Zinc Coordination is Essential for the Function and Activity of the Type II Secretion ATPase EpsE

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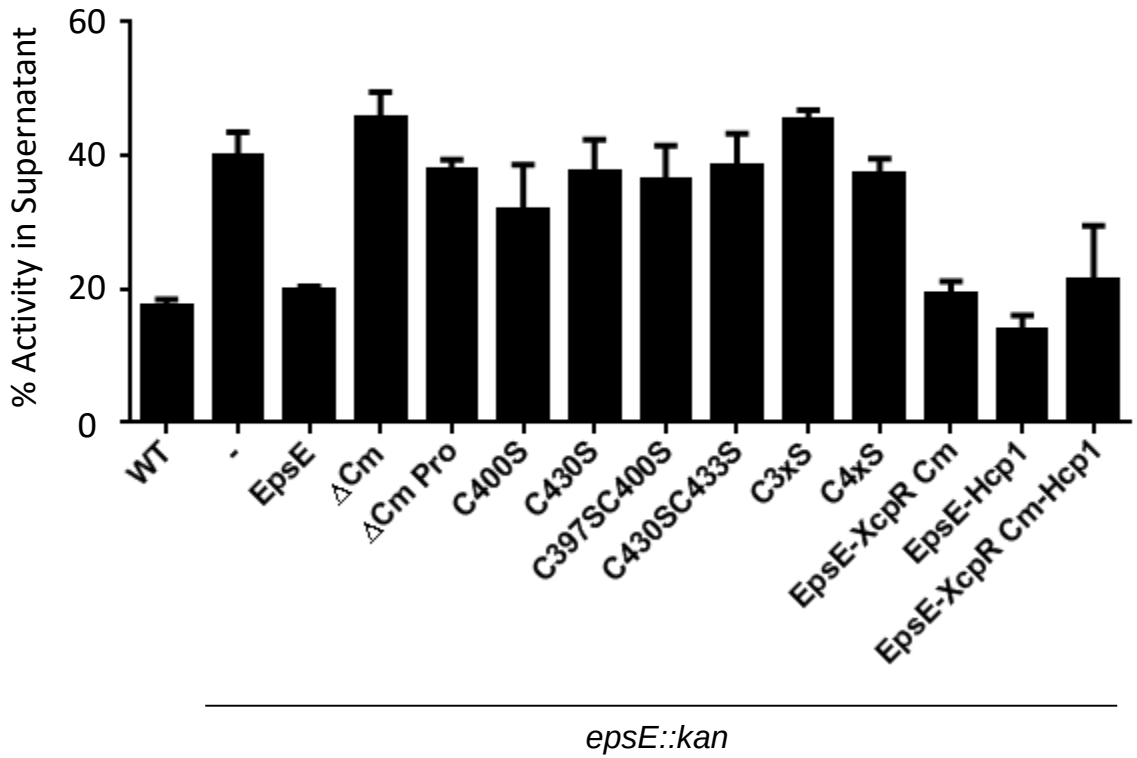
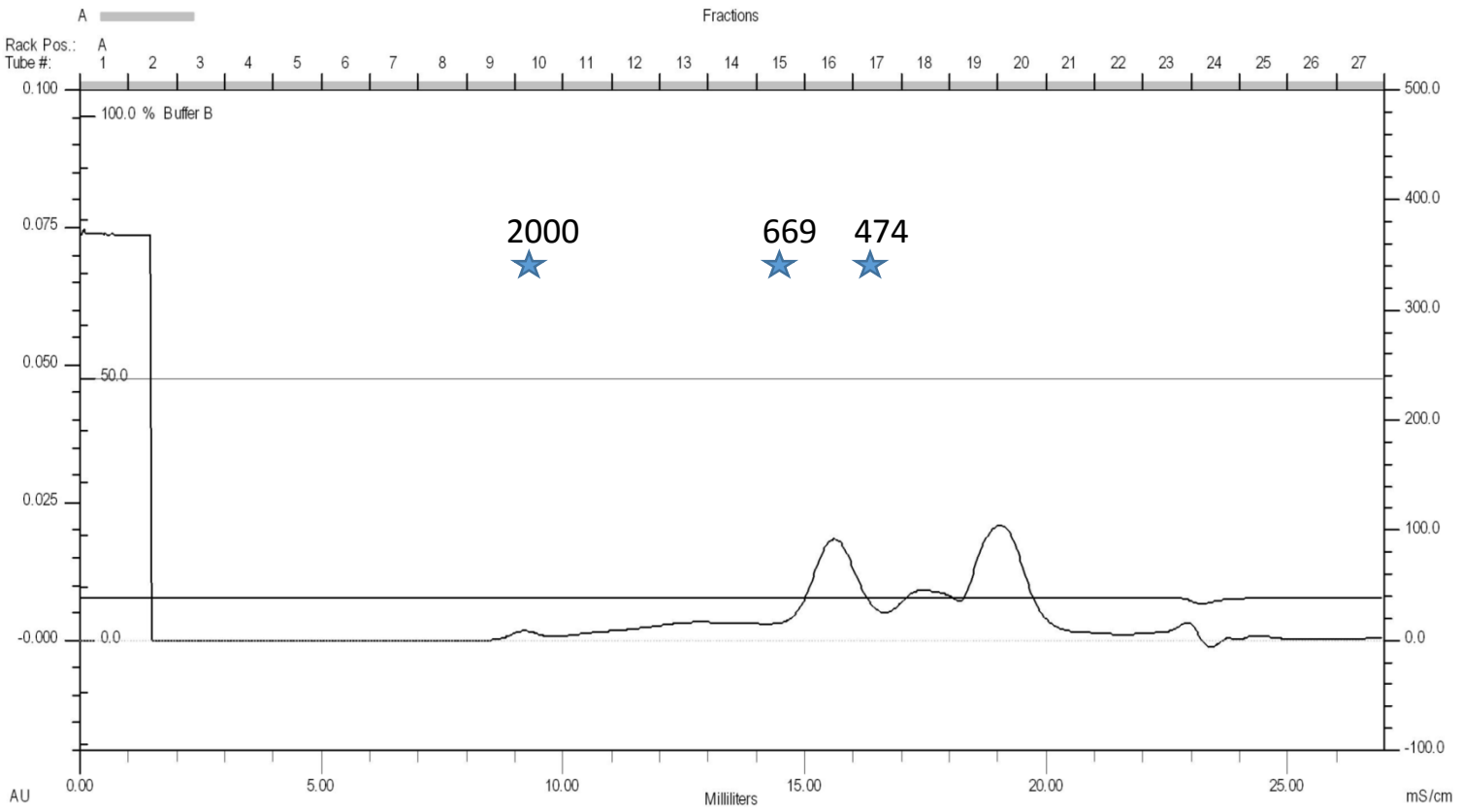
SUPPORTING INFORMATION

