Supplementary Table 1: *Te*CpcS Identity and Similarity Scores

	*S*CpcS-I[a]	*S*CpcU-I	*N*CpcS-III	*P*CpeS-II	*Fd*CpeS-I
*Te*CpcS-III Identity Scores (%)	45.5	28.9	64.1	25.1	43.0
*Te*CpcS-III Similarity Scores (%)	60.6	44.7	74.0	45.5	59.7

[a] Species designations are as follows: *Te*CpcS-III from *Thermosynechococcus elongatus*; *S*CpcS-I from *Synechococcus* sp. PCC 7002; *S*CpcU-I from *Synechococcus* sp. PCC 7002; *N*CpcS-III from *Nostoc* sp. PCC 7120; *P*CpeS-II from *Prochlorococcus marinus*; *Fd*CpeS-I from *Fremyella diplosiphon*

Supplementary Figure Legends:

Supplementary Fig. 1. Analysis of purified HT-CpcB chromophorylated by *Te*CpcS in recombinant *E. coli*. **(A)** Absorbance (solid) and fluorescence emission (dashed) spectra of HT-CpcBA purified from recombinant *E. coli* cells containing pCpcBA, p*Te*CpcS and either pPebS (red) or pHy2 (green). **(B)** Coomassie-stained SDS-polyacrylamide gel of HT-CpcBA purified from cells containing pCpcBA, p*Te*CpcS and either pPebS (lane 1), or pHy2 (lane 2). Molecular mass standards were loaded in lane S. **(C)** Zn-enhanced bilin fluorescence of the gel in panel B excited at 532 nm to detect PEB covalent attachment. **(D)** Zn-enhanced bilin fluorescence of the gel in panel B excited at 635 nm to detect PΦB covalent attachment.

Supplementary Fig. 2. Attachment of three different bilins (PEB, PCB or PΦB) to AP by *Te*CpcS. Absorbance (solid) and fluorescence emission (dashed) spectra of HT-ApcAB purified from recombinant *E. coli* cells containing pApcAB, p*Te*CpcS and the bilin reductase genes to produce **(A)** PCB from pPcyA, **(B)** PEB from pPebS or **(C)** PΦB from pHy2. **(D)** Coomassie-stained SDS-polyacrylamide gel of HT-ApcAB purified from cells containing pApcAB, pTeCpcS and either pPcyA (lane 1), pPebS (lane 2), or pHy2 (lane 3). Molecular mass standards were loaded in lane S. **(E)** Zn-enhanced bilin fluorescence of the gel (excitation at 532 nm) in panel D.

Supplementary Fig. 3. Attachment of three different bilins (PEB, PCB or PΦB) to AP-B by *Te*CpcS. Absorbance (solid) and fluorescence emission (dashed) spectra of HT-ApcDB purified from recombinant *E. coli* cells containing pApcDB, p*Te*CpcS and either the bilin reductase genes to produce **(A)** PCB from pPcyA, **(B)** PEB from pPebS or **(C)** PΦB from pHy2. **(D)** Coomassie-stained SDS-polyacrylamide gel of HT-ApcDB purified from cells containing pApcDB, p*Te*CpcS and either pPcyA (lane 1), pPebS (lane 2), or pHy2 (lane 3). Molecular mass standards were loaded in lane S. **(E)** Zn-enhanced bilin fluorescence of the gel (excitation at 532 nm) in panel D.

Supplementary Fig. 4. Attachment of PEB, and/or PCB, or PΦB to ApcF **(A)** Absorbance (solid) and fluorescence emission (dashed) spectra of HT-ApcF purified from recombinant *E. coli* cells containing pApcF, p*Te*CpcS and either pPcyA (blue) or pPebS (red). **(B)** Absorbance spectrum (solid purple) of HT-ApcF purified from recombinant *E. coli* cells containing pApcF, p*Te*CpcS, and both pPcyA and pPebs. Fluorescence emission resulting from excitation of this sample at 490 nm (red-dashed line) is attributed to protein-bound PEB. Fluorescence emission resulting from excitation at 590 nm (blue-dashed line) is attributed to protein-bound PCB. **(C)** Absorbance (solid green) and fluorescence emission (dashed green) spectra of HT-ApcF purified

from recombinant *E. coli* cells containing pApcF, p*Te*CpcS and pHy2. **(D)** Coomassie-stained SDS-polyacrylamide gel of HT-ApcF purified from cells containing pApcF, p*Te*CpcS and either pPcyA (lane 1), pPebS (lane 2), both pPcyA and pPebS (lane 3), or pHy2 (lane 4). Molecular mass standards were loaded in lane S. **(E)** Zn-enhanced bilin fluorescence of the gel (excitation at 532 nm) in panel D.

