S1: Fusion protein sequence used for attaching PEB or PCB.

Fusion sequence with His at position 6:

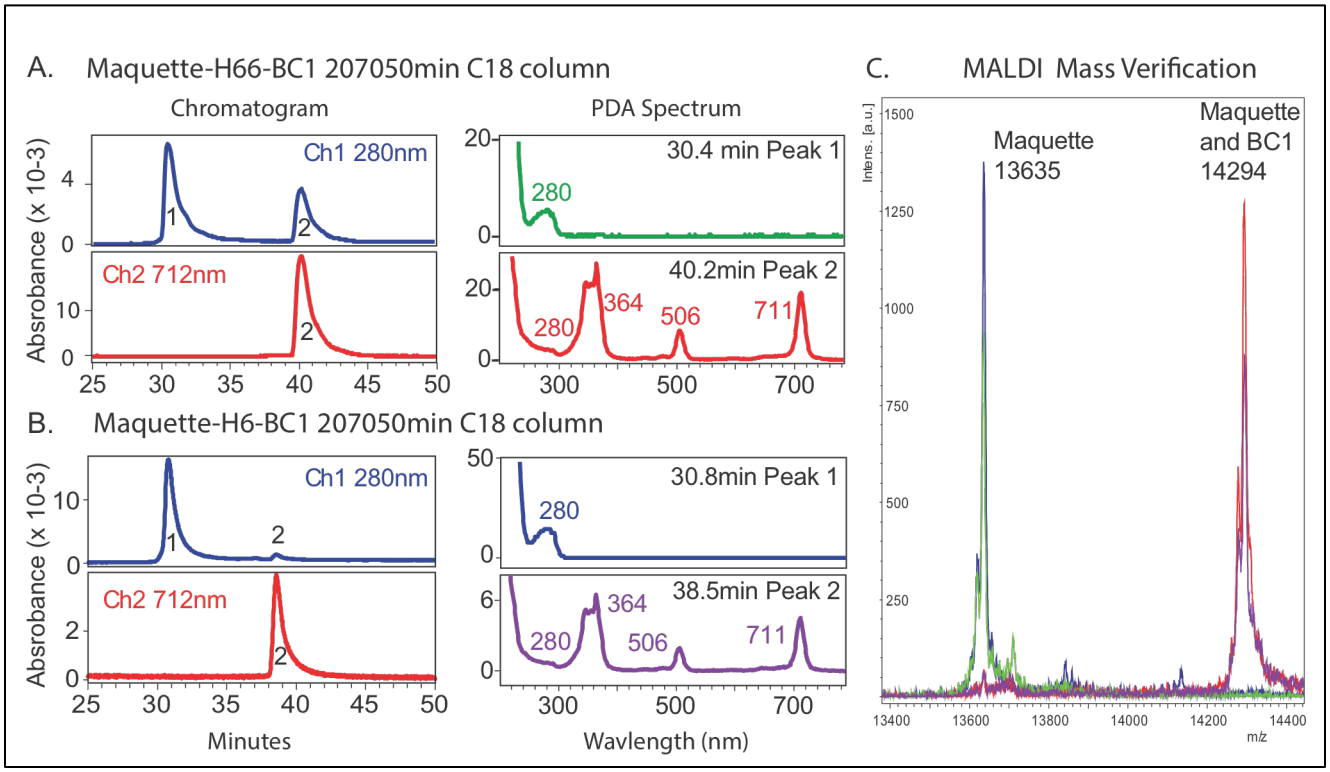
G E I W K Q **H** E D A L Q K F E E A L N Q F E D L K Q L G G C G E I K Q R A E D A L
R K F E E A L K R F E D K K Q K G G S G E I W K Q A E D A L Q K F E E A L N Q F E
D L K Q L G G S G E I K Q R A E D A L R K F E E A L K R F E D L K Q K M K T P L T
E A V S T A D S Q G R F L S S T E L Q I A F G R L R Q A N A G L Q A A K A L T D N
A Q S L V N G A A Q A V Y N K F P Y T T Q T Q G N N F A A D Q R G K D K **C** A R D
I G Y Y L R I V T Y C L V A G G T G P L D E Y L I A G I D E I N R T F D L S P S W Y V
E A L K Y I K A N H G L S G D A R D E A N S Y L D Y A I N A L S

His at position 66:

G E I W K Q A E D A L Q K F E E A L N Q F E D L K Q L G G C G E I K Q R A E D A L
R K F E E A L K R F E D K K Q K G G S G E I W K Q **H** E D A L Q K F E E A L N Q F E
D L K Q L G G S G E I K Q R A E D A L R K F E E A L K R F E D L K Q K M K T P L T
E A V S T A D S Q G R F L S S T E L Q I A F G R L R Q A N A G L Q A A K A L T D N
A Q S L V N G A A Q A V Y N K F P Y T T Q T Q G N N F A A D Q R G K D K **C** A R D
I G Y Y L R I V T Y C L V A G G T G P L D E Y L I A G I D E I N R T F D L S P S W Y V
E A L K Y I K A N H G L S G D A R D E A N S Y L D Y A I N A L S

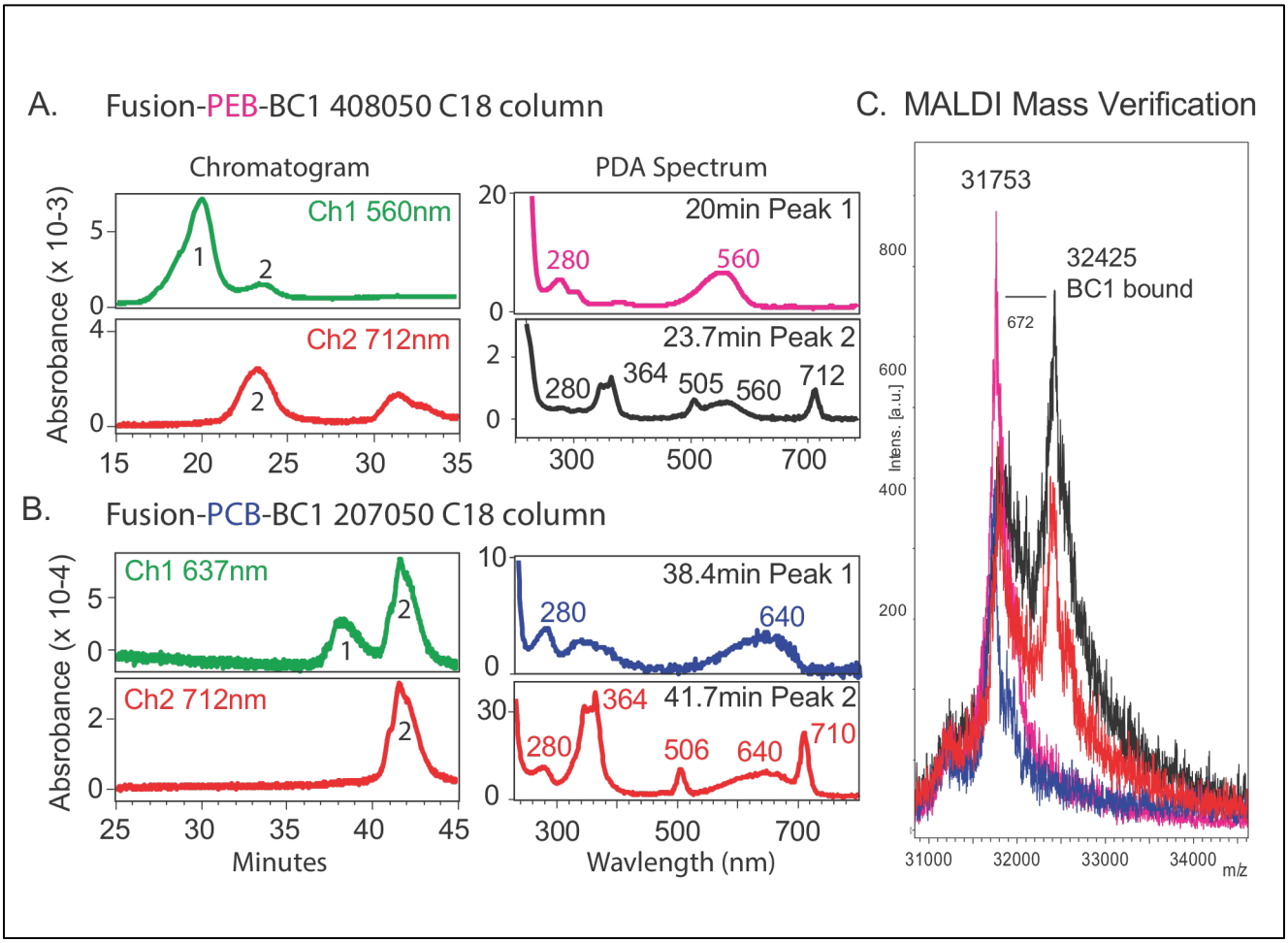
Histidines used for chlorin ligation and cysteines used for bilin attachment are shown in bold red letters. Numbering for His position starts at the beginning of the first helix after initial Glycine.