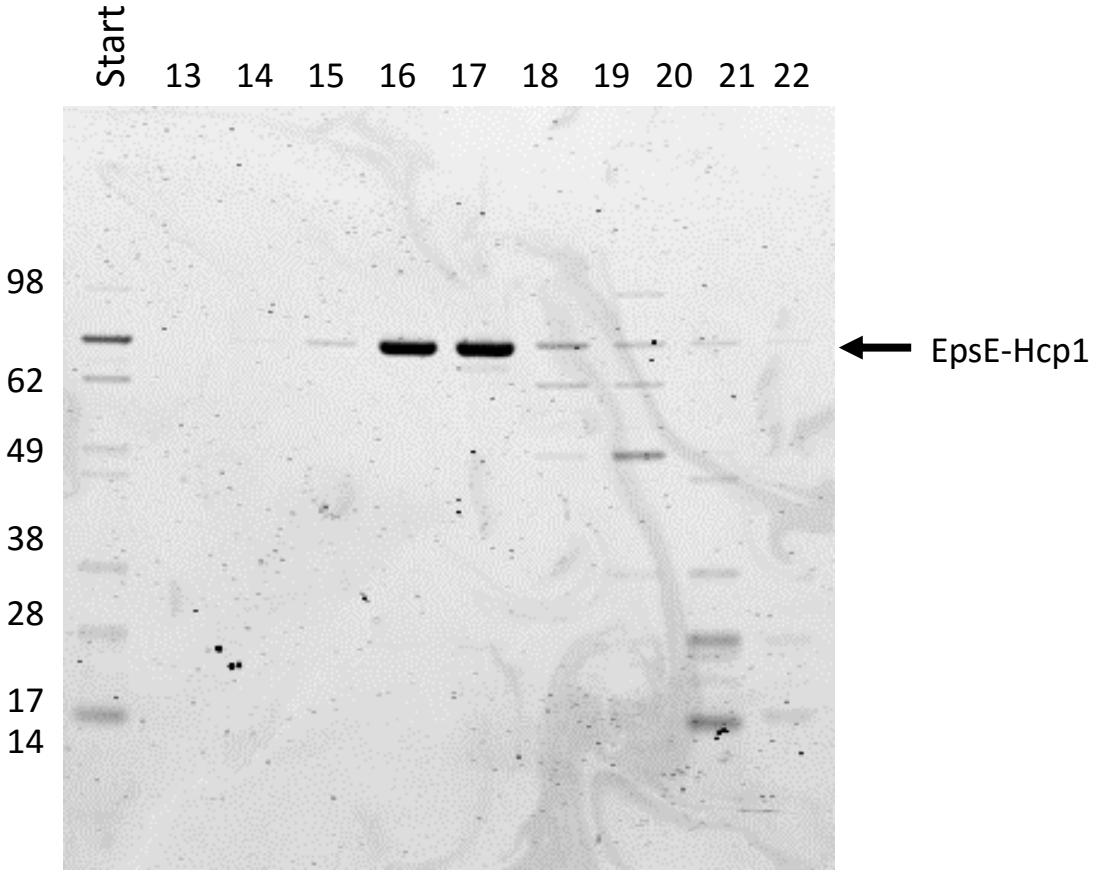


Supplementary Figure 1. Cysteines in EpsE are required for outer membrane stability in *V. cholerae*. Overnight culture supernatants and periplasmic extracts were isolated from WT *V. cholerae* as well as strains of *V. cholerae epsE::kan* containing empty vector or expressing WT and C_M variants of EpsE. β-lactamase activity was measured as described in *Experimental Procedures* and expressed as the percent of total activity in the supernatant. Assays were performed in triplicate, and means and SEM are presented.

A.