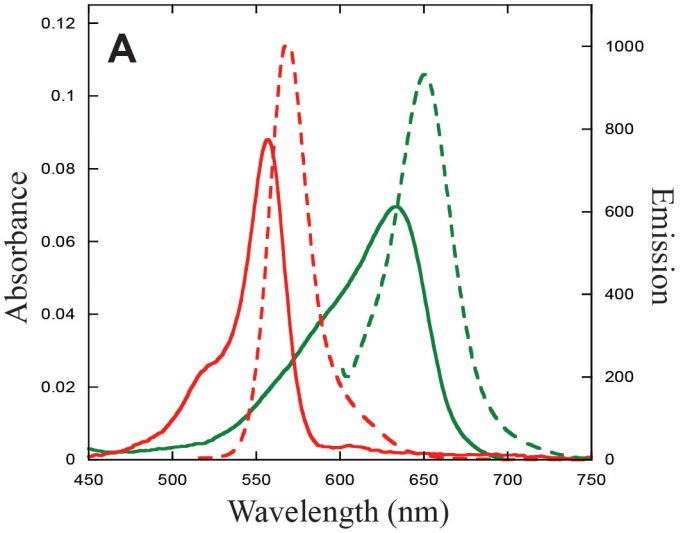
Supplementary Fig. 5. MALDI-TOF/TOF MS analysis of the tryptic digest of purified HT-*Te*CpcS-PEB **(A and B)** and HT-*Te*CpcS-PCB **(C and D)**. The structure of each precursor is presented in the corresponding figure inset. Presented are 1 keV (E_{LAB}) collision-induced dissociation (CID) product ion mass spectra of precursors at: **(A)** m/*z* 2124.9. The product ion at m/*z* 587 was identified as protonated PEB. The peak at m/*z* 1538 was matched with a peptide containing a cysteine at position 169. The sequence of the peptide is FGGFSMASFCSEIR. **(B)** m/*z* 1524.7. The fragment at m/z 938 was matched with a peptide containing a cysteine at position 2 after cleavage of the bilin. The sequence of the peptide is MCIGMDIR. **(C)** m/*z* 2124.9. The product ion at m/*z* 587 was identified as protonated PCB. The peak at m/*z* 1538 was matched with a peptide containing a cysteine at position 169 after cleavage of the bilin. The sequence of the peptide is FGGFSMASFCSEIR. **(D)** m/*z* 1524.7. The fragment at m/*z* 938 was matched with a peptide containing a cysteine at position 2 after cleavage of the bilin. The sequence of the peptide is MCIGMDIR.

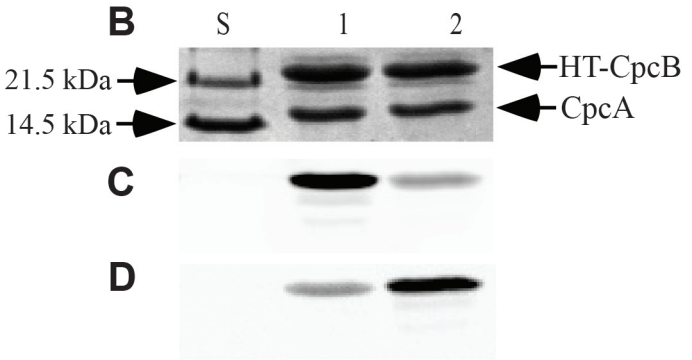
Supplementary Fig. 6. Analysis of *Te*CpcS C2S, C169S, and C2S/C169S variants in the binding and ligation of PCB. **(A)** Absorbance (solid) and fluorescence emission (dashed) spectra of HT-*Te*CpcS purified from recombinant *E. coli* cells containing pPcyA and either pTER13-30 (black), pTER13(C2S) (cyan), pTER13(C169S) (orange), or pTER13(C2S/C169S) (pink). **(B)** Absorbance (solid) and fluorescence emission (dashed) spectra of HT-CpcBA purified from recombinant *E. coli* cells containing pCpcBA and pPcyA with either pTER13-30 (black), pTER13(C2S) (cyan), pTER13(C169S) (orange), or pTER13(C2S/C169S) (pink). **(C)** Coomassie-stained SDS-polyacrylamide gel of HT-*Te*CpcS purified from cells containing pPcyA and either pTER13-30 (lane 1), pTER13(C2S) (lane 2), pTER13(C169S) (lane 3), or pTER13(C2S/C169S) (lane 4); and of HT-CpcBA purified from cells containing pCpcBA and pPcyA with either pTER13-30 (lane 5), pTER13(C2S) (lane 6), pTER13(C169S) (lane 7), or pTER13(C2S/C169S) (lane 8). Molecular mass standards were loaded in lane S. **(E)** Zn-enhanced bilin fluorescence of the gel (excitation at 635 nm) in panel D.

Supplementary Fig. 7. Analysis of *Te*CpcS R151G and S155G variants in the binding and ligation of PCB. **(A)** Absorbance (solid) and fluorescence emission (dashed) spectra of HT-*Te*CpcS purified from recombinant *E. coli* cells containing pPcyA and either pTER13-30 (black),

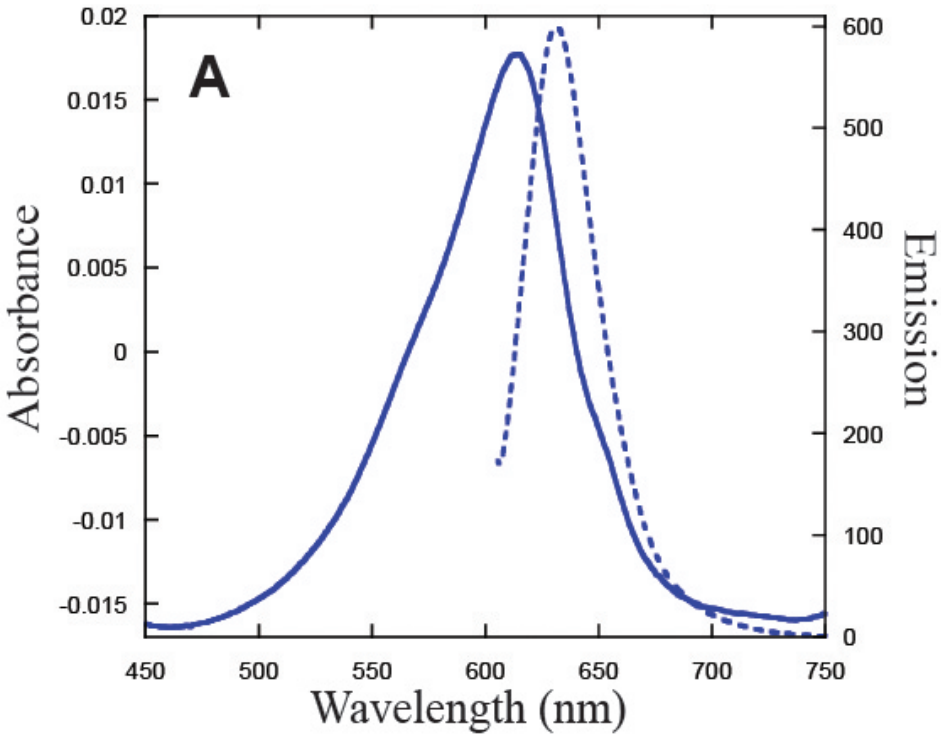
pTER13(R151G) (orange) or pTER13(S155G) (purple). **(B)** Coomassie-stained SDS polyacrylamide gel of HT-*Te*CpcS purified from cells containing pPcyA and pTER13-30 (lane 1), pTER13(R151G) (lane 2) or pTER13(S155G) (lane 3). Molecular mass standards were loaded in lane S. **(C)** Zn-enhanced bilin fluorescence of the gel (excitation at 635 nm) in panel B. **(D)** Absorbance (solid) and fluorescence emission (dashed) spectra of HT-CpcBA purified from recombinant *E. coli* cells containing pCpcBA and pPcyA with pTER13-30 (black), pTER13(R151G) (orange) or pTER13(S155G) (purple). **(E)** Coomassie-stained SDS polyacrylamide gel of HT-CpcBA purified from cells containing pCpcBA and pPcyA with either pTER13-30 (lane 1), pTER13(R151G) (lane 2) or pTER13(S155G) (lane 3). **(F)** Zn-enhanced bilin fluorescence of the gel (excitation at 635 nm) in panel E.

