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Supporting information for article:

Cryo-EM structure of a thermostable bacterial nanocompartment

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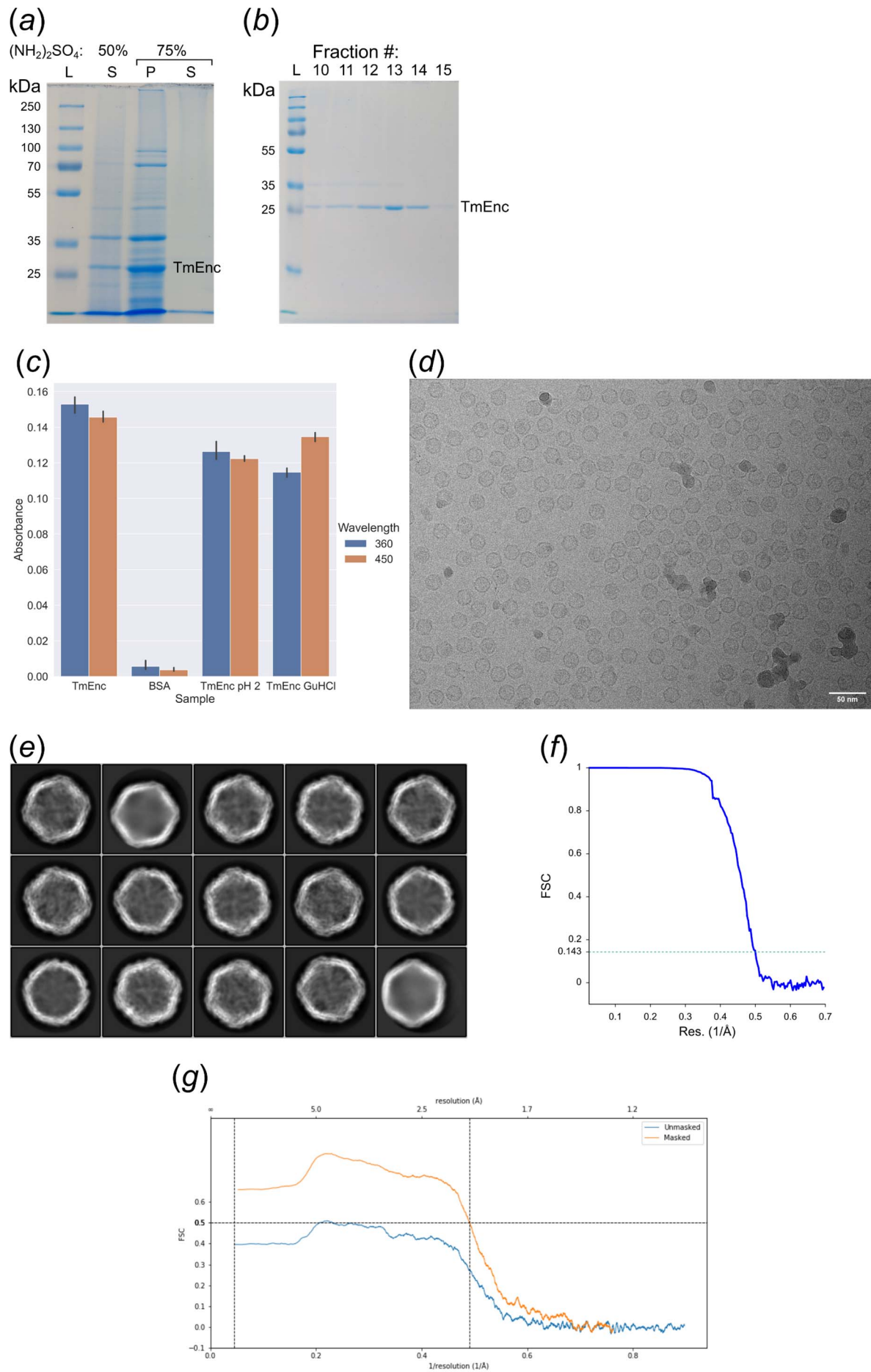


Figure S1 (a) 10% SDS-polyacrylamide gel of TmEnc during ammonium sulfate precipitation step in purification. Percentages above lanes indicate the ammonium sulfate saturation. S refers to the supernatant and P refers to the pellet after incubating the protein solution with ammonium sulfate and centrifuging. (b) 12% SDS-polyacrylamide gel of fractions collected after purifying TmEnc on a HiPrep Sephacryl S-500 column. (c) UV-vis absorption at 360 nm and 450 nm for TmEnc, TmEnc diluted in pH 2.0 buffer, TmEnc diluted in 8 M guanidine hydrochloride, and bovine serum albumin (BSA). Error bars are 95% confidence intervals. (d) Representative micrograph of TmEnc from cryo-EM data collection. The micrograph was low-pass filtered to 20 Å. (e) 2D class averages of *crYOLO*-picked particles calculated by *RELION*. (f) Fourier shell correlation of two half-maps refined independently in *RELION*. (g) Map-to-model Fourier shell correlation after refinement in *PHENIX*.