S2. HPLC and MALDI-MS verification of BC1 attachment to unfused maquette protein.



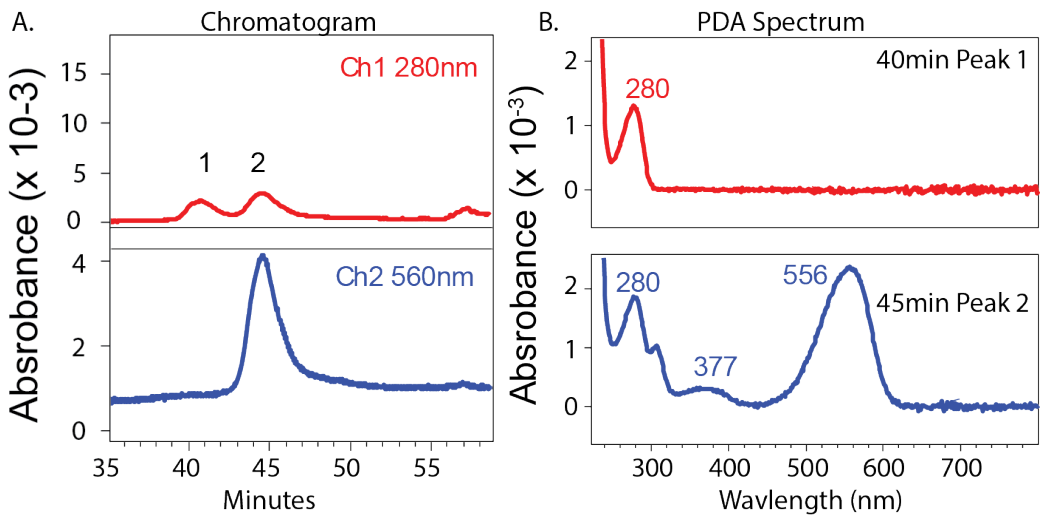
A. HPLC elution profile monitored at 280 nm and 712 nm for maquette H66 with maleimide-anchored BC1. **B.** HPLC elution profile monitored by absorbance at 280 nm and 712 nm for maquette H66 with maleimide-anchored BC1. Absorbance spectra for maxima of peaks shown in **A** and **B** are shown in the panel labeled “photodiode array (PDA) spectrum.” **C.** MALDI mass spectra of the peaks in **A** and **B**; colors correspond to absorbance spectra shown under the panels labeled “PDA Spectrum.”

S3. HPLC and MALDI-MS verification of BC1 anchoring to fusion protein.