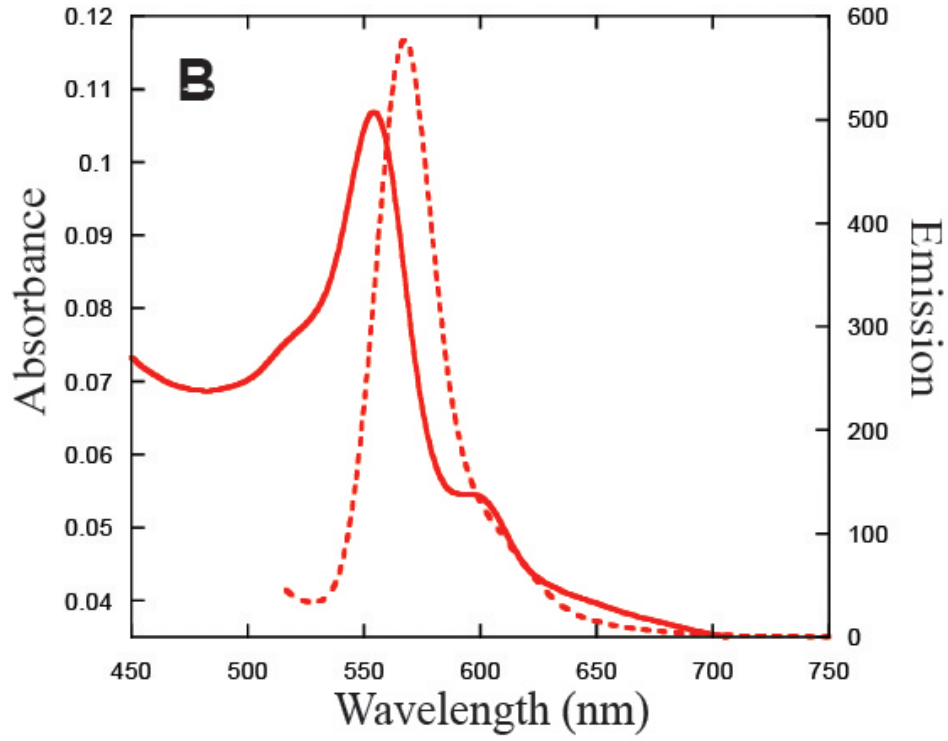
Supplementary Fig. 1

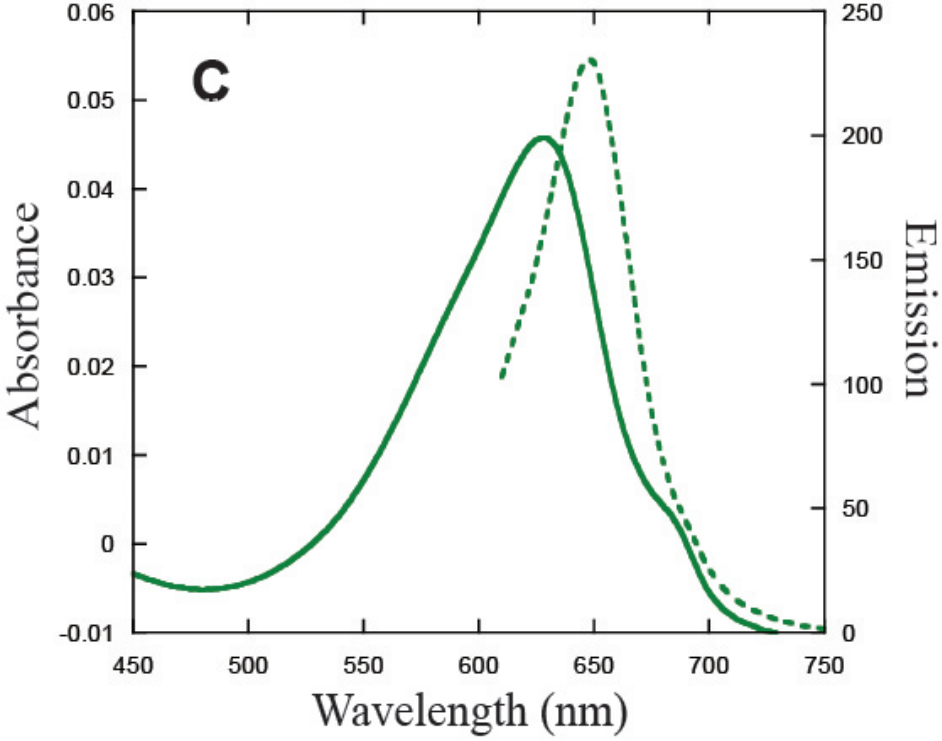


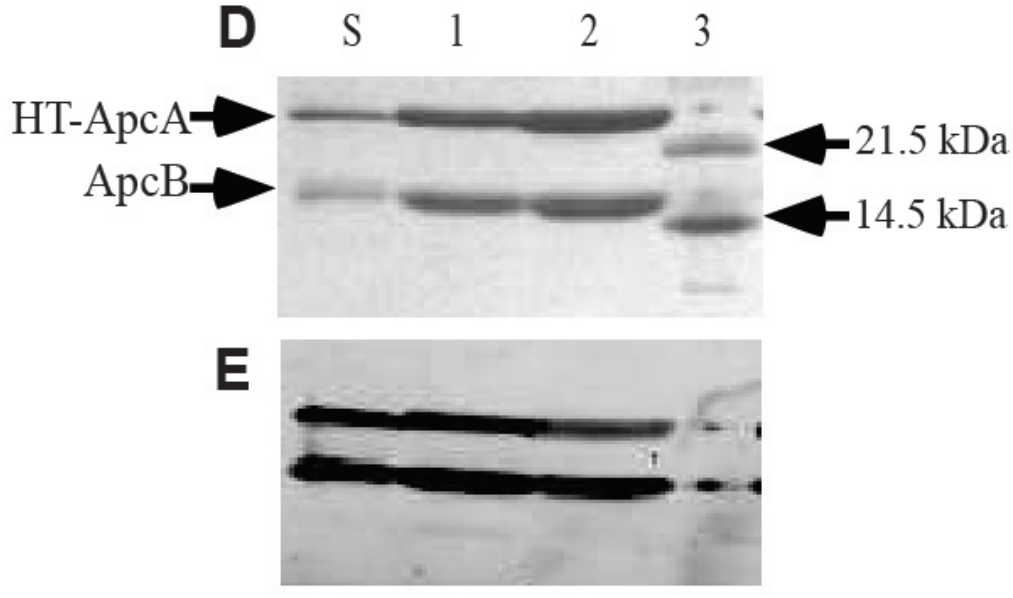