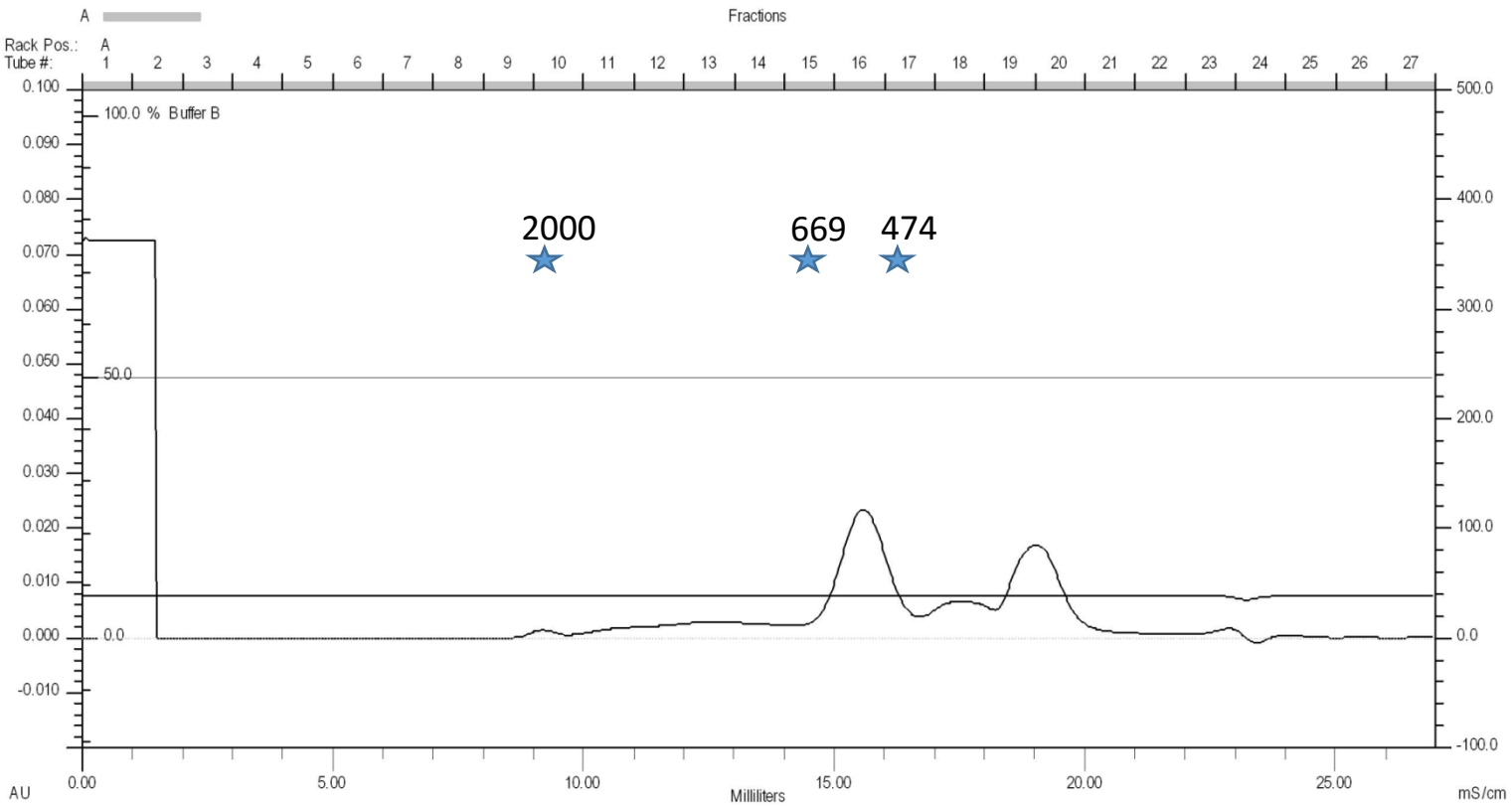

A
Fractions
Rack Pos.: A
Tube #:
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
0.100
0.075
0.050
0.025
-0.000
100.0 % Buffer B
50.0
0.0
2000
669
474
500.0
400.0
300.0
200.0
100.0
0.0
-100.0
AU
0.00
5.00
10.00
15.00
20.00
25.00
Milliliters
mS/cm

B.