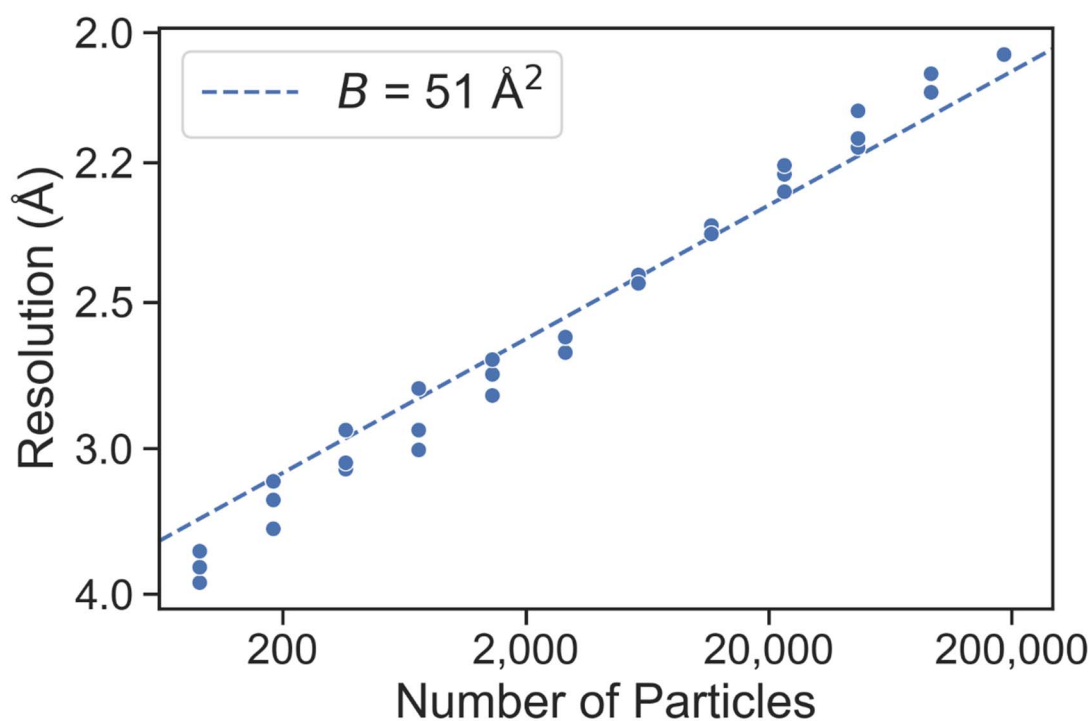


Figure S2 Particle subsets were taken from the final polished particles and refined in *RELION*. The resolution as a function of the number of particles is shown on a Henderson-Rosenthal *B*-factor plot. The estimated *B*-factor from linear regression is 51.3 Å².

Table S1 Amino acid content, expressed in percentages, of TmEnc, MhEnc, SeEnc, and QsEnc.

Name	TmEnc	MhEnc	SeEnc	QsEnc
Uniprot Accession	Q9WZP2	K5BEG2	Q55032	A0A0F5HPP7
PDB ID	7KQ5	3I9G	6X8M	6NJ8
A	4.53	10.19	7.52	6.03
C	1.13	0.75	0.98	0.35
D	5.66	8.68	5.56	6.74
E	13.21	6.79	5.88	8.16
F	5.66	2.64	2.29	5.32
G	7.17	6.42	5.23	6.03
H	0.75	2.64	1.96	2.84
I	6.42	5.66	4.90	6.03
K	7.17	2.26	2.61	3.90
L	12.08	9.43	12.09	10.28
M	0.38	0.38	1.31	3.19
N	2.64	1.51	4.90	5.67
P	3.77	5.66	6.21	4.26
Q	1.89	2.26	4.58	3.90
R	6.79	7.17	7.84	6.03
S	4.53	6.79	5.56	5.67
T	4.91	7.17	6.54	3.90
V	7.17	9.43	9.15	7.45
W	1.89	1.13	1.31	0.71
Y	2.26	3.02	3.59	3.55
Negative (D+E)	18.87	15.47	11.44	14.89
Positive (H+K+R)	14.72	12.08	12.42	12.77
Charged total (D+E+H+K+R)	33.58	27.55	23.86	27.66
Polar uncharged (S+T+N+Q)	13.96	17.74	21.57	19.15
Aliphatic/hydrophobic (A+I+L+M+V)	30.57	35.09	34.97	32.98
Aromatic (F+W+Y)	9.81	6.79	7.19	9.57