Supplementary Fig. 2

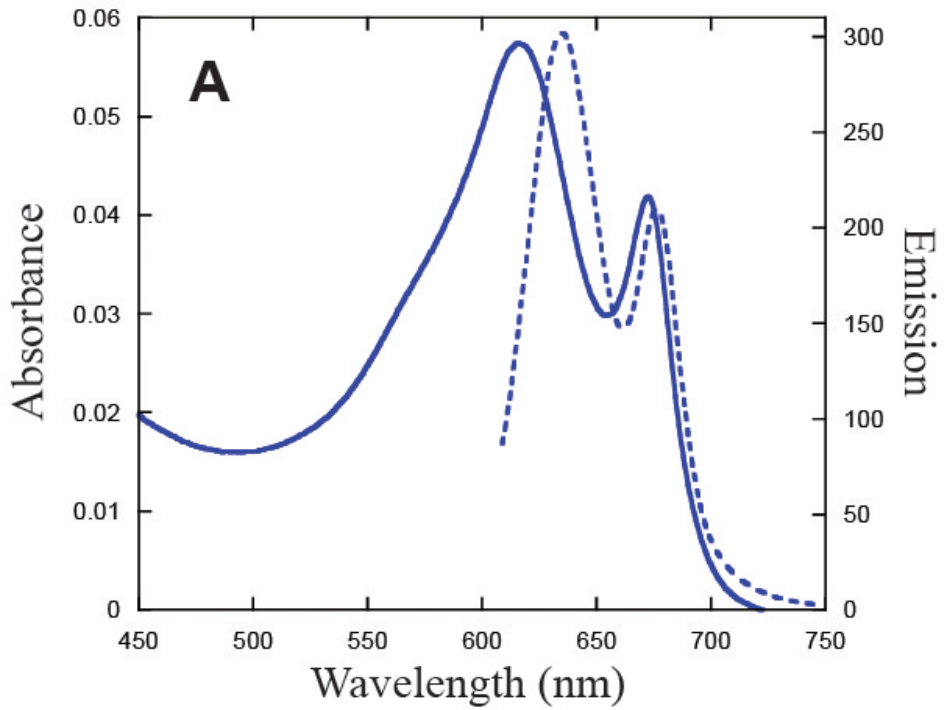


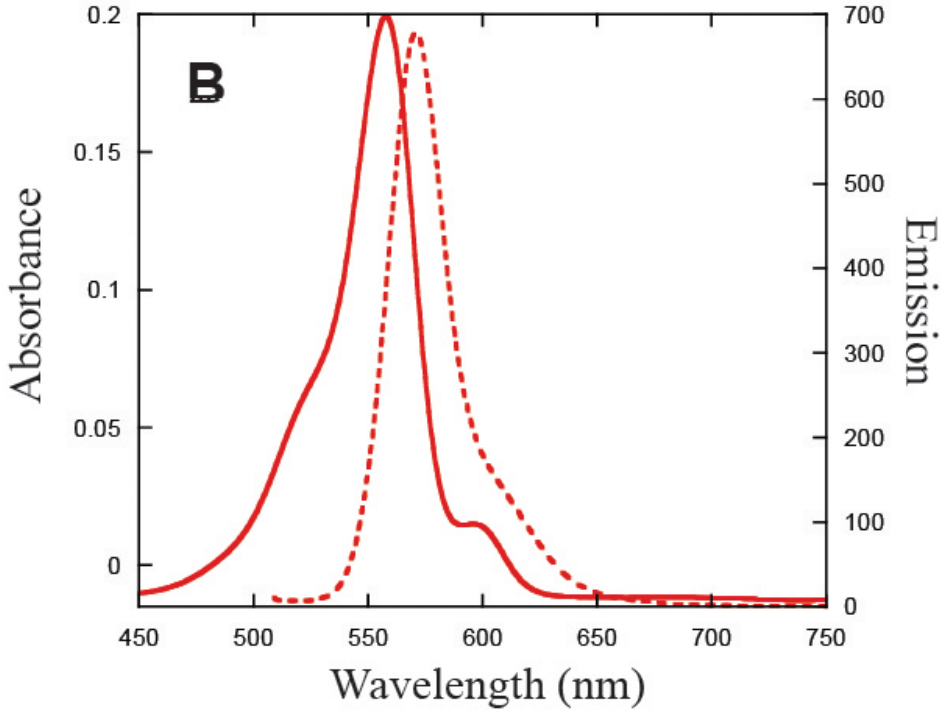


