Supplemental Figures:

Supplemental Fig. 1. Picture of the *E. coli* cell pellets from cells containing HT-CpeA with pPebS and either pCpeYZ (left) or pCpeS (right).

Supplemental Fig. 2. Immunoblot analyses of whole-cell extracts expressing HT-CpeA and HT-CpeB. To determine whether apo-HT-CpeA and apo-HT-CpeB were present in the supernatant (soluble) or in the pellet fraction (as inclusion bodies) of whole-cell extracts of *E. coli* cells grown at either 37 °C or 18 °C, cell pellets were lysed and soluble and insoluble proteins were separated by low-speed centrifugation at 10,000 × *g*. Samples from the supernatant (soluble) and pellet (inclusion bodies and unbroken cells) were separated by SDS-PAGE, transferred to a PVDF membrane, and probed with antibodies raised against CpeA (Panel A) or CpeB (Panel B) from *F. diplosiphon*. **Panel A:** Lanes were loaded as follows: HT-CpeA 37 °C supernatant (lane 1); HT-CpeA 37 °C insoluble pellet (lane 2); HT-CpeA 18 °C supernatant (lane 3); HT-CpeA 18 °C insoluble pellet (lane 4). **Panel B:** Lanes were loaded as follows: HT-CpeB 37 °C supernatant (lane 1); HT-CpeB 37 °C insoluble pellet (lane 2); HT-CpeB 18 °C supernatant (lane 3); HT-CpeB 18 °C insoluble pellet (lane 4). Proteins are identified to the left of each panel and with arrows.

Supplemental Fig. 3. Pull-down assay showing absence of a detectable interaction between CpeY and HT-CpeZ. This figure shows a Coomassie-blue stained SDS-polyacrylamide gel that was loaded with purified HT-CpeZ (lane 1); two different *E. coli* whole-cell extracts containing recombinant CpeY obtained from expression cells with pCpeY (lanes 2 and 3), the flow-through from metal affinity chromatography of an interaction assay (the same assay as shown in lane 2) between HT-CpeZ and CpeY extract (lane 4); and the eluate from this interaction assay between HT-CpeZ and CpeY extract (lane 5). Lane S shows the molecular mass standards at left. Arrows at the right show the expected migration positions of CpeY and HT-CpeZ.

Supplemental Fig. 4. Chromatogram of a tryptic digest of HT-CpeA-PEB purified from cells containing pCpeA, pCpeYZ, and pPebS separated on a C_{18} RP-HPLC column.

Supplemental Fig. 5. Mass spectrometric analyses of low abundance tryptic peptide of HT-CpeA-PEB produced with CpeY/CpeZ **A.** MALDI MS/MS spectrum of the precursor ion at m/*z* 1089, which was deduced to be a peptide fragment with a covalently bound PEB chromophore. This peptide binding PEB was derived from trypsin digestion of the HT-CpeA-PEB produced in the presence of CpeY and CpeZ. The MS/MS spectrum contains a peak of interest at m/*z* 503. The peak, resulting from a neutral loss of 586, was attributed to a peptide containing a cysteine at position 139. The sequence of the peptide is (R) GCAPR (D). The peak corresponding to protonated PEB, which is detected at m/*z* 587, was not detected in the spectrum shown in this figure. Nonetheless when applying a higher acceleration voltage the peak is visible. **B.** Peak assignments of product ion spectrum corresponding to the precursor protonated PEB-peptide (derived from CpeA) complex. A tick mark prior to number, e.g., ‘803, indicates that one hydrogen has been transferred to the departing neutral ion upon cleavage. A tick mark after a number, e.g., 969’, indicates the transfer of one hydrogen to the formed ion. A dot (·) indicates a radical ion.

Supplemental Fig. 6. Tryptic digest of HT-CpeB-PEB purified from cells containing pCpeB, pCpeS, and pPebS. The chromatogram represents sample separated on a C_{18} RP-HPLC column.

Supplemental Fig. 7. A. MALDI MS/MS spectrum of the precursor ion at m/*z* 1250, which was deduced to be a peptide fragment with a covalently bound PEB chromophore, and which was derived from trypsin digestion of the HT-CpeB-PEB produced in the presence of CpeS. The MS/MS spectrum contains two peaks of interest at m/*z* 664 and m/*z* 587. The peak at m/*z* 664 was attributed to a peptide containing a cysteine at position 80. The sequence of the peptide is (R) MAACLR (D). The second peak at m/*z* 587 was attributed to protonated PEB. **B.** Peak assignments of product ion spectrum corresponding to the precursor protonated PEB-peptide (derived from CpeB) complex. A tick mark prior to number, e.g., '964, indicates that one hydrogen has been transferred to the departing neutral ion upon cleavage. A tick mark after a number, e.g., 1129', indicates the transfer of one hydrogen to the formed ion. A dot (·) indicates a radical ion.

Supplemental Fig. 8. Analyses of HT-CpeA-PCB produced in the presence of pPcyA and p*CpeYZ* **A.** Absorbance (solid line) and fluorescence emission (dashed line) spectra of HT-CpeA purified from cells containing pCpeA, pPcyA with pCpeYZ and absorbance (dashed dotted line), fluorescence (dotted line) without pCpeYZ are shown. **B**. Coomassie-blue-stained SDS polyacrylamide gel containing HTCpeA purified from cells containing pCpeA, pPcyA (lane 1) and pCpeA, pPcyA, pCpeYZ (lane 2). Position of a molecular mass standard is indicated to the right. **C**. Zn-enhanced fluorescence image of the gel pictured in panel **B**.