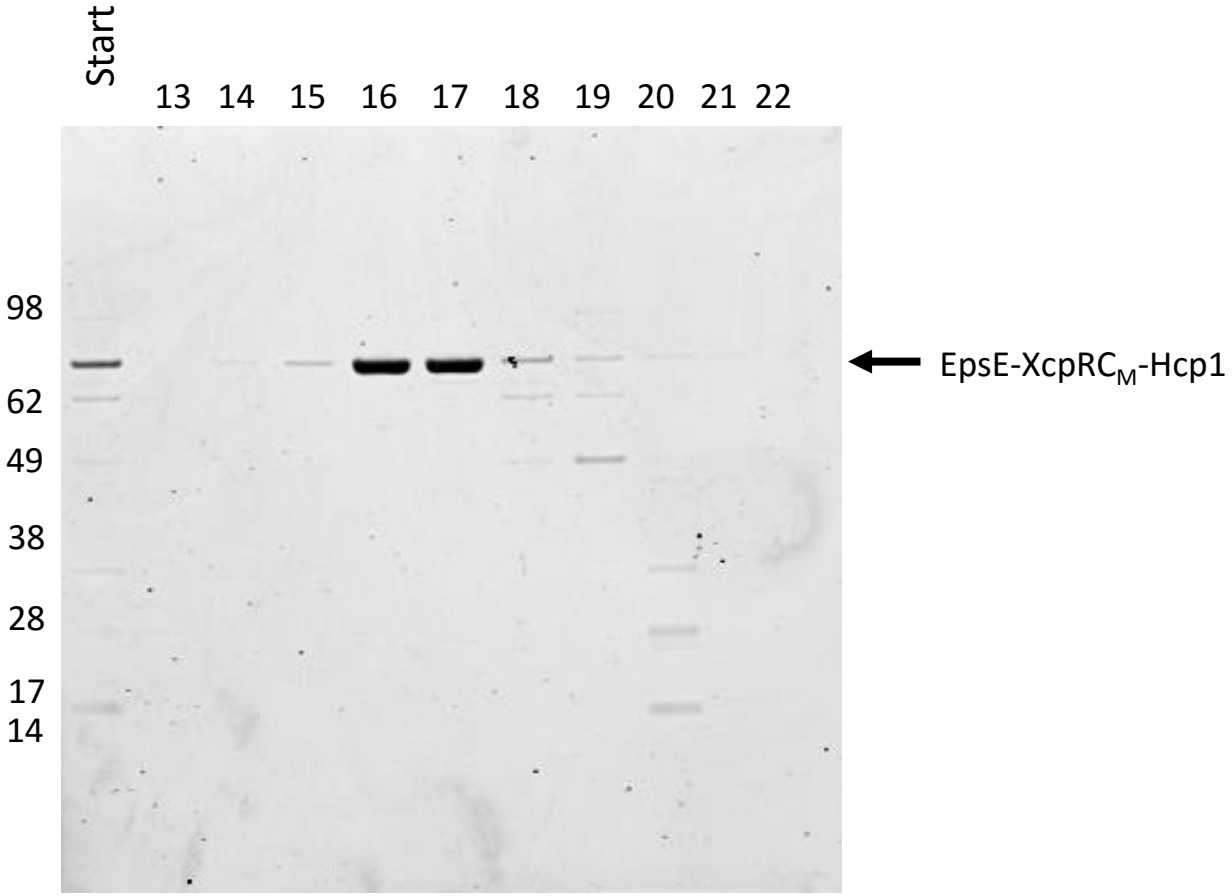


Supplementary Figure 2. Purification of hexameric EpsE-Hcp1. A. EpsE-Hcp1 was purified by metal affinity chromatography and then subjected to gel filtration using a Superose 6 column. Stars represent protein standards with sizes in kDa indicated above. **B**. Fractions containing protein peaks were analyzed via SDS-PAGE and proteins visualized with Coomassie staining. Fractions 16 and 17 were pooled. The size of EpsE-Hcp1 is indicated with an arrow.

A.