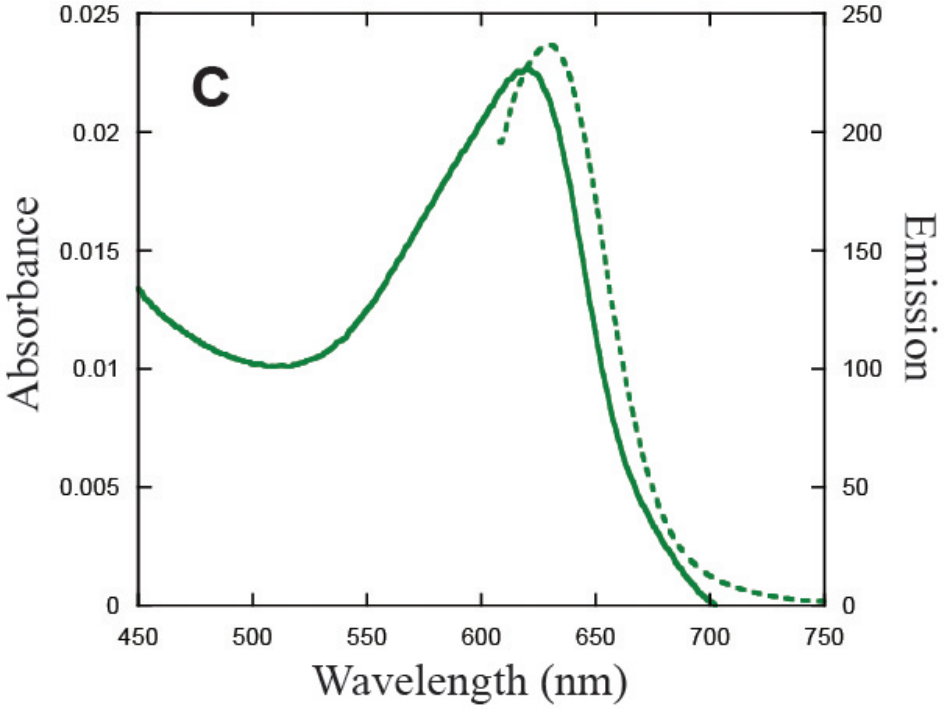


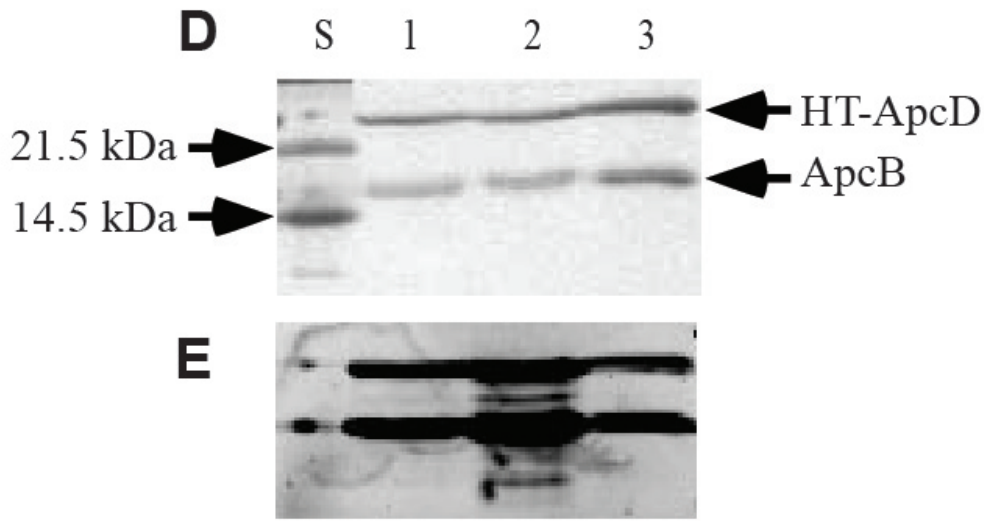


Supplementary Fig. 3

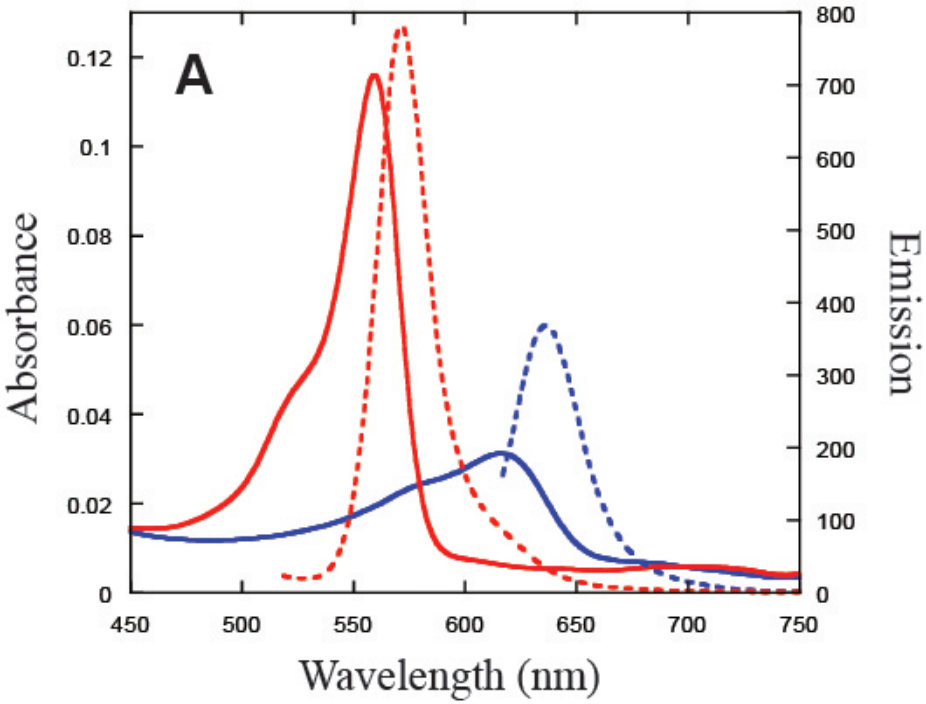


