB-generating plasmid (pACYC-ho1-pcyA) and lyase-generating plasmid (pCDF-cpcE-cpcF, pCDF-pecE-pecF, or derivatives) (Table S5). The triply transformed cells are cultured at 16 °C in LB medium supplemented with kanamycin (20 μg/mL), chloromycetin (17 μg/mL), and streptomycin (25 μg/mL). After induction with isopropyl β-D-thiogalactoside (1 mM) for 16 h, the cells are collected by centrifugation.

Cell pellets containing His-tagged CpcF and excessive CpcE(Δhis tag) lacking the His tag are resuspended in ice-cold potassium phosphate buffer (KPB; 20 mM, pH 7.3) containing 0.5 M NaCl and disrupted by five passages through a chilled French press at 1,200 bar. The suspension is centrifuged at 12,000 × *g* for 40 min at 4 °C. The supernatant containing crude proteins is purified via Ni^{2+}-affinity chromatography and eluted by using KPB (20 mM, pH 7.3) containing 0.5 M NaCl and 0.5 M imidazole. After purification via Ni^{2+}-affinity chromatography (5), the sample of the complex of CpcE and CpcF is dialyzed twice against KPB (20 mM, pH 7.3) containing 150 mM NaCl and subsequently purified further via gel filtration (HiLoad 16/600, Superdex 200; GE Healthcare) with elution of the same buffer at a flow rate of 1.0 mL/min.

Protein Assays. Protein concentrations are determined by the Bradford assay (6) and calibrated with BSA, and SDS/PAGE is performed with the buffer system of Laemmli (7). Proteins are stained with Coomassie brilliant blue, and those containing chromophores are identified by Zn^{2+}-induced fluorescence (8).

Spectral Analyses. All chromoproteins are investigated by UV-Vis absorption spectroscopy (Beckman-Coulter DU 800). Covalently bound chromophores are quantified after denaturation with acidic urea (8 M, pH 1.5) by their absorptions at 662 nm (PCB) by using an extinction coefficient of 35,500 $M^{-1}{\cdot}cm^{-1}$ (9). Fluorescence spectra are recorded at room temperature with a F320 spectrofluorimeter (TianJin GangDong Sci and Tech Development Company).

Oligomerization Analysis. To determine the oligomeric state of the CpcE/F complex and its variants, the proteins are first purified by Ni^{2+}-affinity chromatography. A total of 1.0 mL of the eluate is loaded on a Superdex 200 preparative grade column (60 × 1.6 cm) and developed (1.0 mL/min) with KPB (20 mM, pH 7.3) containing NaCl (0.15 M). The apparent molecular mass is determined by comparison with a marker set (12–443 kDa).

Assay of Enzymatic Activities. Relative enzymatic activities of CpcE/F or PecE/F variants are compared with the respective wild-type lyases by a fluorescence assay, based on the fluorescence of the chromophorylated PBPs, with controls in the absence of the respective lyases and variants (5). Chromophorylated PBPs, PCB^T-CpcA or PVB-PecA, were assembled in BL21(DE3) cells. After assembly, the relative amounts of the products are evaluated by their fluorescence emissions at 643 and 583 nm (10, 11), respectively.

Activity of PCB Binding to Lyases. To evaluate the relative activity of PCB binding, the purified complex of CpcE/F or its variants are incubated with PCB in KPB (0.5 M, pH 7.3) containing NaCl (0.5 M) for 1 h. The lyase is then purified by Ni^{2+}-affinity chromatography, and the amount of bound PCB is quantitated by the fluorescence emission at 646 nm (10).

Assay of Chromophore Detachment Activities. The assay is based on chromophore transfer from covalently bound PCB^T–CpcA to the apo-protein of the truncated phytochrome fragment, Cph1(1–514), of Cph1 from *Synechocystis* PCC 6803 (4), in the presence of NblB, CpcE, CpcF, and variants thereof (12). PCB^T–CpcA (10 μM) and Cph1(1–514) (20 μM) are incubated at 37 °C with CpcE/F or variants (10 μM) in KPB (20 mM, pH 7.3) containing NaCl (150 mM), $MgCl_2$ (5 mM), and mercaptoethanol (5 mM). The transfer is followed by the decrease of absorption of PCB^T–CpcA at 618 nm and the increase of absorption of PCB^T–Cph1(1–514) at 658 nm. For measuring the related enzymatic parameters, the concentration of the donor holoprotein, PCB^T–CpcA, is varied (0.5, 1.0, 2.0, 3.0, and 4.0 μM) in a range well below that of the acceptor protein, Cph1(1–514), at 10 μM and, vice versa, by varying the concentration of the acceptor Cph1(1–514) (0.5, 1.0, 2.0, 3.0, and 4.0 μM) and maintaining the donor at 10 μM, while keeping all other conditions identical.

Crystallization and Data Collection. Initial screening for crystallization conditions of the purified CpcE/F complex is performed by using 96-well triple sitting drop plates (TTP Lab Tech; catalog no. 4150-05800) and commercial screening kits (PEGRx; Hampton Research). Crystallization droplets are prepared by mixing 200 nL of protein solution (15 mg/mL in 20 mM KPB, pH 7.3, and 0.15 M NaCl) with 200 nL of reservoir solution. They are equilibrated against 75 μL of the reservoir solution and stored at 293 K. Best diffracting crystals grow under conditions containing 0.1 M sodium acetate trihydrate (pH 4.8), 13% (wt/vol) PEG 10000 within 2 wk to a final size of 150 × 40 × 20 $μm^3$. Selenomethionine-substituted CpcE/F (CpcE/F–SeMet) is prepared according to the standard protocol (13) via the CpcE/F preparation method. After purification, CpcE/F–SeMet (15 mg/mL in 20 mM KPB, pH 7.3, and 0.15 M NaCl) is crystallized similarly as described above. Best diffracting crystals grow under conditions containing 0.1 M sodium acetate trihydrate, pH 4.8, 10% (wt/vol) PEG 10000, and 20 mM spermidine within 2 wk to a final size of 100 × 30 × 15 $μm^3$.

To cryoprotect CpcE/F crystals, the crystallization drops are covered with mineral oil (Sigma-Aldrich) before a single crystal is retrieved with a litho loop and flash-frozen in liquid nitrogen. Native

and SAD X-ray diffraction data are collected from a single crystal in each case on beamline P11 or P13, DESY, EMBL (Hamburg, Germany). All diffraction data are processed with the XDS program package and scaled by using XSCALE (14). The crystal structure is determined by the SAD method (15) using the selenomethionine-substituted crystals. The initial model is obtained using MRSAD on the Auto-Rickshaw server (16). The structure of native CpcE/F is determined by molecular replacement [Phaser in CCP4 (17) using CpcE/F-SeMet structure as starting model]. The structures of native CpcE/F are refined by iterative cycles of manual refinement using Coot and Refmac5 (18) from CCP4 suite (17). Data refinement statistics and model content are summarized in Table 1. The structure of complex of CpcE and CpcF from *Nostoc* is deposited at the Protein Data Bank (PDB ID code: 5N3U).

Structure Analyses, Modeling and Docking. Figures of protein structures are created by using PyMOL (https://pymol.org/2/) and Biovia Discovery studio (Version 16; accelrys.com/products/collaborative-science/biovia-discovery-studio/). Protein sequence alignments are created by using Clustal (www.clustal.org/).

The structures of PecE/F and of NblB from *Nostoc* are modeled by using the present crystal structure of CpcE/F from *Nostoc* as template (PDB ID code 5N3U), and those of apoproteins CpcA, CpcB, and PecA were modeled by using the respective crystal structures of holoproteins as template on the SWISS-MODEL server (19, 20). The structures were compared with the Swiss-PDBViewer (Version 4.1) (21).

For docking, the following components are used: CpcE/F (this work), PCB (ligand from the CpcT:PCB complex; PDB ID code 4O4S) (5), PecE/F (modeled *Nostoc* structure; this work), PCB^{T}–CpcA [α-subunit of CPC trimer from *T. elongatus* (PDB ID code 4ZIZ) (22)], and PecA modeled from holoprotein PEC from *M. laminosus* (PDB ID code 2J96) (23). The desired components (lyases and PCB) are combined for docking by using AutoDockTools (24), and corresponding docking calculations are performed with AutoDock Vina (Version 1.1.2) (25). For the docking of CpcE/F and PCB, the center coordinates of the search box are 34, 27, −2.5 (x, y, z) defined from the $N_{75}Y_{76}$ of CpcF, and the size of the box is 20 × 20 × 20 $Å^3$. For the docking of PecE/F and PCB, the center coordinates of the search box are 29.5, 30, −0.5 (x, y, z) defined from the $H_{87}C_{88}$ of PecF, and the size of the box is 20 × 20 × 20 $Å^3$. A two-stage approach was used by first docking PCB to a lyase (CpcE/F or PecE/F), followed by docking of this complex to the respective target apoprotein, CpcA, CpcB, or PecA. The docking model of lyases (with PCB or without) and the respective target apoprotein or holoprotein are obtained by Hex (Version 8.0.0; hex.loria.fr/), and settings for docking are selected by default except correlation type (shape and electro) (26).