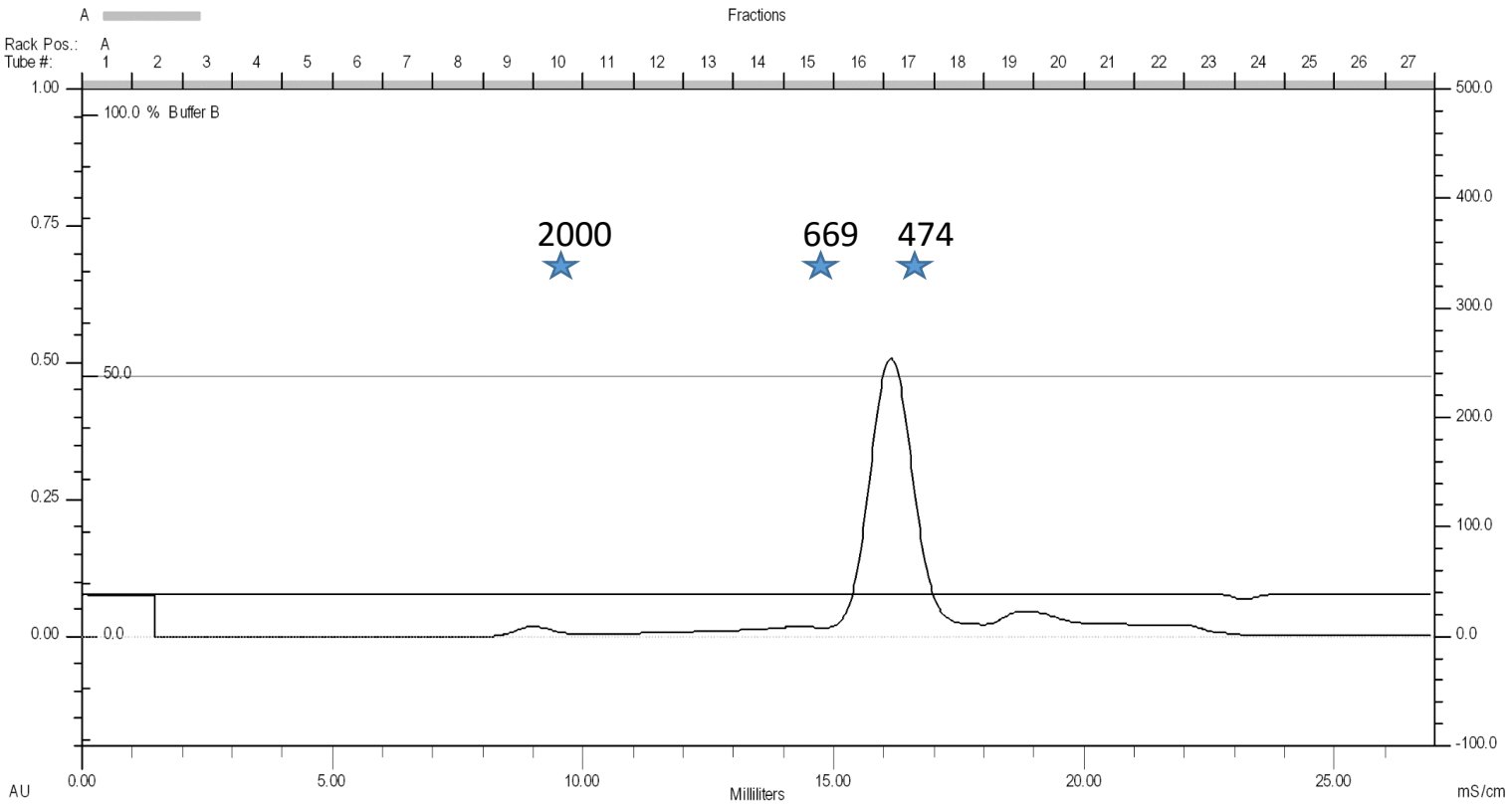

A
Fractions
Rack Pos.: A
Tube #: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
100.0 % Buffer B
2000
669
474
50.0
0.0
AU
Milliliters
mS/cm
0.00
5.00
10.00
15.00
20.00
25.00
0.100
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
-0.010
500.0
400.0
300.0
200.0
100.0
0.0
-100.0

B.