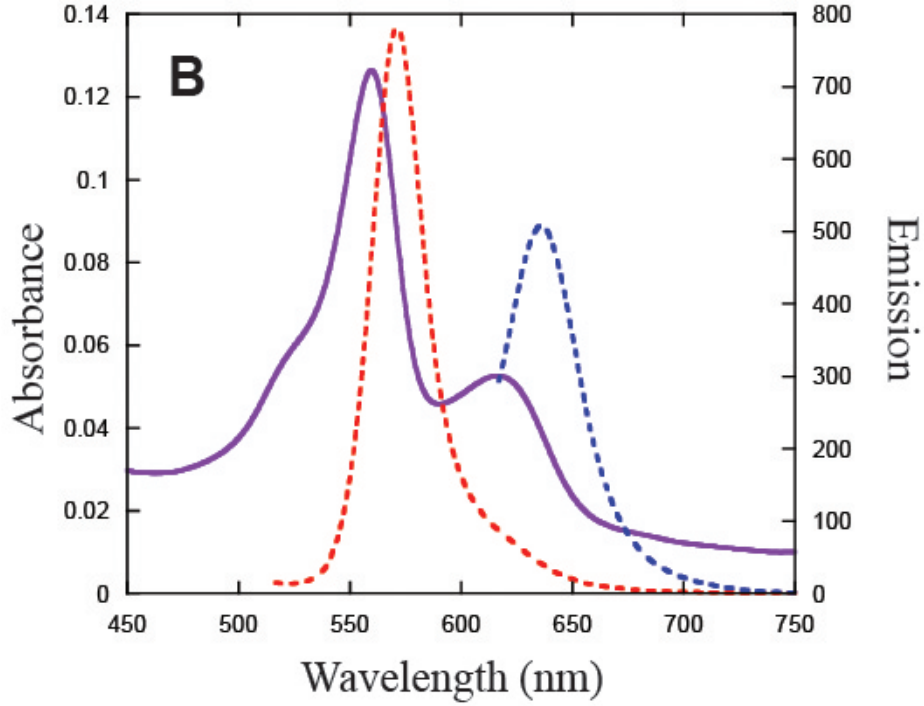


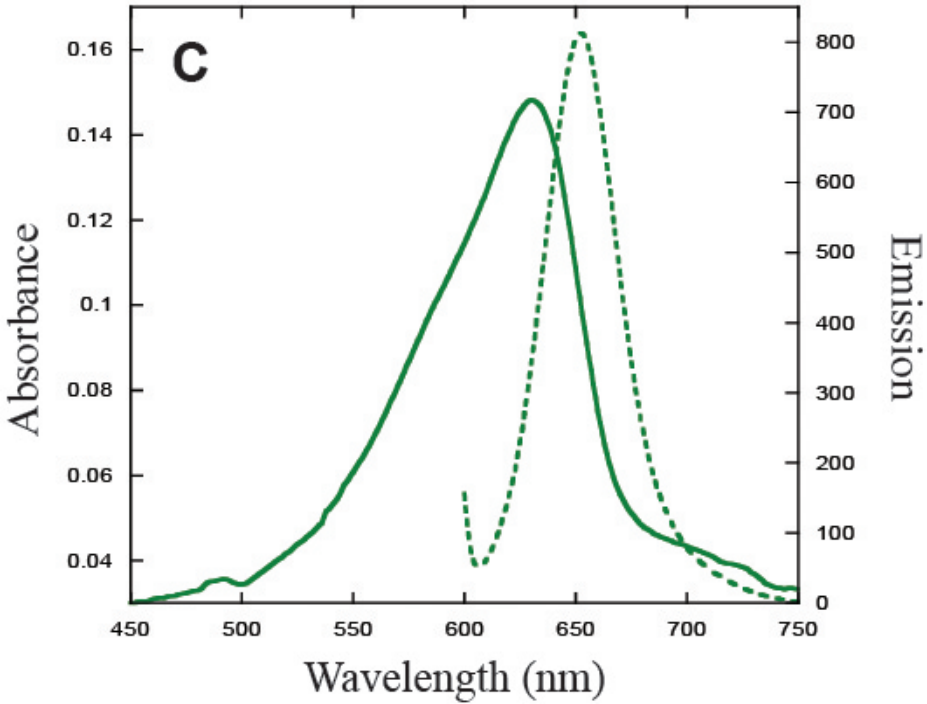


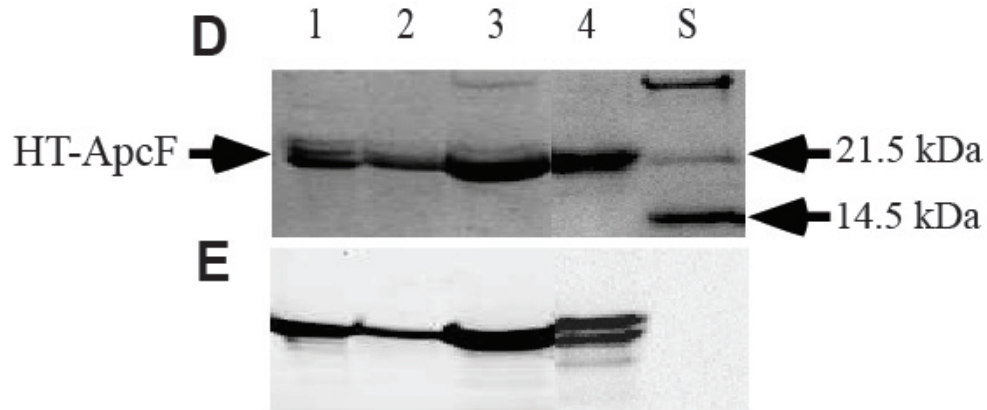


Supplementary Fig. 4