Supplemental Fig. 9. Amino acid sequence alignment between CpeY from *F. diplosiphon* (called Fd in the figure), a fusion of CpcE with CpcF from *Synechocystis* sp. PCC 6803 (called PCC6803 CpcEF), and RpcG from *Synechococcus* WH8102 (called WH8102 RpcG). The CpcE/CpcF proteins were combined to form one concatenated protein. The software used was MacVector 9.0. Dark shading indicates identical residues and light shading indicates similar residues.

Supplemental Table 1: Plasmids used in this study

Plasmid Name	Recombinant proteins produced[a]	Parent vector	Antibiotic[b]	Reference
pPebS	Myovirus HO1 and HT-PebS	pACYCDuet-1	Cm	(1)
pPcyA	PcyA from *Synechococcus* sp. PCC 7002 and Ho1 from *Synechocystis* sp. PCC 6803	pACYCDuet-1	Cm	(2)
pCpeA	*F. diplosiphon* HT-CpeA	pETDuet-1	Ap	This paper
pCpeA:C82S	*F. diplosiphon* HT-CpeA (Cys^{82} mutated to Ser)	pETDuet-1	Ap	This paper
pCpeA:C139S	*F. diplosiphon* HT-CpeA (Cys^{139} mutated to Ser)	pETDuet-1	Ap	This paper
pCpeA:C82S/ C139S	*F. diplosiphon* HT-CpeA (Cys^{82} and Cys^{139} mutated to Ser)	pETDuet-1	Ap	This paper
pCpeB	*F. diplosiphon* HT-CpeB	pETDuet-1	Ap	This paper
pCpeB:C80S	*F. diplosiphon* HT-CpeB (Cys^{80} mutated to Ser)	pETDuet-1	Ap	This paper
pCpeB:C165S	*F. diplosiphon* HT-CpeB (Cys^{165} mutated to Ser)	pETDuet-1	Ap	This paper
pCpeB:C48S/ C59S	*F. diplosiphon* HT-CpeB (Cys^{48} and Cys^{59} mutated to Ser)	pETDuet-1	Ap	This paper
pCpeZ	*F. diplosiphon*,HT-CpeZ	pCOLADuet-1	Km	This paper
pCpeY	*F. diplosiphon* CpeY	pCOLADuet-1	Km	This paper
pCpeYZ	*F. diplosiphon* HT-CpeZ and CpeY	pCOLADuet-1	Km	This paper
pCpeS	*F. diplosiphon* CpeS	pCOLADuet-1	Km	This paper

[a] Proteins produced as Hexa-histidine-tagged fusions are indicated as HT-

[b] Antibiotic resistance used to select for the presence of the plasmid (Ap: ampicillin; Cm: chloramphenicol; Km: kanamycin; Sp: spectinomycin)

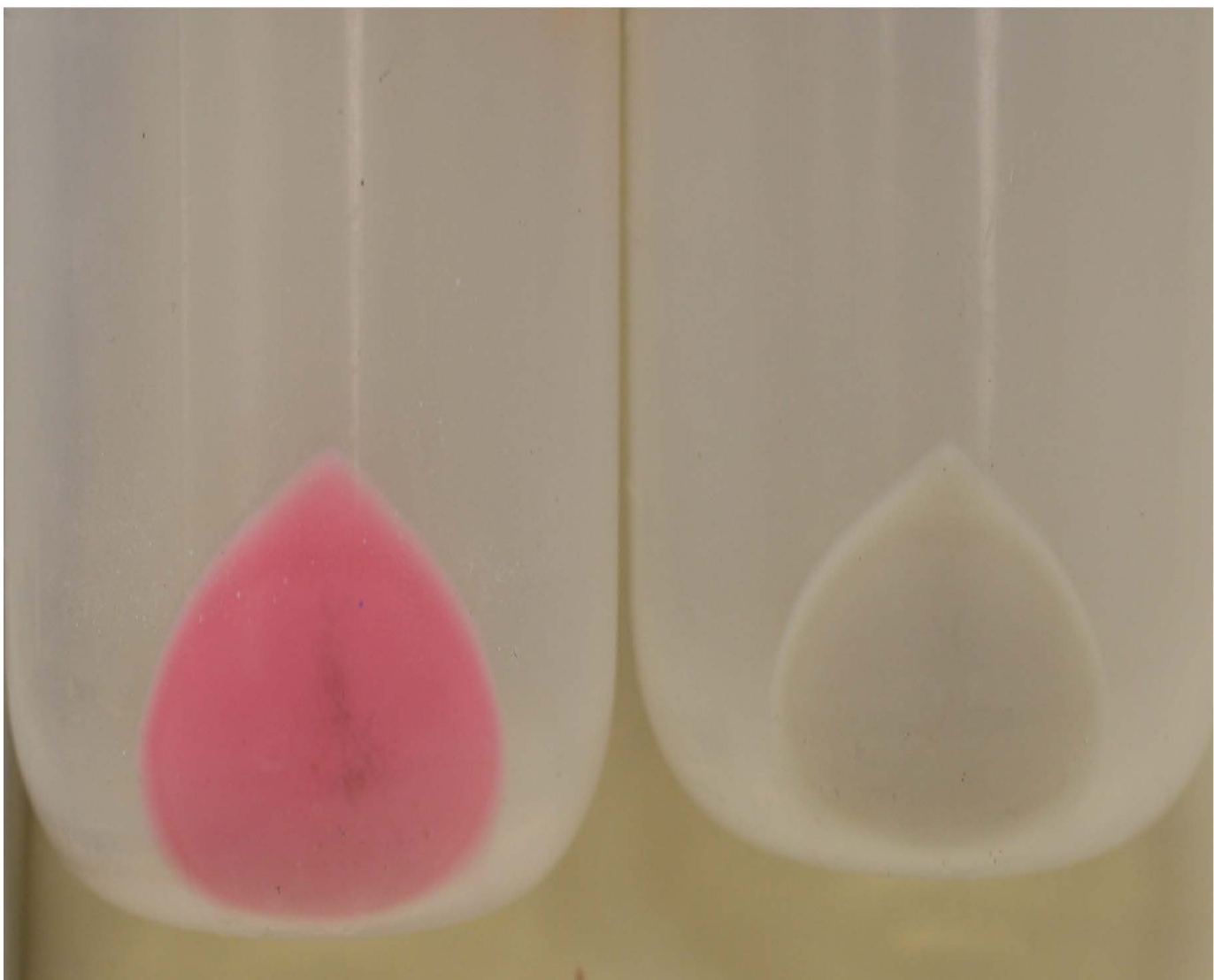
1. Dammeyer, T., Bagby, S. C., Sullivan, M. B., Chisholm, S. W., and Frankenberg-Dinkel, N. (2008) *Curr. Biol.* **18**, 442-448
2. Biswas, A., Vasquez, Y. M., Dragomani, T. M., Kronfel, M. L., Williams, S. R., Alvey, R. M., Bryant, D. A., and Schluchter, W. M. (2010) *Appl. Environ. Microbiol.* **76**, 2729-2739