The docking is a fairly roundabout process. Firstly, the chromophore, PCB, is fit to the lyase, which in case of PecE/F is itself a simulated structure. We have assumed that no major structural change results in either case from chromophore binding. This complex is then docked onto empty CpcA that is modeled from the α-subunit of the reported crystal structure of CPC (22). While there are many structures of chromophorylated PBPs in oligomer, there is only a single structure of an isolated subunit, PVB–PecA (23), and none of an apoprotein lacking the chromophore. The modeled CpcA seems to be slightly less structured than the holoprotein, which is in agreement with spectroscopic data (27, 28): The results of these dockings (Table S2, columns 3–5) reflect the uncertainties of the process. Besides the docking energy, several additional criteria have, therefore, been applied in selecting a likely structure of the complex formed between bilin-loaded lyase and apo-PBP. First, the mutation data locate the chromophore in the cavity formed between the E and F subunits of the lyase. Therefore, the apoprotein was assumed to locate in the region of the large opening of this cavity, preferentially covering it up. Secondly, proton-donating residue(s) are close to the chromophore, thereby assisting the thiol addition. Thirdly, the chromophore is E-configured at the $3,3^1$ double bond (5, 29). The stereochemistry of the product is then determined by which face of the chromophore locates near the binding cysteine. In no case, the best complex selected by these criteria (marked in green in Table S2) is the energetically most favorable one.

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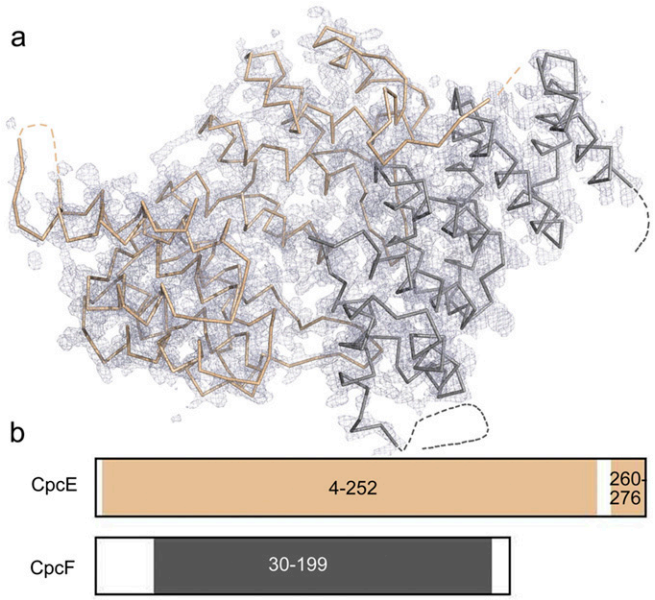


Fig. S1. (*A*) The $2F_o - F_c$ electron density maps (contoured at 2σ) of CpcE/F. (*B*) The electron density is clearly defined for most of the residues of CpcE (96%) and CpcF (82%). Owing to no or weak electron density for terminal or loop regions, 4% sequence of CpcE (N terminus and gap between h14 and h15) and 18% sequence of CpcF (N and C terminus) are not modeled (dashed lines). A total of 266 residues of CpcE (276 aa) and 170 residues of CpcF (208 aa; i.e., 200 aa of CpcF plus 8 aa of C-terminal His tag) of the heterodimer are resolved.

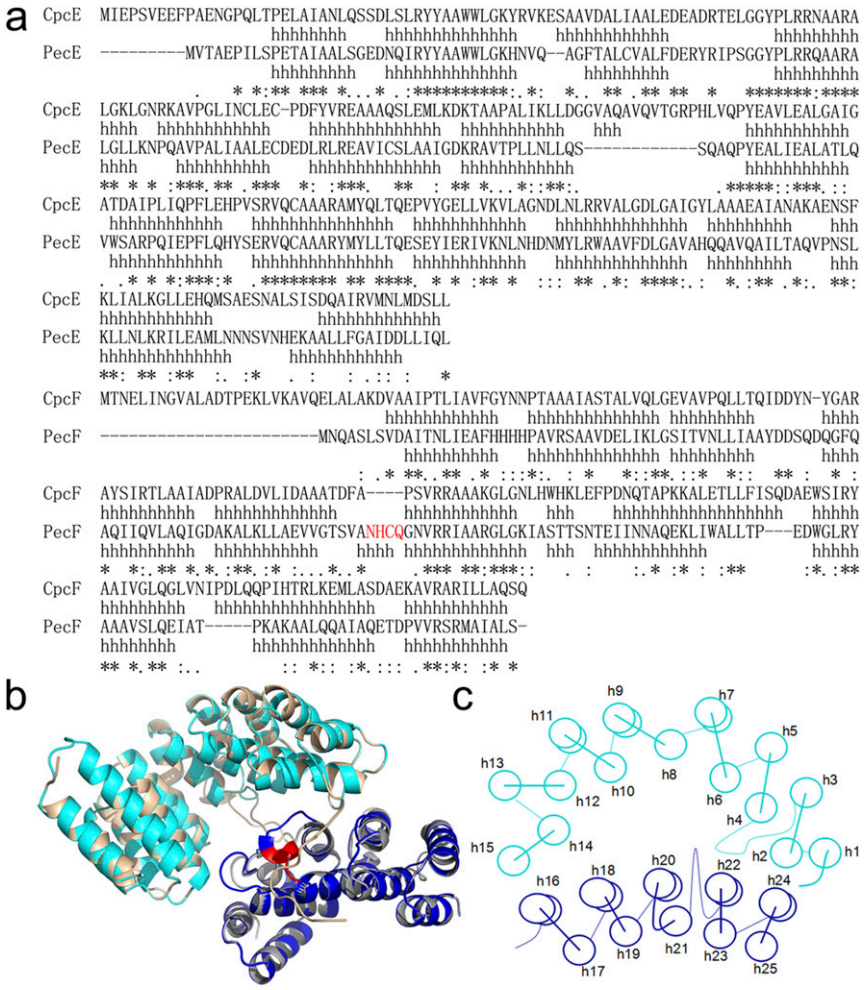


Fig. S2. (*A*) Homology between CpcE/F and PecE/F. (*B*) Structural comparison of CpcE/F (wheat/gray) and the modeled structure of PecE/F (chain A, cyan; chain B, blue). (*C*) Schematic structure, seen from the top into the cavity as in *B*. Curved lines indicate extended structures. The overall structures between CpcE/F and PecE/F overlap well. In the sequences, "h" marks amino acids forming helices. The isomerase motif ($H_{87}C_{88}$) is marked in red in the sequence and the structure. The motif is unique to PecF and, as verified by mutagenesis, necessary for isomerization activity of PecE/F.