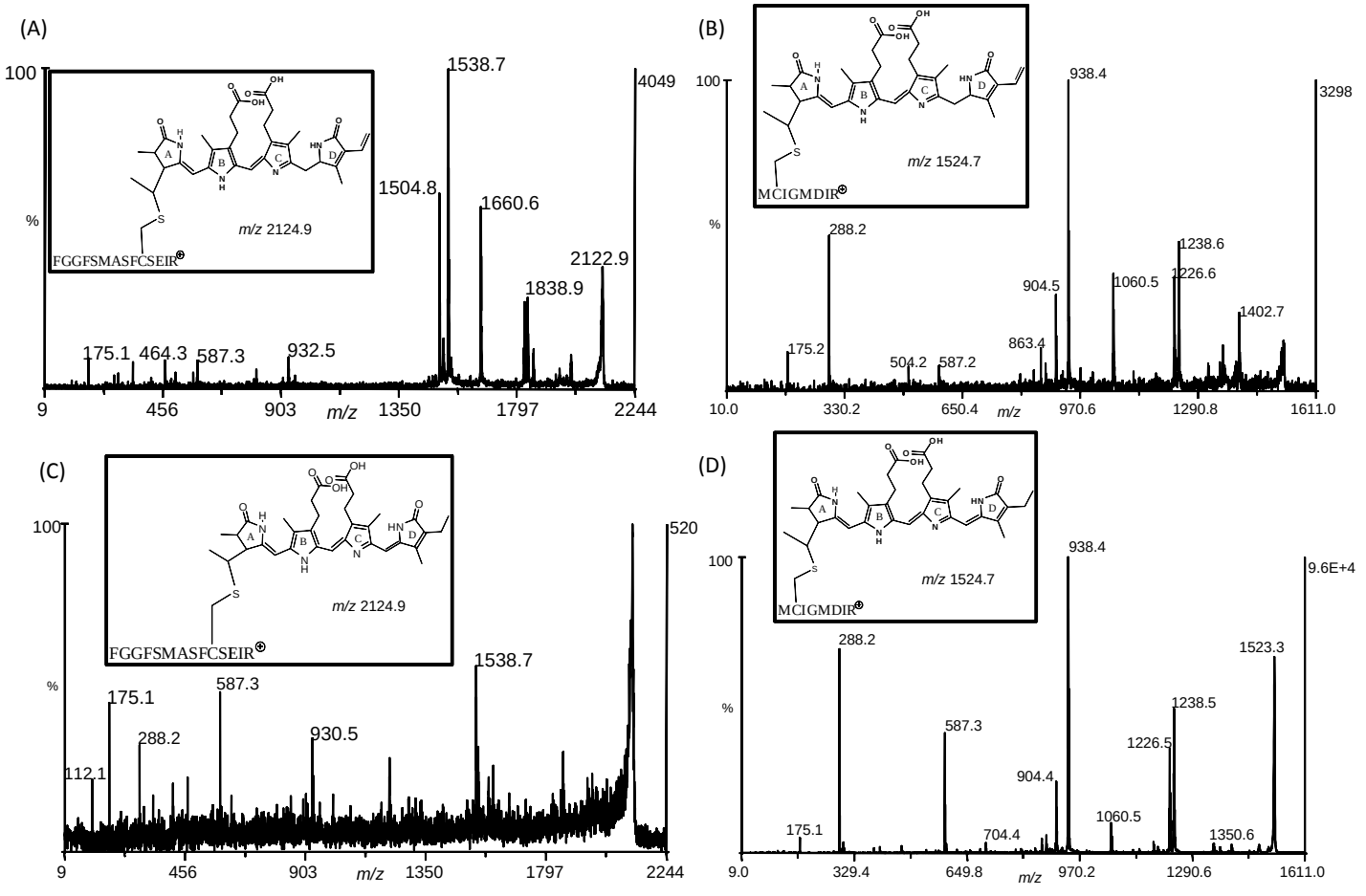








Supplementary Fig. 5



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