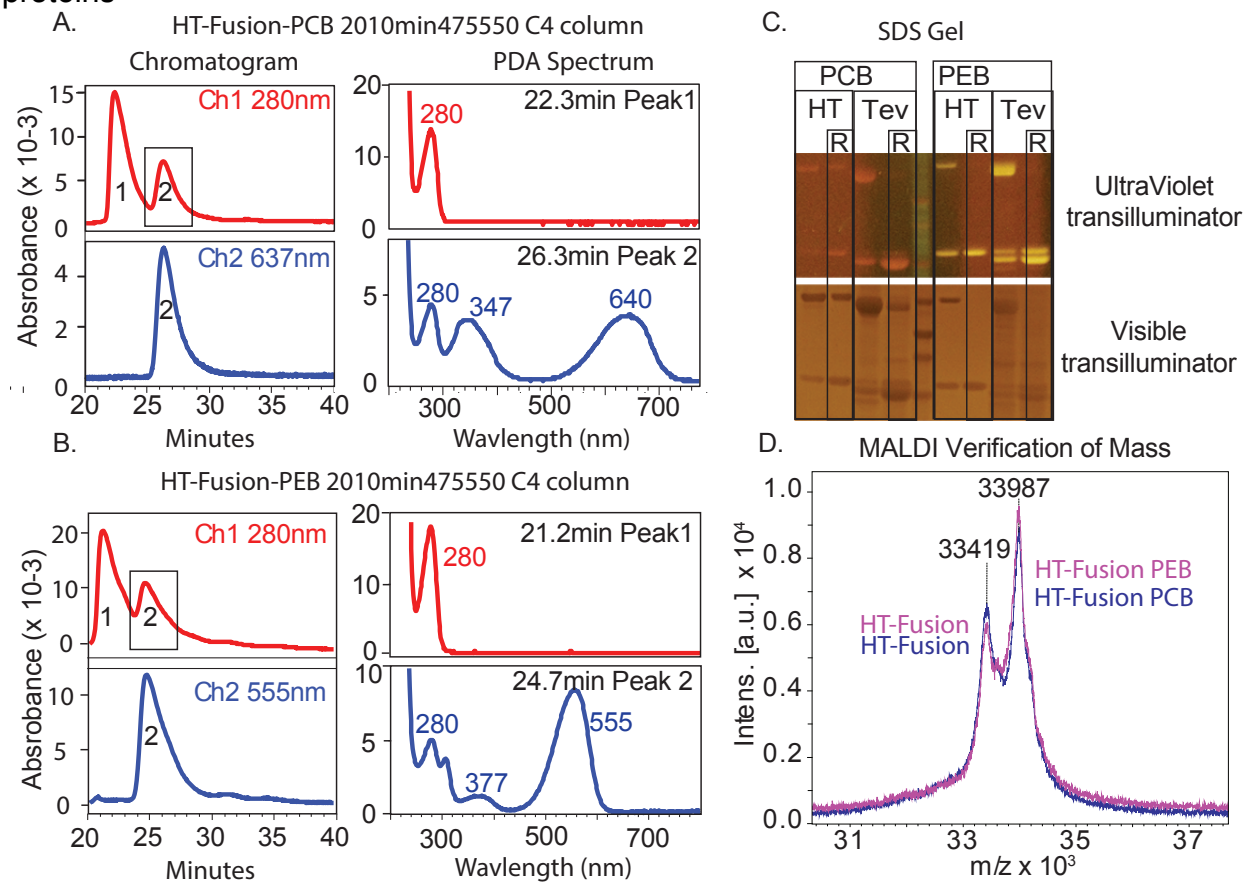
A. HPLC elution profile monitored by absorbance at 560 nm and 712 nm for Fusion-PEB with maleimide-anchored BC1. **B.** HPLC elution profile monitored by absorbance at 637 nm and 712 nm for Fusion-PCB with BC1 attached. Photodiode array (PDA) spectrum: absorbance spectra for maximum of peaks shown in panels **A** and **B**. **C.** MALDI mass spectra of the peaks in **A** and **B**; colors correspond to absorbance spectra recorded by PDA in panels **A** and **B**. The masses of the two peaks are indicated and differ by 672 Da, the mass difference expected when BC1 is bound to the protein.

S4. HPLC estimates of PEB attachment yields to fusion protein



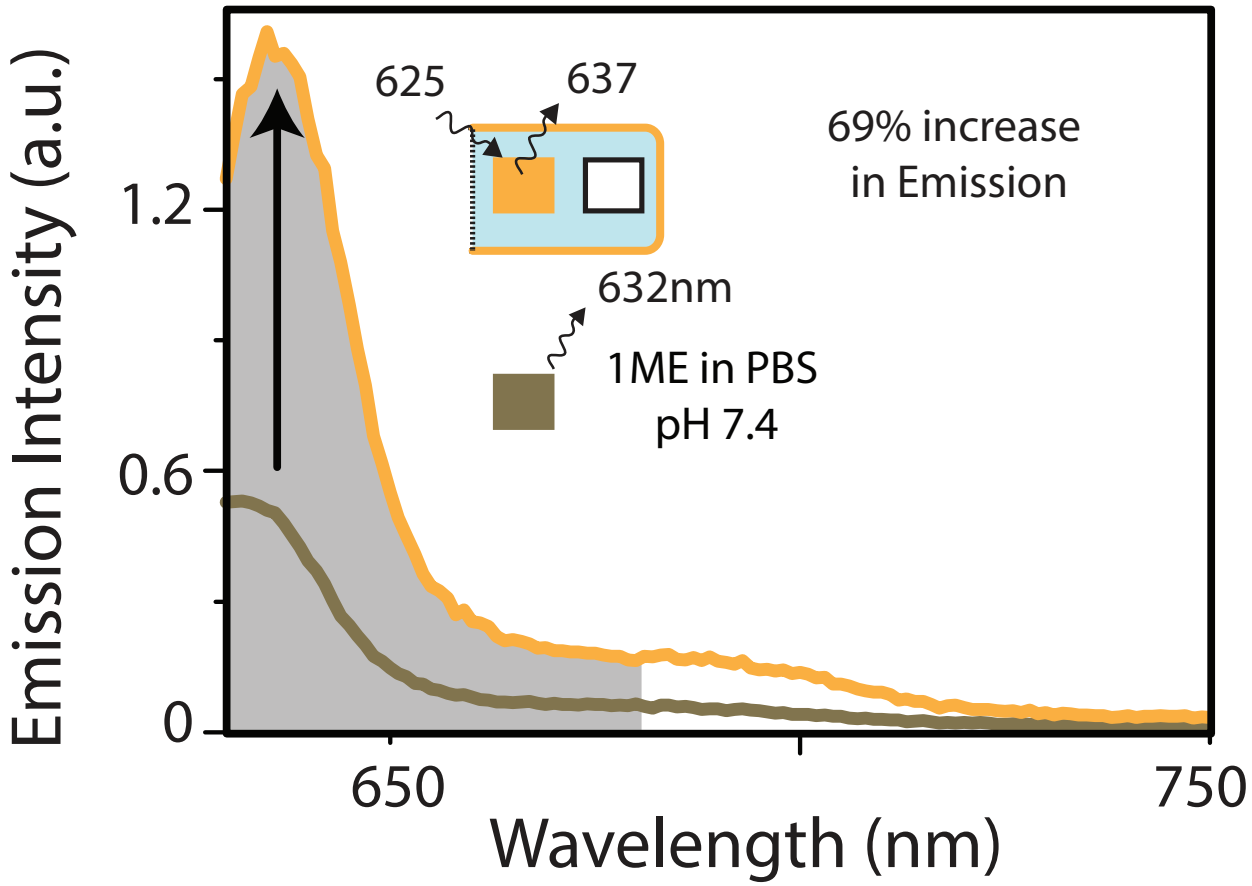
A 500-ml gradient of 40% to 47% acetonitrile (ACN), pH 2, was used with a C4 prep column to purify HT-Fusion with PEB bound (2) from unbound (1) after 20 min reduction in 2-mercaptoethanol. Despite high expression yields, upwards of 50% attachment of bilin to fusion protein can be achieved, as determined by HPLC. Chromatograms are shown in A and photodiode array detector (PDA) spectra for each peak in the chromatogram are shown in B.