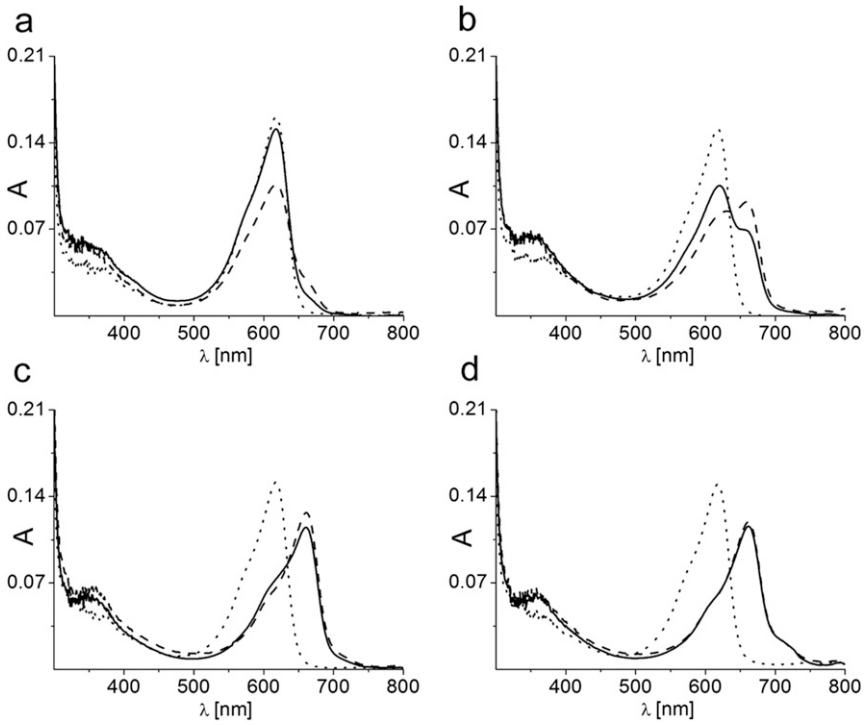


Fig. S3. PCB chromophore transfer from PCB–CpcA to Cph1(1–514). PCB transfer from PCB–CpcA to Cph1(1–514) proceeded under the following reaction conditions: Cph1 (20 μM) and PCB–CpcA (10 μM) in KPB (20 mM, pH 7.3) containing NaCl (150 mM), $MgCl_2$ (5 mM), and mercaptoethanol (5 mM), in the presence of CpcE/F (solid line) or CpcE(Δ2–10)/F (dashed line) (10 μM) or with no lyases (dotted line). Spectra were monitored at $t = 5$ (*A*), 20 (*B*), 60 (*C*), and 100 (*D*) min, indicating nearly complete transfer in the presence of CpcE/F or CpcE(Δ2–10)/F (*D*). The activity of CpcE(Δ2–10)/F (dashed line) was remarkably increased compared with the wild-type lyase (solid line). Therefore, the poorly structured N terminus of CpcE may interfere with the detachment of PCB from PCB^T–CpcA.

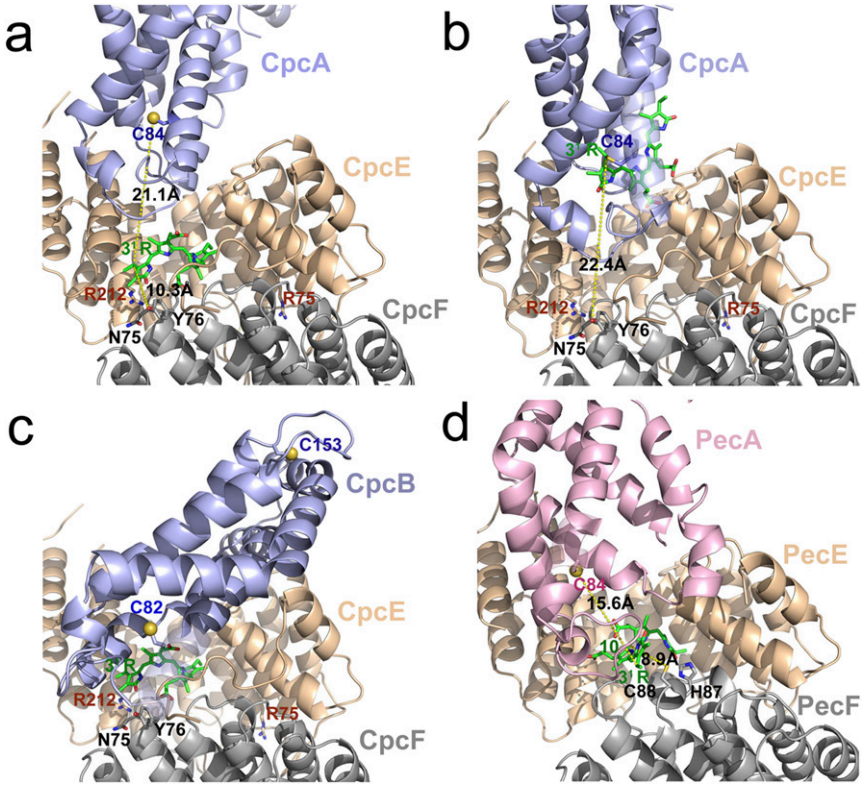


Fig. S4. Details of the docking complexes of CpcE/F (*A*–*C*) and PecE/F (*D*). Starting structures: CpcE/F (*Nostoc*; this work), PecE/F (*Nostoc*; modeled based on the former structure), apo-CpcA and apo-CpcB modeled from holoprotein CPC (PDB ID code 4ZIZ), and apo-PecA modeled from holoprotein PEC (PDB ID code 2J96). (*A*) Docking of PCB:CpcE/F to apo-CpcA; this corresponds to the encounter starting the chromophorylation process. CpcE, light brown; CpcF, gray; CpcA, light blue. (*B*) Docking of PCB^{T}–CpcA (PDB ID code 4ZIZ) on CpcE/F this corresponds to the encounter starting chromophore detachment from PCB^{T}–CpcA. Color coding is as in *A*. (*C*) Docking of PCB:CpcE/F on apo-CpcB; colors are as in *A*. (*D*) Docking of PCB:PecE/F to apo-PecA. This corresponds to the encounter starting chromophorylation of PecA. PecE, light brown; PecF, gray; PecA, purple. Numbers in black give distances (Å) indicated by the dotted lines. The detailed docking information and parameters are shown in Table S2.

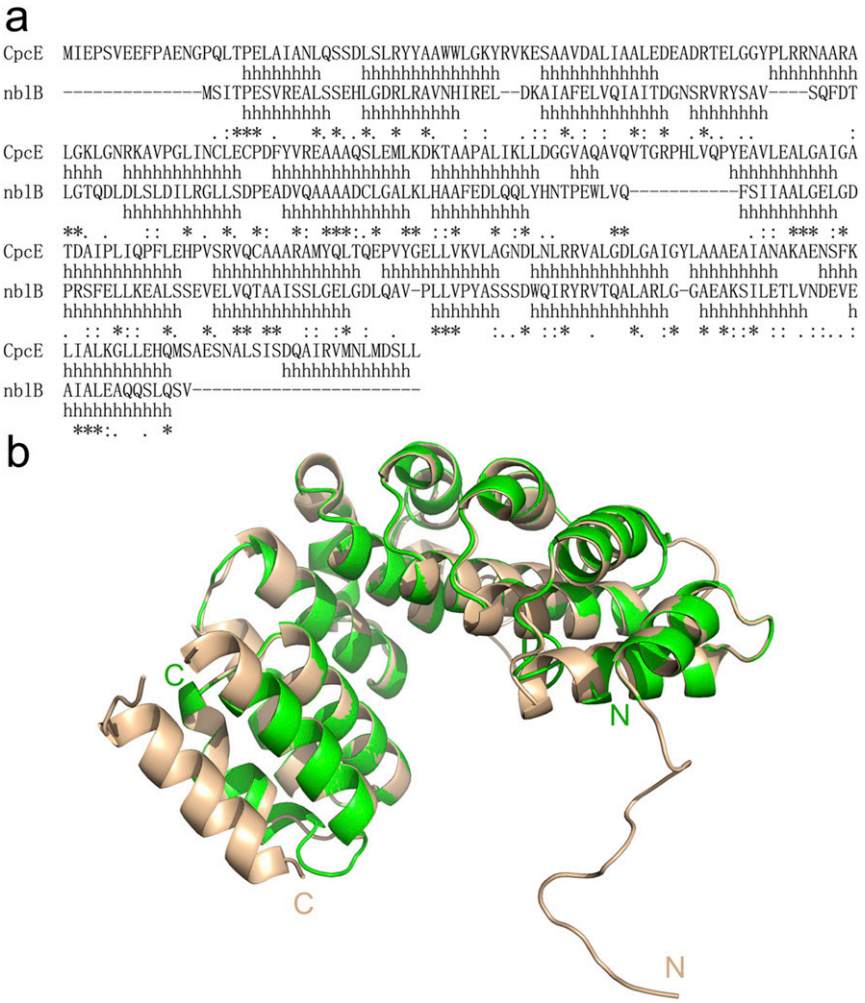


Fig. S5. Simulated structure of NblB (Alr3814) from *Nostoc*. (*A*) Homologies and secondary structure predictions for CpcE and NblB. (*B*) Structure simulation of NblB using CpcE from this work as template. Modeling was done by the SWISS-MODEL server (for details, see *SI Materials and Methods*). The simulated structure of NblB (green) is generally very similar to that of CpcE (brown). It lacks, however, the N-terminal extended stretch (arm), the C-terminal helix 15, and the long loop (Val138–Pro147) between helices 7 and 8.