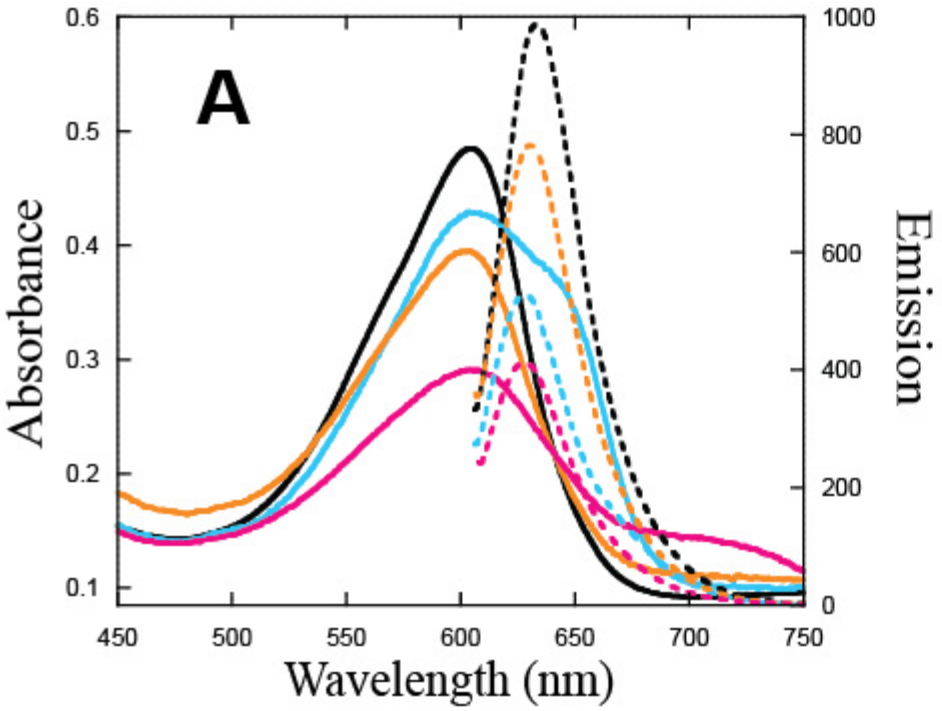

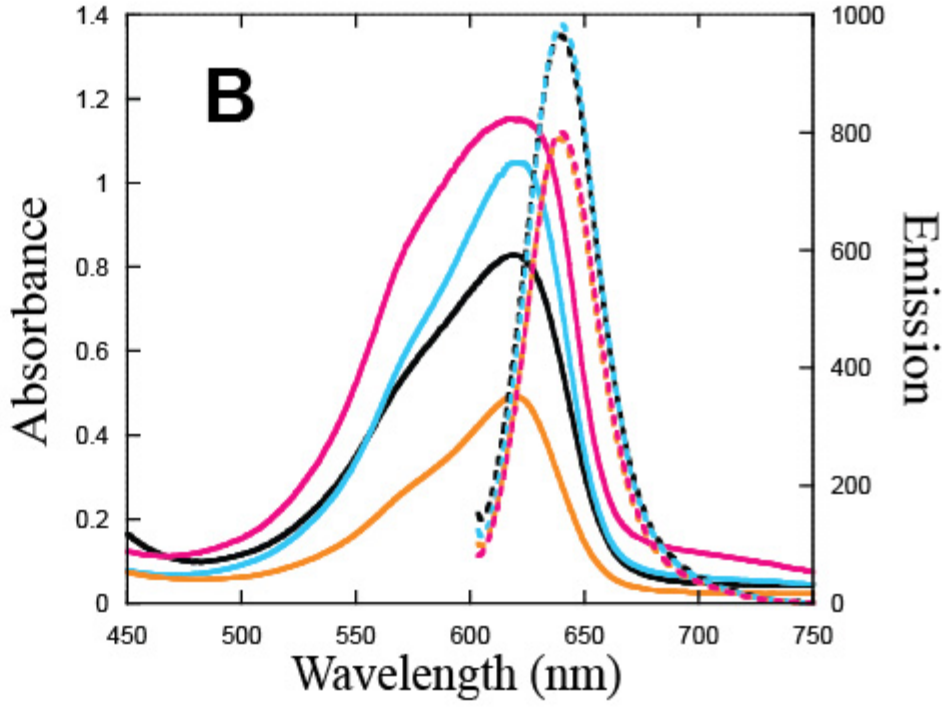

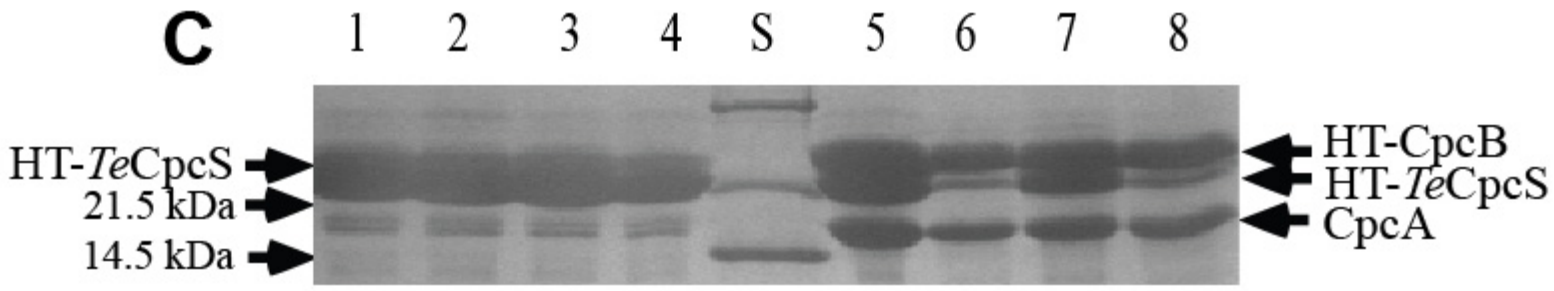

Supplementary Fig. 7