S5: UV illumination of SDS gel and MALDI confirm covalent bilin attachment to fusion proteins



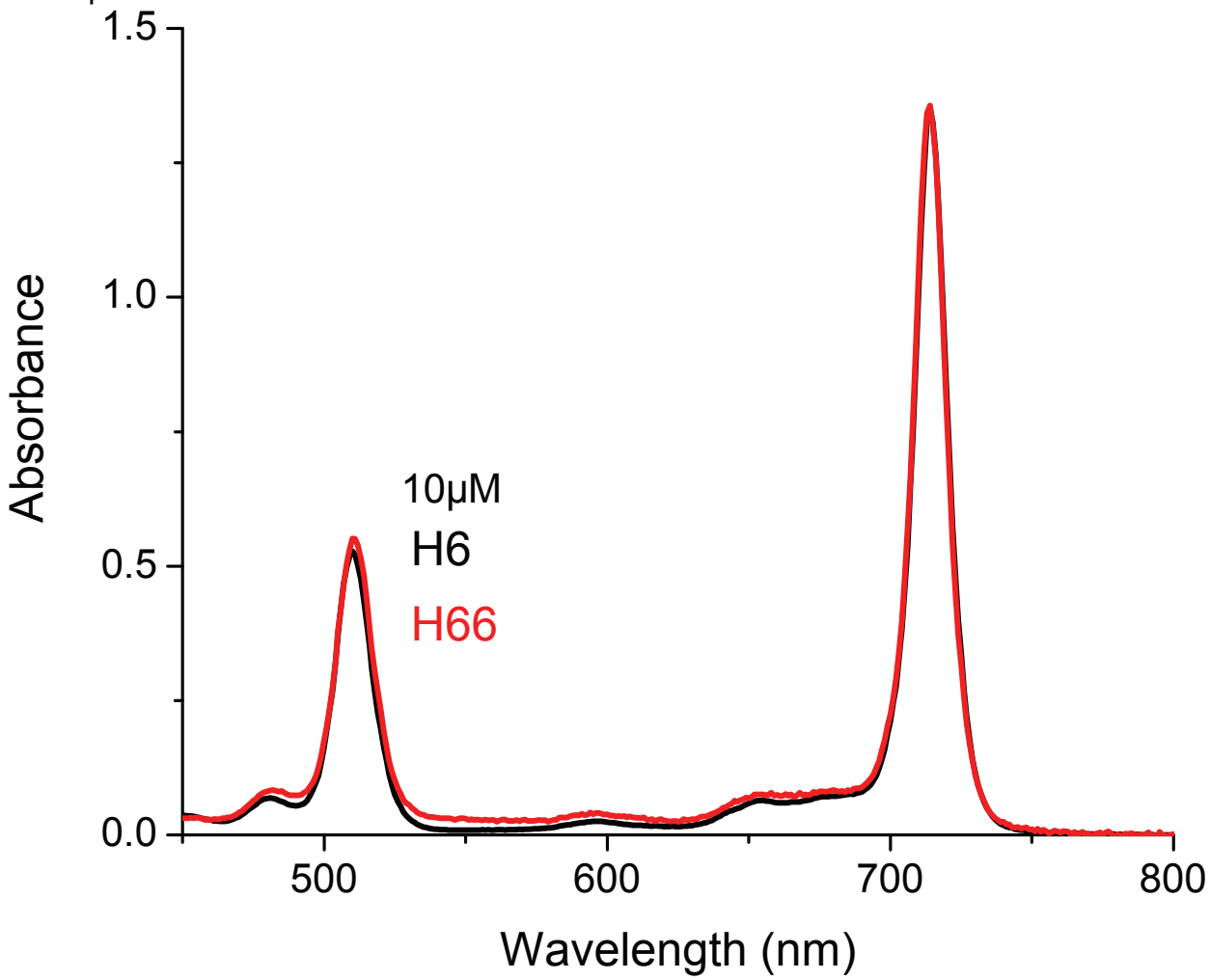
His-tagged fusion protein with either PCB **(A)** or PEB **(B)** was precipitated using 50% saturated ammonium sulfate, resolubilized in acidic 8 M Urea (100 mM HCl) and applied to an HPLC reversed-phase column at pH 2. **C.** SDS-PAGE analysis of recombinant proteins. Proteins carrying bilins were identified by fluorescence excited by UV illumination of the electrophoretically separated proteins. His-tag and TEV-tag cleaved PEB and PCB fusion proteins are shown before and after reduction (R) with 14mM 2-mercaptoethanol. **D**. MALDI-MS shows a major peak at 33987 Da for phycobilin-bound fusion protein. High MALDI-MS laser power appears to be correlated with bilin destruction and generates a 568 Da lower molecular mass fragment.