Supplemental Table 2.

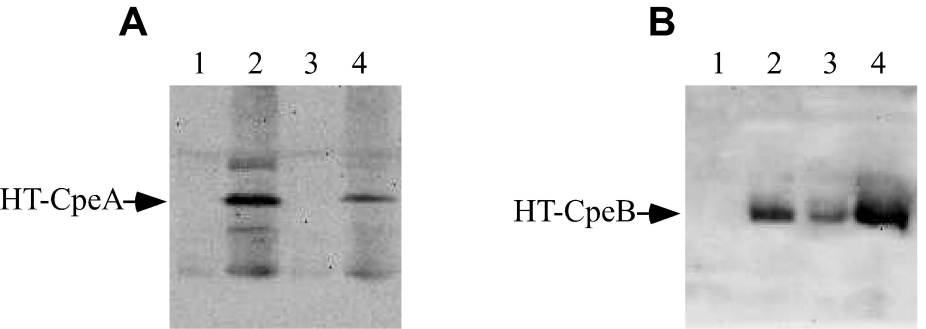
Oligonucleotide primers used in this paper (Engineered restriction enzyme sites are underlined)

Primer Name	Sequences
cpeAF	5'-AAGGATCCGATGAATCAGTTGTTACCACCGT-3'
cpeAR	5'-AAGAATTCCTAGGAGAGAGAGTTAATAGCGTA-3'
cpeBF	5'-AAGGATCCGATGCTTGATGCTTTTTCTAGAGC-3'
cpeBR	5'-CCGAATTCTTAGCTCAAAGCAGAGATTACGCG-3'
cpeZF	5'-CCGGATCCGATGCCGACAACAGAAGAACTATTCCAA-3'
cpeZR	5'-CCGAATTCTTATTTTTCTCCCCGCTGAAACTT-3'
cpeYF	5'-ACAAGGAGCTTGCATATGGATAAGCGCTTTTTT-3'
cpeYR	5'-AACTCGAGGGCTGTGATTTCTTGATTTTTCAGGGT-3'
cpeSF	5'-CAAATAGCTAAAACATATGGAAACCAAAGTGTTG-3'
cpeSR	5'-AACTGCAGCTAGGCACCAGTGTTTATG-3'
CpeA (C82S)	5' CCTTCAAAGCTAAGTCCGCTCGTGACATC-3'
CpeA (C139S)	5'- CGTAACCGTGGTTCTGCACCTCGTGATATG-3'
pETDuet(XhoI del)	5'-ACGTCGGTACCCTCCAGTCTGGTAAAGAAACCGCTG-3'
CpeB (C80S)	5'-CGTATGGCTGCCTCCTTACGCGATGCA-3'
CpeB (C165S)	5'-GTTGAAGATCGTTCCGCTAGCTTAGTT-3'
CpeB (C48S, C59S)	5'-GCTAGCTCCATGGTTTCTGATGCGTAGC
	TGGAATGATCTCCGAAAACCAAGGT-3

Sup. Fig. 1



Sup. Fig.2



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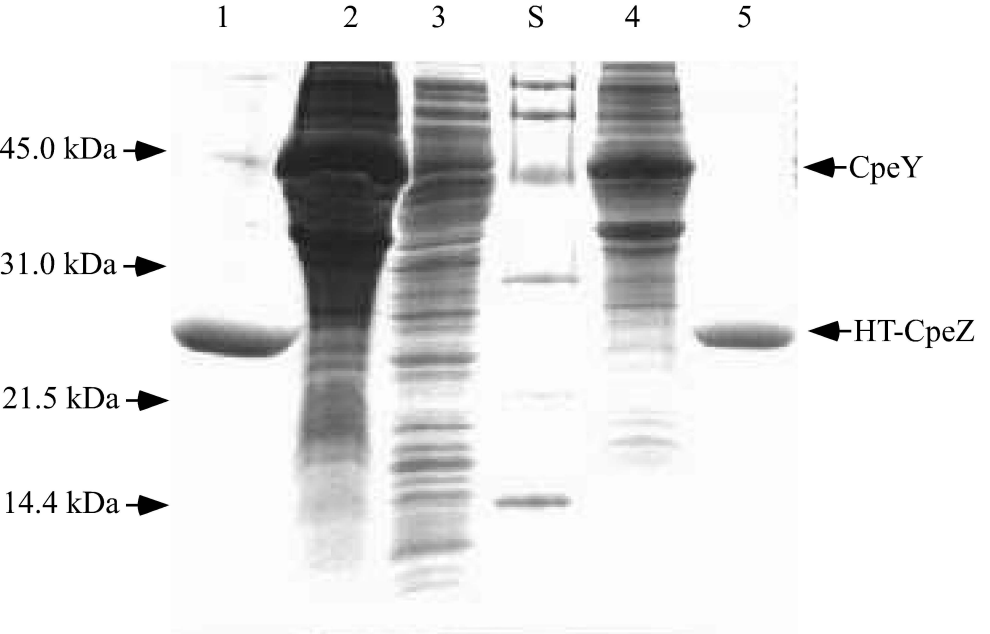