Table S1. Variants of CpcE, CpcF, PecE, and PecF, their relative activities, and oligomerization states

Variants	Oligomerization state, mass (E/F-*n*-mer)	Relative activity		
		For chromo-phorylation	For binding PCB	For detaching PCB
CpcE/F	53 (1)	100	100	100
CpcE/F*	53 (1)	0		
CpcE	37 (1)	0		0
CpcF	27 (1)	0		0
CpcE(Δ2–10)/F	50 (1)	95	95	182
CpcE(Δ2–10/*h15*)/F	33 (1)	0	13	4
NblB	45 (2)	0	51	0
NblB/CpcF	39 (2), 22 (1)	0	54	3
CpcE(E8A)/F		80		
CpcE(F9A)/F		109		
CpcE(L33R)/F		102		
CpcE(R34A)/F		99		
CpcE(R34D)/F		90		
CpcE(Y35A)/F		92		
CpcE(Y35R)/F		98		
CpcE(Y36A)/F		95		
CpcE(Y36R)/F		86		
CpcE(W39A)/F	47 (1)	77	103	
CpcE(W40A)/F		82		
CpcE(K43A)/F		98		
CpcE(K43D)/F		99		
CpcE(Y44A)/F		81		
CpcE(R45A)/F		88		
CpcE(R45D)/F		99		
CpcE(D64R)/F		94		
CpcE(E67A)/F		108		
CpcE(R75A)/F	50 (1)	94	72	
CpcE(R75D)/F	52 (1)	27	10	
CpcE(N76A)/F		81		
CpcE(R79A)/F		98		
CpcE(R79D)/F		71		
CpcE(K83A)/F		103		
CpcE(K83D)/F		94		
CpcE(C96A)/F		92		
CpcE(C99A)/F		82		
CpcE(Y103A)/F		93		
CpcE(E106A)/F		95		
CpcE(Q110A)/F		93		
CpcE(E113A)/F		98		
CpcE(M114A)/F		65		
CpcE(K116A)/F		94		
CpcE(G140F)/F		129		
CpcE(E149A)/F		85		
CpcE(E153A)/F		126		
CpcE(R177A)/F		110		
CpcE(C180A)/F		84		
CpcE(R184A)/F		106		
CpcE(R184D)/F		89		
CpcE(Q188A)/F		90		
CpcE(N209A)/F		86		
CpcE(R212A)/F	50 (1)	62	65	
CpcE(R212D)/F	52 (1)	18	51	
CpcE(D217A)/F		95		
CpcE(F239A)/F		89		
CpcE(I242A)/F		90		
CpcE(K245A)/F		104		
CpcE(L276A)/F		89		
CpcE(L276D)/F		120		
CpcE/F(N44A)		93		
CpcE/F(N45A)		108		
CpcE/F(P46A)		91		

Table S1. Cont.

Variants	Oligomerization state, mass (E/F-*n*-mer)	Relative activity		
		For chromo-phorylation	For binding PCB	For detaching PCB
CpcE/F(D72R)	50 (1)	68	100	
CpcE/F(D73A)		87		
CpcE/F(Y74A)		80		
CpcE/F(N75A)	42 (1), 349 (7)	64	21	
CpcE/F(N75L)	47 (1)	55	54	
CpcE/F(Y76A)	53 (1)	1	91	
CpcE/F(R79A)		73		
CpcE/F(I83A)		111		
CpcE/F(R84A)		112		
CpcE/F(R93A)		89		
CpcE/F(L95A)		117		
CpcE/F(D96R)		104		
CpcE/F(D100A)		89		
CpcE/F(T104A)		75		
CpcE/F(T104R)		100		
CpcE/F(D105R)	50 (1)	59	87	
CpcE/F(F106A)	50 (1)	60	100	
CpcE/F(S109A)		109		
CpcE/F(R112D)		91		
CpcE/F(K116A)		100		
CpcE/F(K137D)		100		
CpcE/F(E140R)		97		
CpcE/F(F144D)		114		
CpcE/F(W151A)		97		
CpcE/F(Y155A)		95		
PecE/F		100		
PecE(W29A)/F		85		
PecE(R63A)/F		75		
PecE(C87A)/F		86		
PecE(C157A)/F		57		
PecE(W189A)/F		63		
PecE/F(S51A)		73		
PecE/F(Q52A)		94		
PecE/F(D53A)		78		
PecE/F(Q54A)		85		
PecE/F(Q57A)		72		
PecE/F(N86A)		120		
PecE/F(H87A)		0		
PecE/F(C88A)		1		
PecE/F(C88S)		0		
PecE/F(Q89A)		77		

The enzymatic activities of variants of CpcE, CpcF, PecE, or PecF relative to the wild-type proteins (=100%) were evaluated by chromophorylation of CpcA or PecA with PCB in *E. coli*; the PCB-binding activities of variants of CpcE, CpcF, PecE, or PecF relative to the wild-type proteins (=100%) were evaluated by the efficiency of their binding of PCB in vitro; and the PCB-detaching activities of variants of CpcE/F relative to the wild-type proteins (=100%) were evaluated by the transfer of PCB from PCB^{T}–CpcA to Cph1(1–514) in vitro. For details, see *Materials and Methods*.

*In this experiment, CpcA had a C-terminal his_6-tag that impedes chromophorylation.

Table S2. The dockings of lyases with apo-PBPs

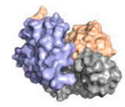
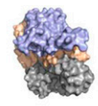
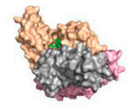
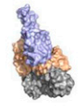
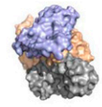
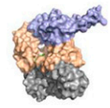
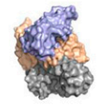
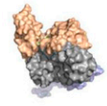
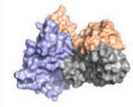
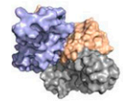
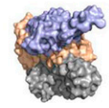
Model	PCB and CpcE/F[a]	PCB and PecE/F[b]	PCB/CpcE/F and CpcA[c]	PCB/CpcE/F and CpcB[d]	PCB/PecE/F and PecA[e]
Model 1 ΔG, R/S	-8.3	-5.6, S	-159, R 	-174, R 	-170, R 
Model 2 ΔG, R/S	-8.1, S	-5.5	-143, R 	-168, R 	-164, R 
Model 3 ΔG, R/S	-7.5	-5.3, S	-138, R 	-166, R 	-158, R 
Model 4 ΔG, R/S	-7.0, S	-5.2, S	-134, R 	-165, R 	-157, R 
Model 5 ΔG, R/S	-7.0, R	-5.1, R	-133, R 	-161, R 	-153, R 
Model 6	-7.0, R	**-5.1, R**	-133, R	-160, R	-152, R

Table S2. Cont.

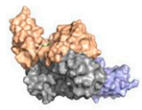
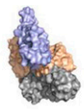
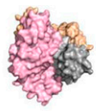
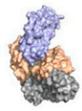
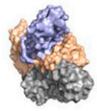
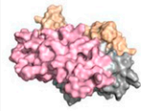
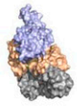
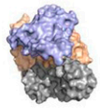
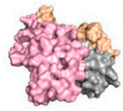
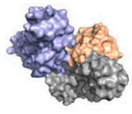
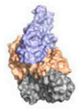
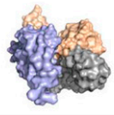
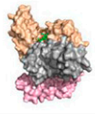
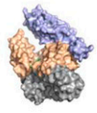
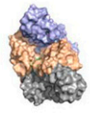
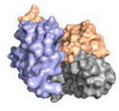
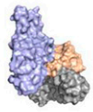
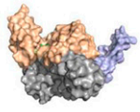
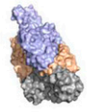
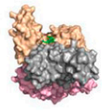
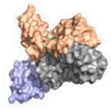
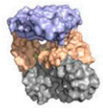
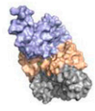
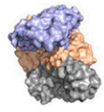
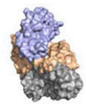
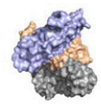
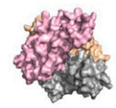
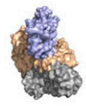
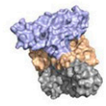
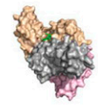
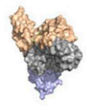
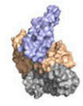
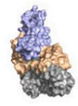
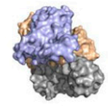
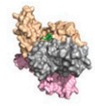
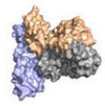
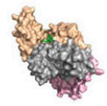
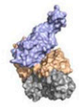
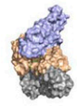
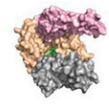
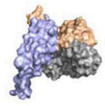
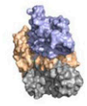
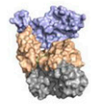
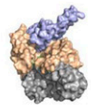
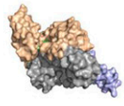
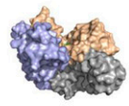
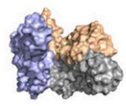
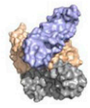
ΔG, R/S					
Model 7 ΔG, R/S	-6.9, R	-5.1, S	**-133, R** 	-158, R 	-151, R 
Model 8 ΔG, R/S	**-6.8, R**	-5.1, S	-133, R 	-154, R 	-149, R 
Model 9 ΔG, R/S	-6.8	-5.1, S	-132, R 	-153, R 	-148, R 
Model 10 ΔG, R/S			-130, R 	-153, R 	-148, R 
Model 11 ΔG, R/S			-130, R 	-153, R 	-148, R 
Model 12 ΔG, R/S			-129, R 	-153, R 	-146, R 
Model 13 ΔG, R/S			-129, R 	-152, R 	-146, R 
Model 14 ΔG, R/S			-128, R 	-152, R 	-145, R 
Model 15 ΔG, R/S			-128, R 	-151, R 	-144, R 
Model 16 ΔG, R/S			-128, R	-151, R	-144, R