S6. His ligation increases ZnC fluorescence

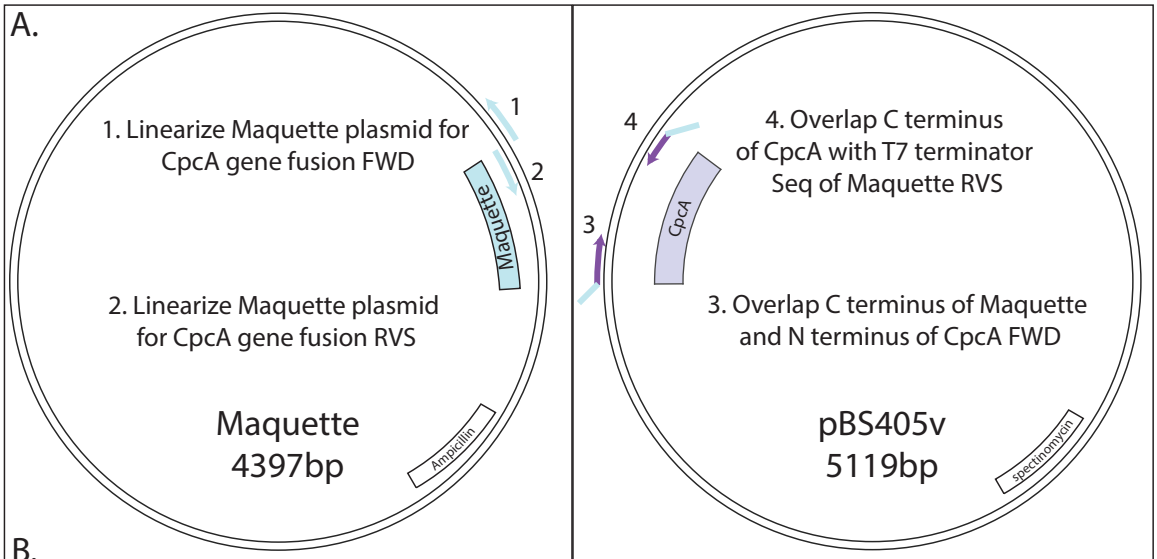


His ligation of ZnC in maquettes increases ZnC fluorescence by ~69% compared to the same concentration in aqueous buffer. Maquette and ZnC concentration were 1µM. Grey area indicates range of fluorescence emission integration used for determining percent increase in fluorescence emission.

S7. Absorption spectra of unfused maquette with bound BC1 are similar for His at either position 6 or 66.