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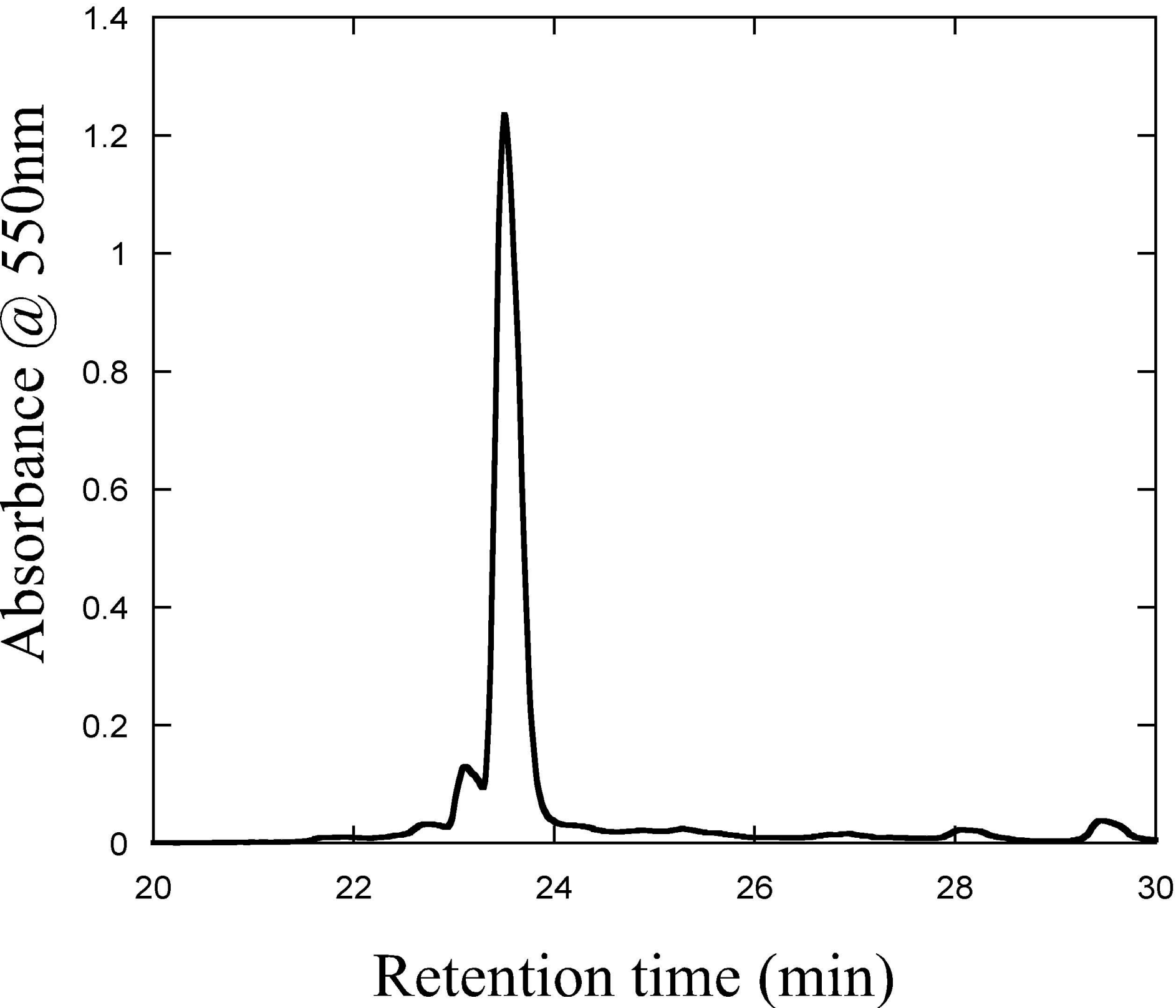
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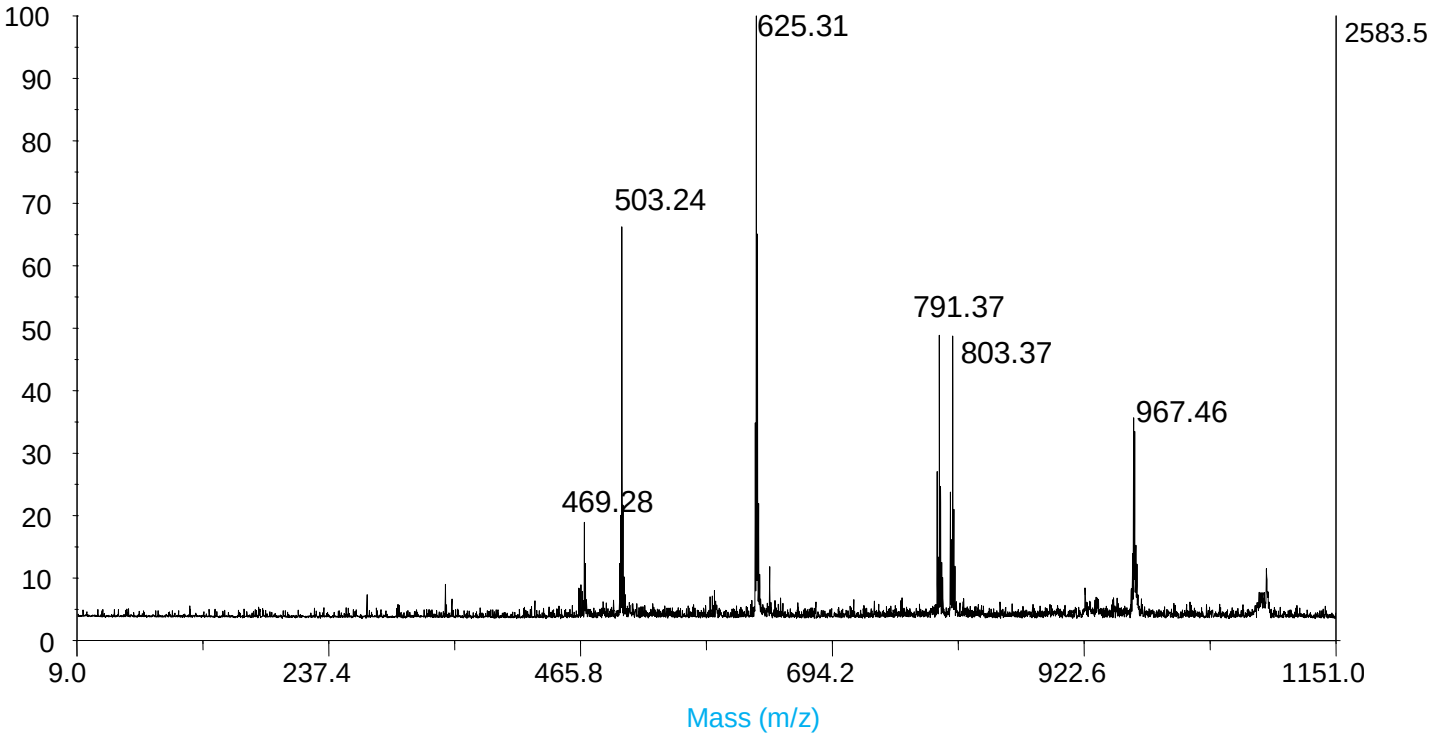
1.2
1
0.8
0.6
0.4
0.2
0
20
22
24
26
28
30
Retention time (min)