Table S2. Cont.

					
Model 17 ΔG, R/S			-126, R 	-150, R 	-144, R 
Model 18 ΔG, R/S			-126, R 	-149, R 	-144, R 
Model 19 ΔG, R/S			-126, R 	-149, R 	-143, R 
Model 20 ΔG, R/S			-126, R 	-148, R 	-143, R 
Model 21 ΔG, R/S			-126, R 	-148, R 	-142, R 
Model 22 ΔG, R/S			-125, R 	-147, R 	-141, R 
Model 23 ΔG, R/S			-125, R 	-146, R 	-141, R 
Model 24 ΔG, R/S			-125, R 	-146, R 	**-141, R** 
Model 25 ΔG, R/S			-125, R 	**-146, R** 	-141, R 

Free energy gain upon docking (ΔG in kcal/Mol) and structures of the docked complex of the 25 best solutions. In the small figures giving an overview of the docking complex, the E and F subunits of the lyase are oriented similar as in Fig. 1, but tilted by ~30° to the front, allowing a look into the cavity. Color coding: E subunits, light brown; F subunits, gray; CpcA and CpcB, blue; PecA, purple. The models shown in bold green were the accepted ones detailed in Fig. S4. Besides the energy, the following factors were taken into consideration: (*i*) the R-configuration of $C3^1$ of PCB^T after chromophorylation; (*ii*) the coincidence of the active site (Fig. S4) with the mutation data (Table S1); and (*iii*) a position of the docked substrate near the upper opening of the cavity of the lyase. Models were discarded because (*i*) models 1–7 conflict with the mutation data; (*ii*) models 1–2 and 4–5 conflict with the mutation data, and model 3 results in S configuration; (*iii*) models 1–6 conflict with the mutation data; (*iv*) models 1–24 conflict with the mutation data; and (*v*) models 1–23 conflict with the mutation data.

Table S3. Pseudo first-order enzyme kinetic parameters of CpcE or CpcE(Δ2–10), in combination with CpcF, for transferring the PCB chromophore from PCB^{T}-CpcA to Cph1(1-514)

	Substrate PCB^{T}-CpcA			Substrate Cph1(1-514)		
Lyase complex	K_m, μM	k_{cat}, min^{-1}	k_{cat}/K_m, $min^{-1}\cdot\mu M^{-1}$	K_m, μM	k_{cat}, min^{-1}	k_{cat}/K_m, $min^{-1}\cdot\mu M^{-1}$
CpcE/F	3.10	2.53×10^{-3}	0.82×10^{-3}	9.94	6.28×10^{-3}	0.63×10^{-3}
CpcE(Δ2–10)/F	26.9	20.2×10^{-3}	0.75×10^{-3}	1.99	3.51×10^{-3}	1.76×10^{-3}

Table S4. Primers for *cpcA*, *cpcE*, *cpcF*, *pecA*, *pecE*, *pecF*, and *nblB* from *Nostoc*, and their site-directed mutants

Primer	Sequence	DNA
P1	5′ CCGTCGTCATATGATAGAACCCAGT 3′	*cpcE*
P2	5′ CTGCTCGAGCAACAATGAATCC 3′	
P3	5′ CGCTCGGCATATGACTAATGAACT 3′	*cpcF*
P4	5′ GTTCTCGAGTTGACTTTGAGCCAAT 3′	
P5	5′ TAACTCGAGCACCACCACCACCACCACT 3′	*cpcE(Δhis-tag)*
P6	5′ CAACAATGAATCCATAAGGTTCATGACC 3′	
P7	5′ TAACTCGAGCACCACCACCACCACCACT 3′	*cpcF(Δhis-tag)*
P8	5′ TTGACTTTGAGCCAATAGAATGCGGGCG 3′	
P9	5′ ACTCCCATGGCTATGGTTAAAACCCCCATT 3′	*cpcA*
P10	5′ CAGCTCGAGCTAGCTGAGAGCGTTGATAGC 3′	
P11	5′ GCACCATGGCTATGAAAACACCTTTGACCGA 3′	*pecA*
P12	5′ CTGCTCGAGTTAACTTAAAGCGTTAATTGCA 3′	
P13	5′ GACCCATGGGCGTGACTGCTGAACCAATTCT 3′	*pecE*
P14	5′ GACCTGCAGTTAAAGTTGAATTAATAAATCA 3′	
P15	5′ GGTTCAGCATATGAATCAAGCTTCATTGAGC 3′	*pecF*
P16	5′ GACCTCGAGCTAACTCAAGGCGATCGCCATA 3′	
P17	5′ CCTTTCCCGCAGAGAACGGGCCACAGCTAAC 3′	*cpcE(E8A)*
P18	5′ CTTCCACACTGGGTTCTATCATTGCCATGGT 3′	
P19	5′ CCCGCAGAGAACGGGCCACAGCTAACACCAG 3′	*cpcE(F9A)*
P20	5′ GGCTTCTTCCACACTGGGTTCTATCATATG 3′	
P21	5′ CGCTATTATGCTGCTTGGTGGTTAGGTAAGT 3′	*cpcE(L33R)*
P22	5′ GCGACTTAAGTCTGATGATTGCAGATTAGCT 3′	
P23	5′ GCCTATTATGCTGCTTGGTGGTTAGGTAAGT 3′	*cpcE(R34A)*
P24	5′ GAGACTTAAGTCTGATGATTGCAGATTAGCT 3′	
P25	5′ GACTATTATGCTGCTTGGTGGTTAGGTAAGT 3′	*cpcE(R34D)*
P26	5′ GAGACTTAAGTCTGATGATTGCAGATTAGCT 3′	
P27	5′ GCCTATGCTGCTTGGTGGTTAGGTAAGTATCG 3′	*cpcE(Y35A)*
P28	5′ GCGGAGACTTAAGTCTGATGATTGCAGATTA 3′	
P29	5′ CGCTATGCTGCTTGGTGGTTAGGTAAGTATC 3′	*cpcE(Y35R)*
P30	5′ GCGGAGACTTAAGTCTGATGATTGCAGATTA 3′	
P31	5′ GCTGCTTGGTGGTTAGGTAAGTATCGGGTGA 3′	*cpcE(Y36A)*
P32	5′ GGCATAGCGGAGACTTAAGTCTGATGATTGC 3′	
P33	5′ GCTGCTTGGTGGTTAGGTAAGTATCGGGTGA 3′	*cpcE(Y36R)*
P34	5′ CCGATAGCGGAGACTTAAGTCTGATGATTGC 3′	
P35	5′ GCCTGGTTAGGTAAGTATCGGGTGAAAGAAAG 3′	*cpcE(W39A)*
P36	5′ AGCAGCATAATAGCGGAGACTTAAGTCTGATGA 3′	
P37	5′ GCCTTAGGTAAGTATCGGGTGAAAGAAAGTGC 3′	*cpcE(W40A)*
P38	5′ CCAAGCAGCATAATAGCGGAGACTTAAGTCT 3′	
P39	5′ GCCTATCGGGTGAAAGAAAGTGCTGCTGTTGA 3′	*cpcE(K43A)*
P40	5′ ACCTAACCACCAAGCAGCATAATAGCGGAGA 3′	
P41	5′ GTGACTATCGGGTGAAAGAAAGTGCTGCTGT 3′	*cpcE(K43D)*
P42	5′ CTAACCACCAAGCAGCATAATAGCGGAGACT 3′	
P43	5′ CGGGTGAAAGAAAGTGCTGCTGTTGATGCTT 3′	*cpcE(Y44A)*
P44	5′ GGCCTTACCTAACCACCAAGCAGCATAATAG 3′	
P45	5′ GTGAAAGAAAGTGCTGCTGTTGATGCTTTAA 3′	*cpcE(R45A)*
P46	5′ CGCATACTTACCTAACCACCAAGCAGCATAA 3′	
P47	5′ GTGAAAGAAAGTGCTGCTGTTGATGCTTTAA 3′	*cpcE(R45D)*
P48	5′ GTCATACTTACCTAACCACCAAGCAGCATAA 3′	
P49	5′ CGTAGAACTGAACTTGGTGGTTATCCTTTGC 3′	*cpcE(D64R)*
P50	5′ GGCTTCATCCTCTAACGCCGCAATTAAAGCA 3′	
P51	5′ CTTGGTGGTTATCCTTTGCGGCGTAACGCAG 3′	*cpcE(E67A)*
P52	5′ CGCAGTTCTATCGGCTTCATCCTCTAACGCC 3′	
P53	5′ GCTAACGCAGCCAGAGCATTAGGGAAATTGG 3′	*cpcE(R75A)*
P54	5′ CCGCAAAGGATAACCACCAAGTTCAGTTCTA 3′	
P55	5′ GACAACGCAGCCAGAGCATTAGGGAAATTGG 3′	*cpcE(R75D)*
P56	5′ CCGCAAAGGATAACCACCAAGTTCAGTTCTA 3′	
P57	5′ GCAGCCAGAGCATTAGGGAAATTGGGCAATC 3′	*cpcE(N76A)*
P58	5′ GGCACGCCGCAAAGGATAACCACCAAGTTCA 3′	
P59	5′ GCAGCATTAGGGAAATTGGGCAATCGTAAAG 3′	*cpcE(R79A)*
P60	5′ GGCTGCGTTACGCCGCAAAGGATAACCACCA 3′	
P61	5′ GACGCATTAGGGAAATTGGGCAATCGTAAAGC 3′	*cpcE(R79D)*
P62	5′ GGCTGCGTTACGCCGCAAAGGATAACCACCA 3′	