S8: Plasmid construction



B.

ATGCATCACCATCATCATCATCACCACGGCGGCGACGGCGA
AAATCTGTATTTTCAAGGTGAGATCTGGAAACAACACGAGG
ACGCGCTGCAGAAATTTGAAGAAGCACTGAACCAGTTCGA
GGATCTGAAGCAATTGGGTGGTTGCGGTGAGATTAAGCAG
CGTCACGAAGATGCGCTGCGTAAGTTCGAAGAGGCGCTGA
AGCGCTTTGAGGACCTGAAACAAAAGGGTGGCAGCGGCG
AGATTTGGAAACAGCATGAAGATGCACTGCAGAAGTTCGA
GGAAGCCCTGAACCAATTTGAGGATTTGAAACAACTGGGT
GGTAGCGGTGAGATCAAGCAGCGCCACGAAGATGCTCTGC
GTAAATTCGAAGAAGCGCTGAAACGTTTCGAGGACTTGAA
GCAGAAA

ATGAAAACCCCTTTAACTGAAGCCGTTTCCACCGCTGACTC
TCAAGGTCGCTTTCTGAGCAGCACCGAATTGCAAATTGCTT
TCGGTCGTCTACGTCAAGCTAATGCTGGTTTGCAAGCCGCT
AAAGCTCTGACCGACAATGCCCAGAGCTTGGTAAATGGTG
CTGCCCAAGCCGTTTATAACAAATTCCCCTACACCACCCAA
ACCCAAGGCAACAACTTTGCTGCGGATCAACGGGGTAAAG
ACAAGTGTGCCCGGGACATCGGCTACTACCTCCGCATCGTT
ACCTACTGCTTAGTTGCTGGTGGTACCGGTCCTTTGGATGA
GTACTTGATCGCCGGTATTGATGAAATCAACCGCACCTTTG
ACCTCTCCCCCAGCTGGTATGTTGAAGCTCTGAAATACATCA
AAGCTAACCACGGCTTGAGTGGCGATGCCCGTGACGAAGC
TAATTCCTACCTCGATTACGCCATCAATGCTCTGAGCTAG

C.

Met H H H H H H H H G G D G E N L Y F Q G E I W K Q H E
D A L Q K F E E A L N Q F E D L K Q L G G C G E I K Q R H E
D A L R K F E E A L K R F E D L K Q K G G S G E I W K Q H E
D A L Q K F E E A L N Q F E D L K Q L G G S G E I K Q R H E
D A L R K F E E A L K R F E D L K Q K

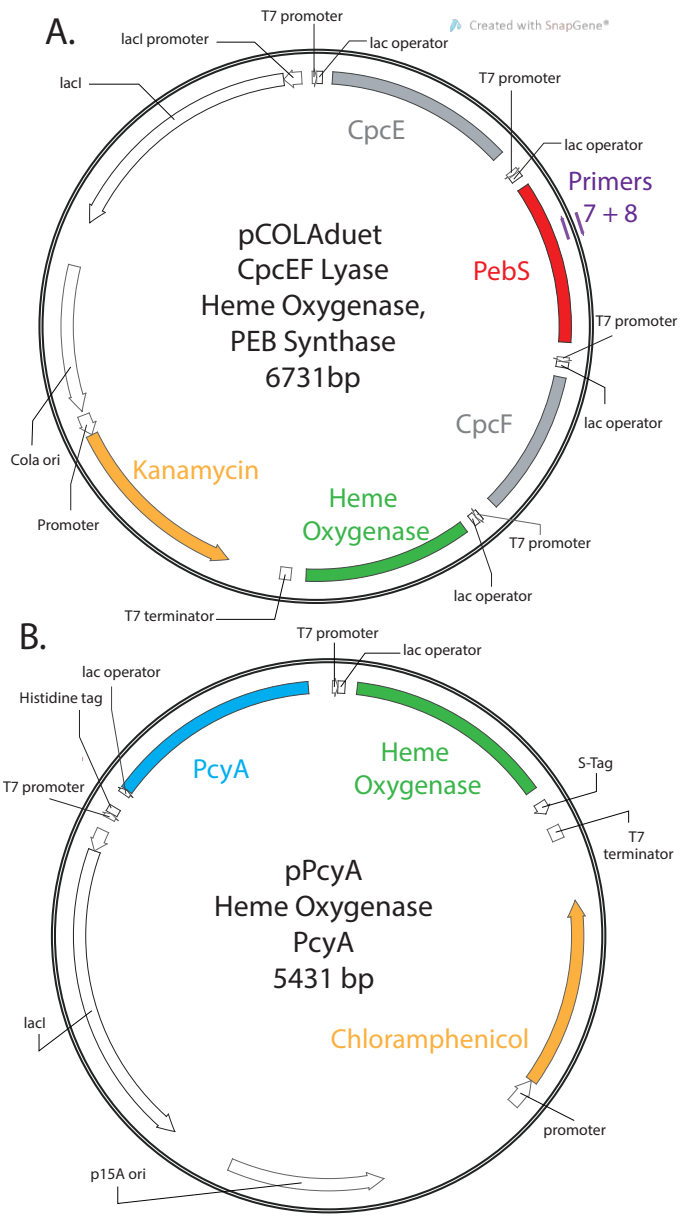
M K T P L T E A V S T A D S Q G R F L S S T E L Q I A F G R L
R Q A N A G L Q A A K A L T D N A Q S L V N G A A Q A V Y
N K F P Y T T Q T Q G N N F A A D Q R G K D K C A R D I G Y
Y L R I V T Y C L V A G G T G P L D E Y L I A G I D E I N R T F
D L S P S W Y V E A L K Y I K A N H G L S G D A R D E A N S
Y L D Y A I N A L S Stop