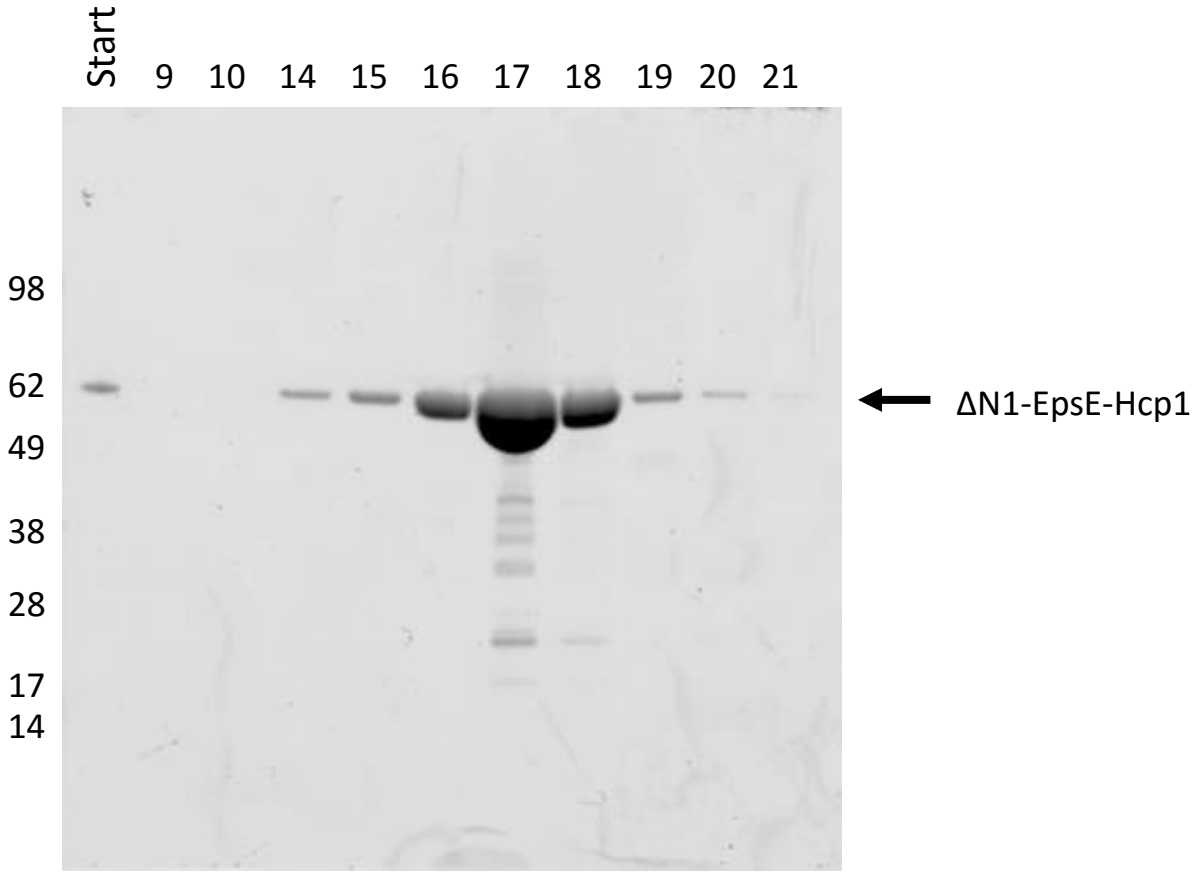


Supplementary Figure 3. Purification of hexameric EpsE-XcpRC$_M$-Hcp1. A. EpsE-XcpRC$_M$-Hcp1 was purified by metal affinity chromatography and then subjected to gel filtration using a Superose 6 column. Stars represent protein standards with sizes in kDa indicated above. **B**. Fractions containing protein peaks were analyzed via SDS-PAGE and proteins visualized with Coomassie staining. Fractions 16 and 17 were pooled. The size of EpsE-XcpRC$_M$-Hcp1 is indicated with an arrow.

A.


A
Fractions
Rack Pos.: A
Tube #: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
100.0 % Buffer B
2000
669
474
50.0
0.0
1.00
0.75
0.50
0.25
0.00
500.0
400.0
300.0
200.0
100.0
0.0
-100.0
AU
0.00
5.00
10.00
15.00
20.00
25.00
Milliliters
mS/cm

B.



Supplementary Figure 4. Purification of hexameric ΔN1-EpsE-Hcp1. A. ΔN1-EpsE-Hcp1 was purified by metal affinity chromatography and then subjected to gel filtration using a Superose 6 column. Stars represent protein standards with sizes in kDa indicated above. **B**. Fractions containing protein peaks were analyzed via SDS-PAGE and proteins visualized with Coomassie staining. Fraction 17 was used for further analyses. The size of ΔN1-EpsE-Hcp1 is indicated with an arrow.