Table S4. Cont.

Primer	Sequence	DNA
P63	5′ GCATTGGGCAATCGTAAAGCCGTGCCAGGCT 3′	*cpcE(K83A)*
P64	5′ CCCTAATGCTCTGGCTGCGTTACGCCGCAAA 3′	
P65	5′ GACTTGGGCAATCGTAAAGCCGTGCCAGGCT 3′	*cpcE(K83D)*
P66	5′ CCCTAATGCTCTGGCTGCGTTACGCCGCAAA 3′	
P67	5′ GCCTTGGAGTGTCCTGACTTTTACGTGCGTG 3′	*cpcE(C96A)*
P68	5′ GTTAATTAAGCCTGGCACGGCTTTACGATTG 3′	
P69	5′ GCTCCTGACTTTTACGTGCGTGAAGCAGCAG 3′	*cpcE(C99A)*
P70	5′ CTCCAAGCAGTTAATTAAGCCTGGCACGGCT 3′	
P71	5′ GTGCGTGAAGCAGCAGCCCAATCGCTGGAAA 3′	*cpcE(Y103A)*
P72	5′ GGCAAAGTCAGGACACTCCAAGCAGTTAATT 3′	
P73	5′ GCAGCAGCCCAATCGCTGGAAATGCTCAAAG 3′	*cpcE(E106A)*
P74	5′ GGCACGCACGTAAAAGTCAGGACACTCCAAG 3′	
P75	5′ GCATCGCTGGAAATGCTCAAAGACAAAACAG 3′	*cpcE(Q110A)*
P76	5′ GGCTGCTGCTTCACGCACGTAAAAGTCAGGA 3′	
P77	5′ GCAATGCTCAAAGACAAAACAGCAGCACCAG 3′	*cpcE(E113A)*
P78	5′ CAGCGATTGGGCTGCTGCTTCACGCACGTAA 3′	
P79	5′ CTCAAAGACAAAACAGCAGCACCAGCACTCA 3′	*cpcE(M114A)*
P80	5′ GGCTTCCAGCGATTGGGCTGCTGCTTCACGC 3′	
P81	5′ GCAGACAAAACAGCAGCACCAGCACTCATCA 3′	*cpcE(K116A)*
P82	5′ GAGCATTTCCAGCGATTGGGCTGCTGCTTCA 3′	
P83	5′ CGACCTCATTTAGTCCAACCCTACGAAGCAG 3′	*cpcE(G140F)*
P84	5′ AAATGTTACTTGCACGGCCTGTGCGACTCCC 3′	
P85	5′ GCAGCAGTATTAGAAGCGTTAGGAGCTATTG 3′	*cpcE(E149A)*
P86	5′ GTAGGGTTGGACTAAATGAGGTCGCCCTGTT 3′	
P87	5′ GCGTTAGGAGCTATTGGTGCTACTGATGCCA 3′	*cpcE(E153A)*
P88	5′ GGCTAATACTGCTTCGTAGGGTTGGACTAAA 3′	
P89	5′ GTGCAGTGCGCCGCCGCTAGGGCAATGTACC 3′	*cpcE(R177A)*
P90	5′ GGCTGAGACTGGATGCTCTAGAAATGGCTGA 3′	
P91	5′ GCCGCCGCCGCTAGGGCAATGTACCAACTGA 3′	*cpcE(C180A)*
P92	5′ CTGCACTCGTGAGACTGGATGCTCTAGAAAT 3′	
P93	5′ GCAATGTACCAACTGACACAAGAACCAGTAT 3′	*cpcE(R184A)*
P94	5′ CGCAGCGGCGGCGCACTGCACTCGTGAGACT 3′	
P95	5′ GCAATGTACCAACTGACACAAGAACCAGTAT 3′	*cpcE(R184D)*
P96	5′ GTCAGCGGCGGCGCACTGCACTCGTGAGACT 3′	
P97	5′ GCACTGACACAAGAACCAGTATATGGAGAGC 3′	*cpcE(Q188A)*
P98	5′ GTACATTGCCCTAGCGGCGGCGCACTGCACT 3′	
P99	5′ GCCCTACGACGCGTTGCTTTAGGTGACTTGG 3′	*cpcE(N209A)*
P100	5′ GAGGTCGTTACCTGCTAACACTTTTACCAGC 3′	
P101	5′ GTTGCTTTAGGTGACTTGGGTGCAATTGGGT 3′	*cpcE(R212A)*
P102	5′ GGCTCGTAGGTTGAGGTCGTTACCTGCTAAC 3′	
P103	5′ GTTGCTTTAGGTGACTTGGGTGCAAAAGGGT 3′	*cpcE(R212D)*
P104	5′ GTCTCGTAGGTTGAGGTCGTTACCTGCTAAC 3′	
P105	5′ CCTTGGGTGCAATTGGGTACTTGGCAGCAGC 3′	*cpcE(D217A)*
P106	5′ CACCTAAAGCAACGCGTCGTAGGTTGAGGTC 3′	
P107	5′ GCCAAACTCATTGCCCTCAAAGGATTGCTAG 3′	*cpcE(F239A)*
P108	5′ GCTGTTTTCGGCTTTGGCGTTAGCGATCGCT 3′	
P109	5′ GCCCTCAAAGGATTGCTAGAACATCAGATGT 3′	*cpcE(I242A)*
P110	5′ GGCGAGTTTGAAGCTGTTTTCGGCTTTGGCG 3′	
P111	5′ GGATTGCTAGAACATCAGATGTCAGCAGAGT 3′	*cpcE(K245A)*
P112	5′ GGCGAGGGCAATGAGTTTGAAGCTGTTTTCG 3′	
P113	5′ GCCTAACTGCAGGTCGACAAGCTTGCGGCC 3′	*cpcE(L276A)*
P114	5′ CAATGAATCCATAAGGTTCATGACCCGGAT 3′	
P115	5′ GACTAACTGCAGGTCGACAAGCTTGCGGCC 3′	*cpcE(L276D)*
P116	5′ CAATGAATCCATAAGGTTCATGACCCGGAT 3′	
P117	5′ CAACCCAACAGCAGCCGCAATTGCCTCTACT 3′	*cpcF(N44A)*
P118	5′ GCATAACCAAACACAGCTATTAGGGTGGGAA 3′	
P119	5′ CCAACAGCAGCCGCAATTGCCTCTACTGCCT 3′	*cpcF(N45A)*
P120	5′ GGCGTTATAACCAAACACAGCTATTAGGGTG 3′	
P121	5′ GCAACAGCAGCCGCAATTGCCTCTACTGCCT 3′	*cpcF(P46A)*
P122	5′ GTTGTTATAACCAAACACAGCTATTAGGGTG 3′	
P123	5′ GACTACAACTATGGCGCACGGGCTTATTCGA 3′	*cpcF(D72R)*
P124	5′ CCGTATTTGTGTGAGTAACTGGGGAACTGCT 3	

Table S4. Cont.