Sup. Fig. 5

A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90
80
70
60
50
40
30
20
10
0
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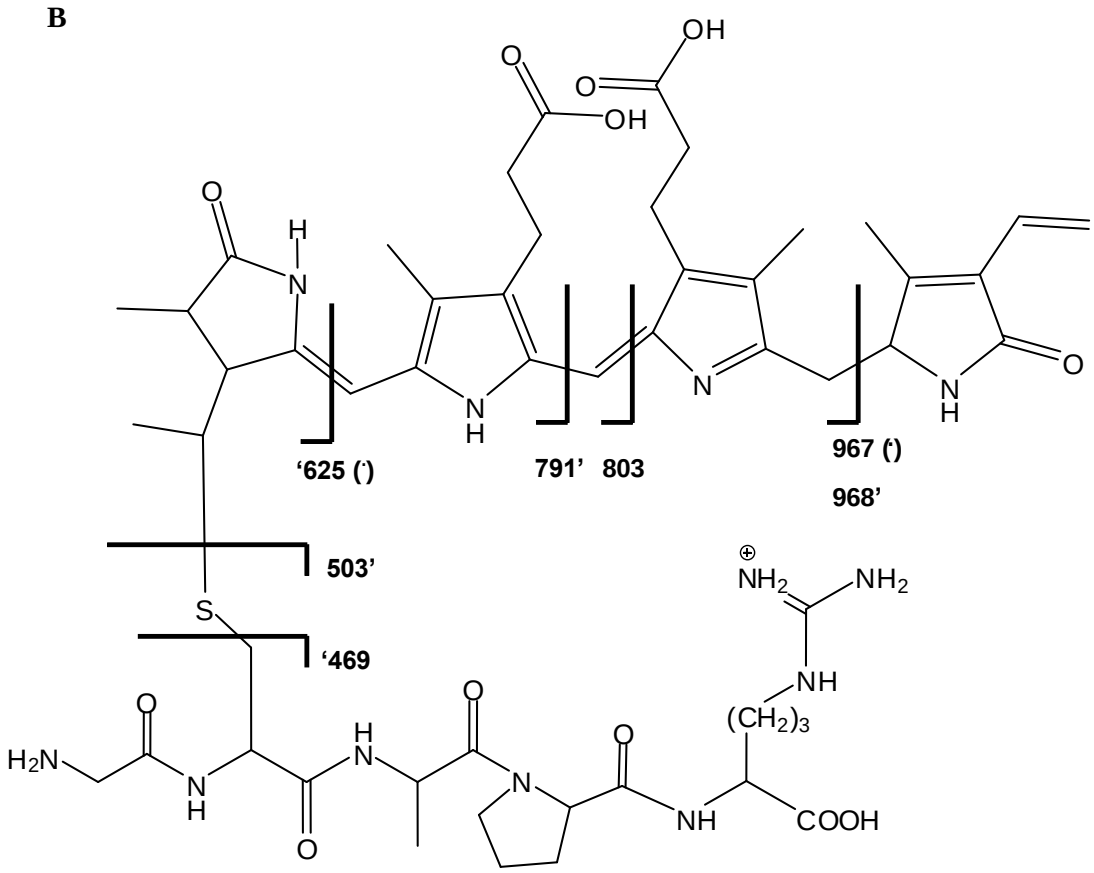
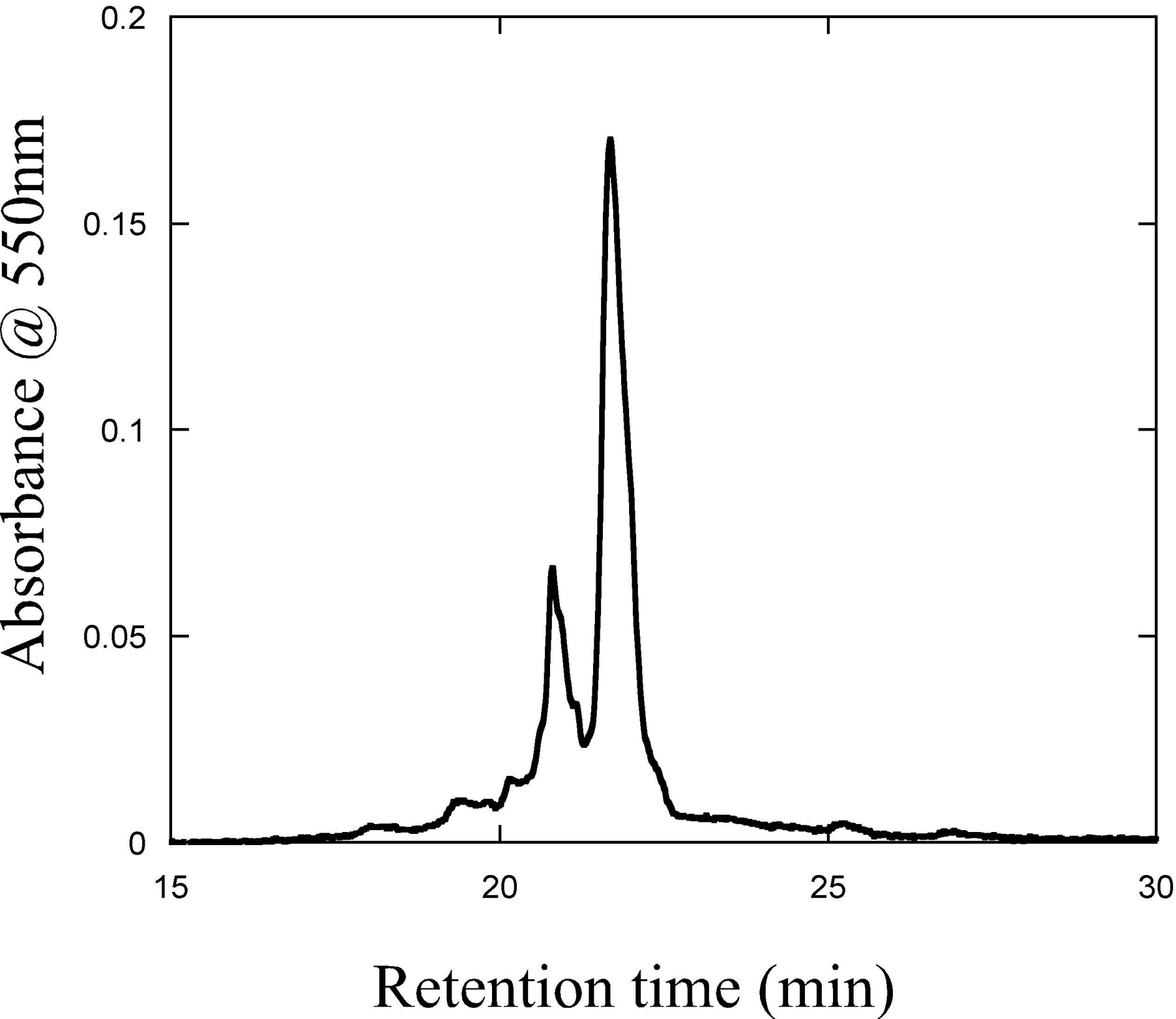
503.24
791.37
803.37
967.46
469.28
9.0
237.4
465.8
694.2
922.6
1151.0
Mass (m/z)