Part A shows the plasmids for each component and the primers that were used to amplify the gene. Gibson Assembly was used to attach the *cpcA* gene and maquette vector. **Part B** shows the DNA sequence for each gene. **Part C** shows the translation of each DNA sequence.

Table 1: Primers used

Primer	Purpose	Sequence 5' to 3'
1	Linearize Maquette plasmid for CpcA gene fusion FWD	CCCCCTAGCATAACCCCT
2	Linearize Maquette plasmid for CpcA gene fusion RVS	TTTCTGCTTCAAGTCCTCG
3	Overlap C terminus of Maquette and N terminus of CpcA FWD	AGGACTTGAAGCAGAAAATGAAAACCCCTTTAACTGAAG
4	Overlap C terminus of CpcA with T7 terminatorSeq	GGTTATGCTAGGGGGCTAGCTCAGAGCATTGATG
5	changing non ligating Cystiene to Alanine in CpcA FWD	CGCATCGTTACCTACGCGTTAGTTGCTGGTGGT
6	changing non ligating Cystiene to Alanine in CpcA RVS	ACCACCAGCAACTAACGCGTAGGTAACGATGCG
7	delofMDLfromPEBsFWD	CTGCCTTGTTTTGGTATGAAGTTTAGTGAT
8	delofMDLfromPEBsRVS	ATCACTAAACTTCATACCAAAACAAGGCAG
9	Maquette H6A FWD	GAGATCTGGAAACAAGCTGAGGACGCGCTGCAG
10	Maquette H6A RVS	CTGCAGCGCGTCCTCAGCTTGTTTCCAGATCTC
11	Maquette H36A FWD	GAGATTAAGCAGCGTGCCGAAGATGCGCTGCG
12	Maquette H36A RVS	CGCAGCGCATCTTCGGCACGCTGCTTAATCTC
13	Maquette H66A FWD	GAGATTTGGAAACAGGCTGAAGATGCACTGCA
14	Maquette H66A RVS	TGCAGTGCATCTTCAGCCTGTTTCCAAATCTC
15	Maquette H96A FWD	GAGATCAAGCAGCGCGCAGAAGATGCTCTGCGT
16	Maquette H96A RVS	ACGCAGAGCATCTTCTGCGCGCTGCTTGATCTC

S9: Bilin biosynthetic machinery plasmids



Panel A shows machinery for PEB production and attachment. Primers 7 and 8 were used to make PebS non-functional so plasmid in **Panel B** could be transformed alongside that shown in **Panel A** to allow for PCB attachment to the Fusion protein. The pCOLAduet and pACYCH plasmids each contain T7 promoters [1]. The PCOLADuet plasmid under the selection by kanamycin is used to express CpcE/CpcF, PebS, and Ho1 in *E. coli*. These are the proteins responsible for the production and attachment of PEB to the Maquette/CpcA gene fusion. The pACYCH plasmid is maintained under selection by chloramphenicol and produces PcyA for synthesis of PCB from biliverdin in *E. coli* (see Ref. 1).

References:

1. Alvey, R. M., Biswas, A., Schluchter, W. M. & Bryant, D. A. 2011 Attachment of noncognate chromophores to CpcA of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 by heterologous expression in *Escherichia coli*. *Biochemistry* **50**, 4890–4902. (doi:10.1021/bi200307s)