Primer	Sequence	DNA
P125	5′ CCTACAACTATGGCGCACGGGCTTATTCGAT 3′	*cpcF(D73A)*
P126	5′ CATCTATTTGTGTGAGTAACTGGGGAACTGC 3′	
P127	5′ GCCAACTATGGCGCACGGGCTTATTCGATTC 3′	*cpcF(Y74A)*
P128	5′ GTCATCTATTTGTGTGAGTAACTGGGGAA 3′	
P129	5′ GCCTATGGCGCACGGGCTTATTCGATTCGCA 3′	*cpcF(N75A)*
P130	5′ GTAGTCATCTATTTGTGTGAGTAACTGGGGA 3′	
P131	5′ CTCTATGGCGCACGGGCTTATTCGATTCGCA 3′	*cpcF(N75L)*
P132	5′ GTAGTCATCTATTTGTGTGAGTAACTGGGGA 3′	
P133	5′ GGCGCACGGGCTTATTCGATTCGCACTCTCG 3′	*cpcF(Y76A)*
P134	5′ GGCGTTGTAGTCATCTATTTGTGTGAGTAAC 3′	
P135	5′ GCTTATTCGATTCGCACTCTCGCGGCGATCG 3′	*cpcF(R79A)*
P136	5′ CGCTGCGCCATAGTTGTAGTCATCTATTTGT 3′	
P137	5′ CGCACTCTCGCGGCGATCGCTGACCCCCGTG 3′	*cpcF(I83A)*
P138	5′ GGCCGAATAAGCCCGTGCGCCATAGTTGTAG 3′	
P139	5′ CACTCTCGCGGCGATCGCTGACCCCCGTGCT 3′	*cpcF(R84A)*
P140	5′ CGAATCGAATAAGCCCGTGCGCCATAGTTGT 3′	
P141	5′ GCTGCTTTAGATGTATTAATCGATGCTGCTG 3′	*cpcF(R93A)*
P142	5′ GGGGTCAGCGATCGCCGCGAGAGTGCGAATC 3′	
P143	5′ GATGTATTAATCGATGCTGCTGCTACAGACT 3′	*cpcF(L95A)*
P144	5′ GGCAGCACGGGGGTCAGCGATCGCCGCGAGA 3′	
P145	5′ GTATTAATCGATGCTGCTGCTACAGACTTTG 3′	*cpcF(D96R)*
P146	5′ CCGTAAAGCACGGGGGTCAGCGATCGCCGCG 3′	
P147	5′ GCTGCTGCTACAGACTTTGCTCCCAGTGTGC 3′	*cpcF(D100A)*
P148	5′ GGCGATTAATACATCTAAAGCACGGGGGTCA 3′	
P149	5′ GACTTTGCTCCCAGTGTGCGCCGGGCTGCGG 3′	*cpcF(T104A)*
P150	5′ TGCAGCAGCAGCATCGATTAATACATCTAAA 3′	
P151	5′ GACTTTGCTCCCAGTGTGCGCCGGGCTGCGG 3′	*cpcF(T104R)*
P152	5′ CCGAGCAGCAGCATCGATTAATACATCTAAA 3′	
P153	5′ CTTTGCTCCCAGTGTGCGCCGGGCTGCGGCT 3′	*cpcF(D105R)*
P154	5′ CGTGTAGCAGCAGCATCGATTAATACATCTA 3′	
P155	5′ GCTCCCAGTGTGCGCCGGGCTGCGGCTAAAG 3′	*cpcF(F106A)*
P156	5′ GGCGTCTGTAGCAGCAGCATCGATTAATACA 3′	
P157	5′ GTGCGCCGGGCTGCGGCTAAAGGATTAGGAA 3′	*cpcF(S109A)*
P158	5′ GGCGGGAGCAAAGTCTGTAGCAGCAGCATCG 3′	
P159	5′ GCTGCGGCTAAAGGATTAGGAAACTTACATT 3′	*cpcF(R112D)*
P160	5′ GTCGCGCACACTGGGAGCAAAGTCTGTAGCA 3′	
P161	5′ GGATTAGGAAACTTACATTGGCACAAGCTAG 3′	*cpcF(K116A)*
P162	5′ GGCAGCCGCAGCCCGGCGCACACTGGGAGCA 3′	
P163	5′ GCTTTGGAAACACTGCTGTTTATTTCTCAAG 3′	*cpcF(K137D)*
P164	5′ GTCTTTTGGTGCTGTTTGGTTGTCAGGAAAC 3′	
P165	5′ CGCACACTGCTGTTTATTTCTCAAGATGCAG 3′	*cpcF(E140R)*
P166	5′ CAAAGCTTTTTTTGGTGCTGTTTGGTTGTCA 3′	
P167	5′ GACATTTCTCAAGATGCAGAATGGTCGATTC 3′	*cpcF(F144D)*
P168	5′ CAGCAGTGTTTCCAAAGCTTTTTTTGGTGCT 3′	
P169	5′ GTCGATTCGTTATGCAGCGATCGTCGGTCTG 3′	*cpcF(W151A)*
P170	5′ GCTTCTGCATCTTGAGAAATAAACAGCAGTG 3′	
P171	5′ GCAGCGATCGTCGGTCTGCAAGGTTTAGTAA 3′	*cpcF(Y155A)*
P172	5′ GGCACGAATCGACCATTCTGCATCTTGAGAA 3′	
P173	5′ CCTGGTTGGGTAAACATAACGTACAGGCTGG 3′	*pecE(W29A)*
P174	5′ CAGCAGCATAATAACGAATTTGGTTATCCTC 3′	
P175	5′ CAGGCTGCACGGGCTTTAGGATTGTTGAAAA 3′	*pecE(R63A)*
P176	5′ GGCACGCAGAGGATACCCTCCTGATGGGATG 3′	
P177	5′ GATGAGGATTTGCGTCTGAGGGAAGCAGTAA 3′	*pecE(C87A)*
P178	5′ GGCTTCCAATGCTGCAATTAAGGCTGGTACA 3	
P179	5′ GCAGCCGCTAGATATATGTATCTCCTCACCC 3′	*pecE(C157A)*
P180	5′ GGCTTGCACACGCTCAGAATAATGCTGAAGA 3	
P181	5′ GCGGCGGTATTTGATTTGGGTGCAGTTGCTC 3′	*pecE(W189A)*
P182	5′ GGCACGTAAATACATATTGTCATGATTGAGA 3′	
P183	5′ CAAGATCAAGGTTTTCAAGCCCAGATCATTC 3′	*pecF(S51A)*
P184	5′ GGCATCATCATAAGCAGCAATTAACAAATTA 3′	
P185	5′ GATCAAGGTTTTCAAGCCCAGATCATTCAGG 3′	*pecF(Q52A)*
P186	5′ GGCAGAATCATCATAAGCAGCAATTAACAAA 3′	

Table S4. Cont.