B


OH
O
O
OH
O
H
N
N
H
N
N
H
O
'625 (')
791'
803
967 (')
968'
503'
S
'469
H2N
O
N
H
O
H
N
O
N
O
NH
COOH
NH2
NH2
NH
(CH2)3

Sup. Fig. 6


0.2
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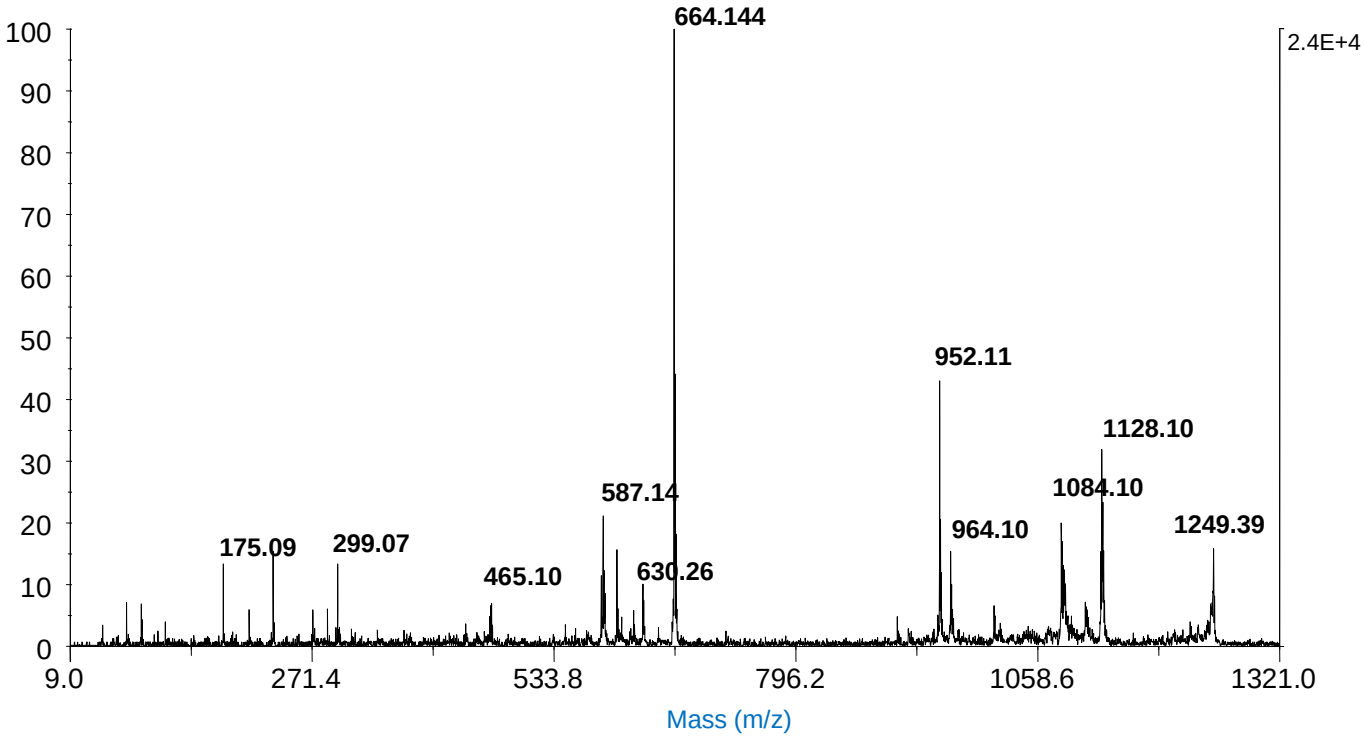
0.05
0
Absorbance @ 550nm
15
20
25
30
Retention time (min)

Sup. Fig. 7

A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2.4E+4
952.11
1128.10
1084.10
587.14
964.10
1249.39
175.09
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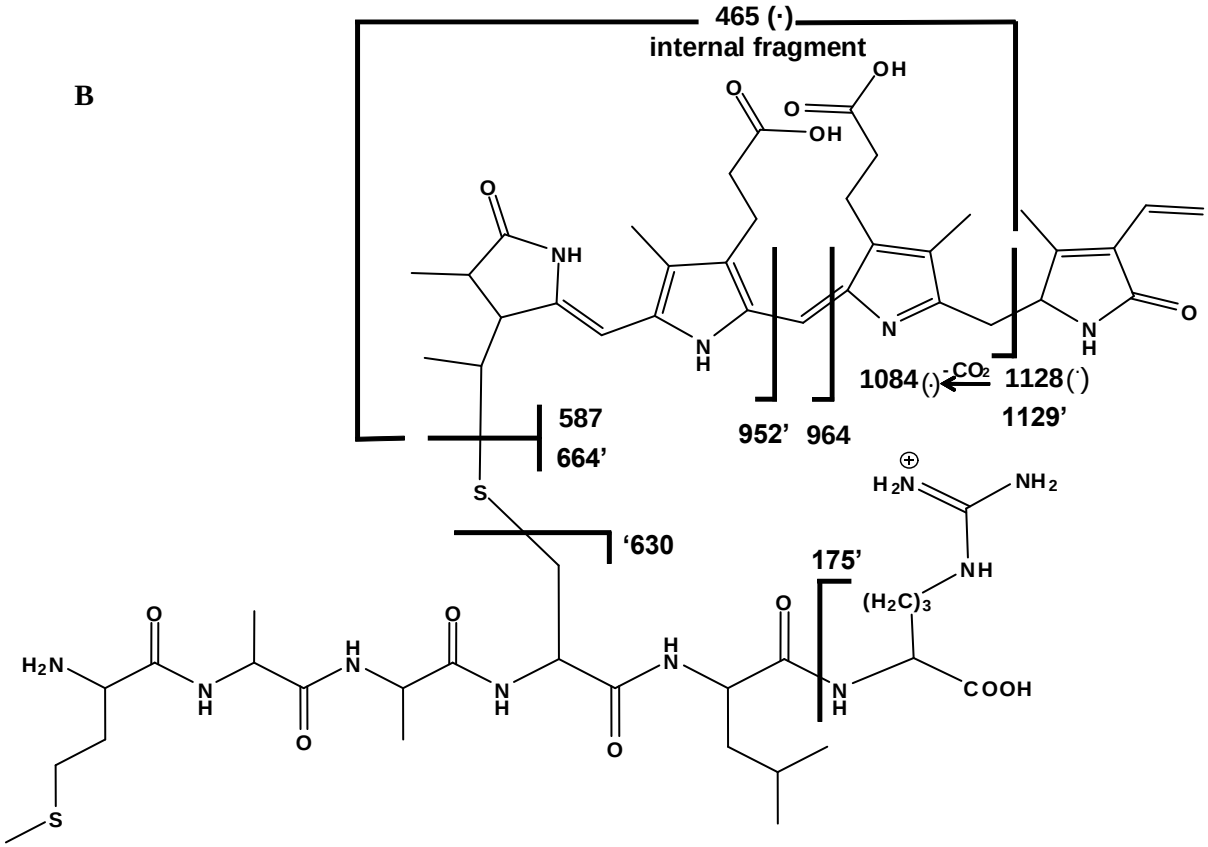
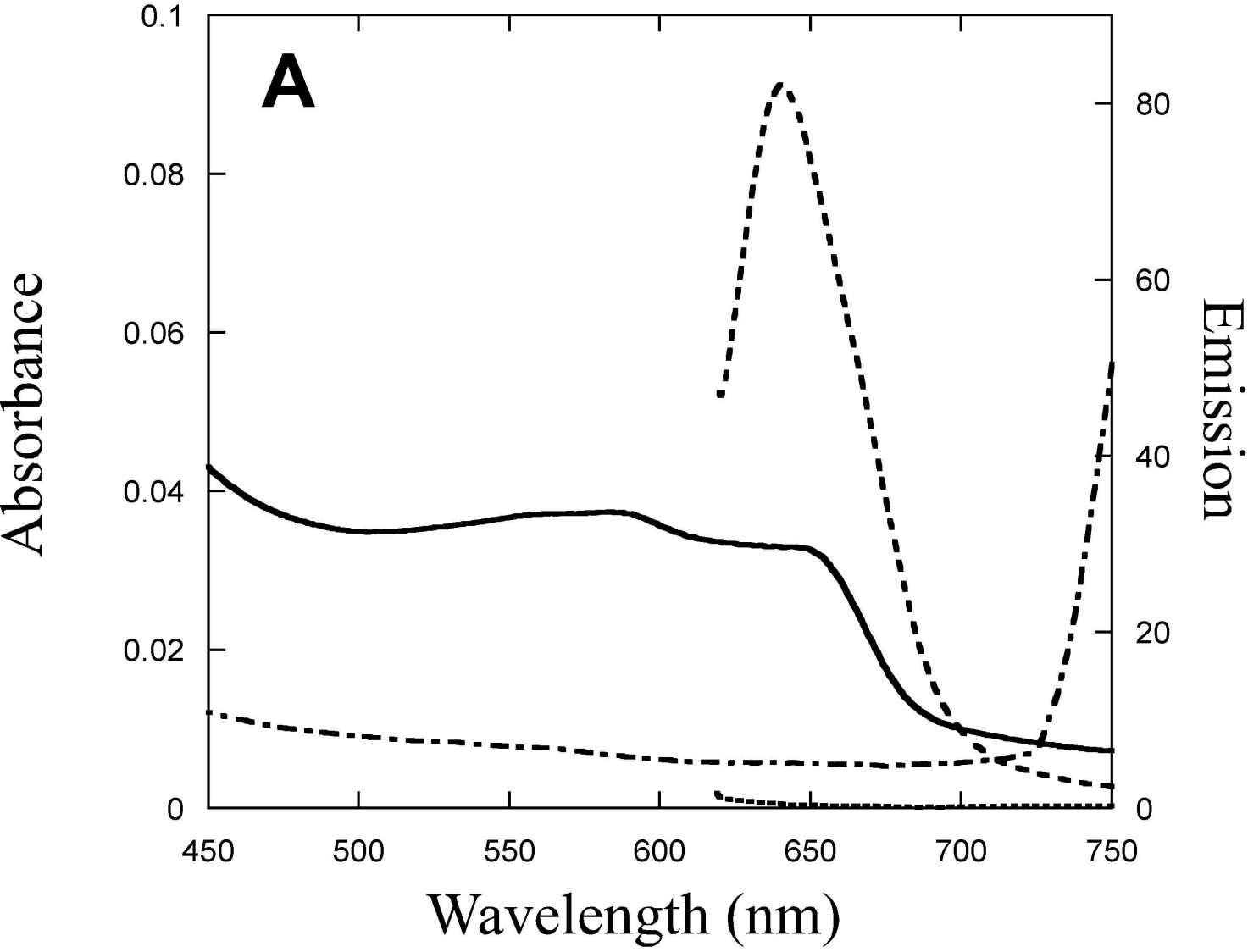
465.10
630.26
100
90
80
70
60
50
40
30
20
10
0
9.0
271.4
533.8
796.2
1058.6
1321.0
Mass (m/z)