Primer	Sequence	DNA
P187	5′ CAAGGTTTTCAAGCCCAGATCATTCAGGTTT 3′	*pecF(D53A)*
P188	5′ GGCTTGAGAATCATCATAAGCAGCAATTAAC 3′	
P189	5′ GGTTTTCAAGCCCAGATCATTCAGGTTTTAG 3′	*pecF(Q54A)*
P190	5′ GGCATCTTGAGAATCATCATAAGCAGCAATT 3′	
P191	5′ GCCCAGATCATTCAGGTTTTAGCTCAAATTG 3′	*pecF(Q57A)*
P192	5′ GGCAAAACCTTGATCTTGAGAATCATCATAA 3′	
P193	5′ CACTGTCAAGGTAATGTCCGCCGCATTGCTG 3′	*pecF(N86A)*
P194	5′ GGCGGCTACACTTGTCCCCACCACCTCGGCT 3′	
P195	5′ CTGTCAAGGTAATGTCCGCCGCATTGCTGCA 3′	*pecF(H87A)*
P196	5′ GCATTGGCTACACTTGTCCCCACCACCTCGG 3′	
P197	5′ GCCCAAGGTAATGTCCGCCGCATTGCTGCAC 3′	*pecF(C88A)*
P198	5′ GTGATTGGCTACACTTGTCCCCACCACCTCG 3′	
P199	5′ CAAGGTAATGTCCGCCGCATTGCTGCACGAG 3′	*pecF(C88S)*
P200	5′ CGAGTGATTGGCTACACTTGTCCCCACCACC 3′	
P201	5′ GGTAATGTCCGCCGCATTGCTGCACGAGGGT 3′	*pecF(Q89A)*
P202	5′ GGCACAGTGATTGGCTACACTTGTCCCCACC 3′	
P203	5′ CCAGATCCATATGAGTATTACTCCTGAGTCT 3′	*nblB*
P204	5′ GCACTCGAGAACTGATTGCAAAGATTGTTGA 3′	
P205	5′ GCAGAGAACGGGCCACAGCTAACACCAGAAC 3′	*cpcE(*Δ2–10)
P206	5′ CATATGTATATCTCCTTCTTAAAGTTAAACA 3′	
P207	5′ GCTCGAGCACCACCACCACCACCACTGAG 3′	*cpcE(Δ254–276)*
P208	5′ GACATCTGATGTTCTAGCAATCCTTTGAGG 3′	*i.e. cpcE(Δh15)*

Table S5. Plasmids used

Antibiotic resistance	pACYCDuet derivatives	pCDFDuet derivatives	pET30 derivatives
Kanamycin			pET-histag-cpcA
			pET-cpcA-histag
			pET-pecA
			pET-cpcE
			pET-cpcE(Δhis-tag)
			pET-cpcE(Δ2–10/his-tag)
			pET-cpcE(Δ2–10/h15)
			pET-cpcE(W39A/Δhis-tag)
			pET-cpcE(R75A/Δhis-tag)
			pET-cpcE(R75D/Δhis-tag)
			pET-cpcE(R212A/Δhis-tag)
			pET-cpcE(R212D/Δhis-tag)
			pET-cpcF
			pET-cpcF(D72R)
			pET-cpcF(N75A)
			pET-cpcF(N75L)
			pET-cpcF(Y76A)
			pET-cpcF(D105R)
			pET-cpcF(F106A)
			pET-cpcF(Δhis-tag)
			pET-nblB
			pET-cph1
Streptomycin		pCDF-cpcE-cpcF	
		pCDF-cpcE(Δ2–10)-cpcF	
		pCDF-cpcE(Δ2–10/h15)-cpcF	
		pCDF-cpcE(E8A)-cpcF	
		pCDF-cpcE(F9A)-cpcF	
		pCDF-cpcE(L33R)-cpcF	
		pCDF-cpcE(R34A)-cpcF	
		pCDF-cpcE(R34D)-cpcF	
		pCDF-cpcE(Y35A)-cpcF	
		pCDF-cpcE(Y35R)-cpcF	
		pCDF-cpcE(Y36A)-cpcF	
		pCDF-cpcE(Y36R)-cpcF	
		pCDF-cpcE(W39A)-cpcF	
		pCDF-cpcE(W40A)-cpcF	
		pCDF-cpcE(K43A)-cpcF	
		pCDF-cpcE(K43D)-cpcF	
		pCDF-cpcE(Y44A)-cpcF	
		pCDF-cpcE(R45A)-cpcF	
		pCDF-cpcE(R45D)-cpcF	
		pCDF-cpcE(D64R)-cpcF	
		pCDF-cpcE(E67A)-cpcF	
		pCDF-cpcE(R75A)-cpcF	
		pCDF-cpcE(R75D)-cpcF	
		pCDF-cpcE(N76A)-cpcF	
		pCDF-cpcE(R79A)-cpcF	
		pCDF-cpcE(R79D)-cpcF	
		pCDF-cpcE(K83A)-cpcF	
		pCDF-cpcE(K83D)-cpcF	
		pCDF-cpcE(C96A)-cpcF	
		pCDF-cpcE(C99A)-cpcF	
		pCDF-cpcE(Y103A)-cpcF	
		pCDF-cpcE(E106A)-cpcF	
		pCDF-cpcE(Q110A)-cpcF	
		pCDF-cpcE(E113A)-cpcF	
		pCDF-cpcE(M114A)-cpcF	
		pCDF-cpcE(K116A)-cpcF	
		pCDF-cpcE(G140F)-cpcF	
		pCDF-cpcE(E149A)-cpcF	
		pCDF-cpcE(E153A)-cpcF	
		pCDF-cpcE(R177A)-cpcF	
		pCDF-cpcE(C180A)-cpcF	

Table S5. Cont.

Antibiotic resistance	pACYCDuet derivatives	pCDFDuet derivatives	pET30 derivatives
		pCDF-cpcE(R184A)-cpcF	
		pCDF-cpcE(R184D)-cpcF	
		pCDF-cpcE(Q188A)-cpcF	
		pCDF-cpcE(N209A)-cpcF	
		pCDF-cpcE(R212A)-cpcF	
		pCDF-cpcE(R212D)-cpcF	
		pCDF-cpcE(D217A)-cpcF	
		pCDF-cpcE(F239A)-cpcF	
		pCDF-cpcE(I242A)-cpcF	
		pCDF-cpcE(K245A)-cpcF	
		pCDF-cpcE(L276A)-cpcF	
		pCDF-cpcE(L276D)-cpcF	
		pCDF-cpcE-cpcF(N44A)	
		pCDF-cpcE-cpcF(N45A)	
		pCDF-cpcE-cpcF(P46A)	
		pCDF-cpcE-cpcF(D72R)	
		pCDF-cpcE-cpcF(D73A)	
		pCDF-cpcE-cpcF(Y74A)	
		pCDF-cpcE-cpcF(N75A)	
		pCDF-cpcE-cpcF(N75L)	
		pCDF-cpcE-cpcF(Y76A)	
		pCDF-cpcE-cpcF(R79A)	
		pCDF-cpcE-cpcF(I83A)	
		pCDF-cpcE-cpcF(R84A)	
		pCDF-cpcE-cpcF(R93A)	
		pCDF-cpcE-cpcF(L95A)	
		pCDF-cpcE-cpcF(D96R)	
		pCDF-cpcE-cpcF(D100A)	
		pCDF-cpcE-cpcF(T104A)	
		pCDF-cpcE-cpcF(T104R)	
		pCDF-cpcE-cpcF(D105R)	
		pCDF-cpcE-cpcF(F106A)	
		pCDF-cpcE-cpcF(S109A)	
		pCDF-cpcE-cpcF(R112D)	
		pCDF-cpcE-cpcF(K116A)	
		pCDF-cpcE-cpcF(K137D)	
		pCDF-cpcE-cpcF(E140R)	
		pCDF-cpcE-cpcF(F144D)	
		pCDF-cpcE-cpcF(W151A)	
		pCDF-cpcE-cpcF(Y155A)	
		pCDF-pecE-pecF	
		pCDF-pecE(W29A)-pecF	
		pCDF-pecE(R63A)-pecF	
		pCDF-pecE(C87A)-pecF	
		pCDF-pecE(C157A)-pecF	
		pCDF-pecE(W189A)-pecF	
		pCDF-pecE-pecF(S51A)	
		pCDF-pecE-pecF(Q52A)	
		pCDF-pecE-pecF(D53A)	
		pCDF-pecE-pecF(Q54A)	
		pCDF-pecE-pecF(Q57A)	
		pCDF-pecE-pecF(N86A)	
		pCDF-pecE-pecF(H87A)	
		pCDF-pecE-pecF(C88A)	
		pCDF-pecE-pecF(C88S)	
		pCDF-pecE-pecF(Q89A)	
		pCDF-nblB	
		pCDF-nblB-cpcF	
Chloramphenicol	pACYC-ho1-pcyA		

The plasmids pACYCDuet, pCDFDuet, pET28, and pET30, from Novagen, are T7 promoter expression vectors. pACYCDuet and pCDFDuet are designed to coexpress two target proteins in *E. coli*. By using the three vector derivatives together with compatible replicons and antibiotic resistance, five proteins could be coexpressed in the same cell, thereby generating the respective designed PC or PEC in *E. coli*.