B


465 (·)
internal fragment
OH
O
O
OH
NH
O
N
N
H
N
H
O
1084 (·)
-CO2
1128 (·)
1129'
952'
964
587
664'
S
'630
175'
H2N
NH2
NH
(H2C)3
COOH
H2N
O
N
H
H
N
O
S

Sup. Fig. 8


A
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0.08
0.06
0.04
0.02
0
Absorbance
80
60
40
20
0
Emission
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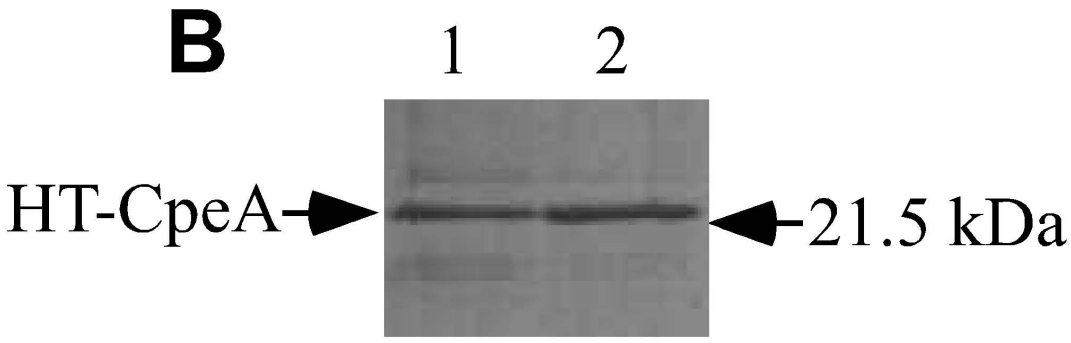
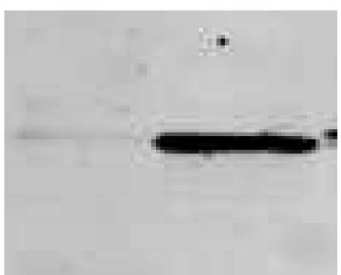
500
550
600
650
700
750
Wavelength (nm)


B
1
2
HT-CpeA
21.5 kDa


C

Supplemental Fig. 9