Structural Characterization of a Synthetic Tandem-domain Bacterial Microcompartment Shell Protein Capable of Forming Icosahedral Shell Assemblies

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Supplementary Material

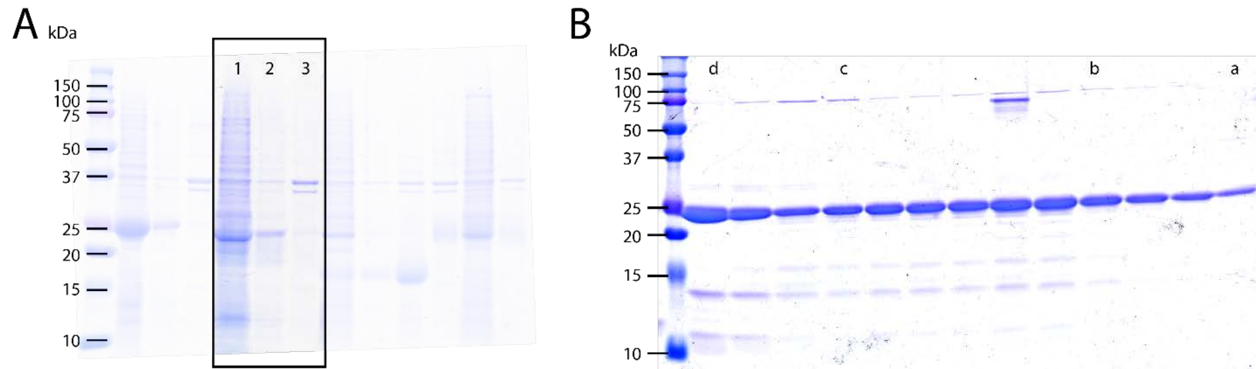

Figure S1. A: SDS-PAGE analysis of soluble; consecutive washes with Triton (lanes 1 and 2) and insoluble (lane 3) fractions of *E. coli* cells expressing BMC-H^2. B: SDS-PAGE of fractions from (B).

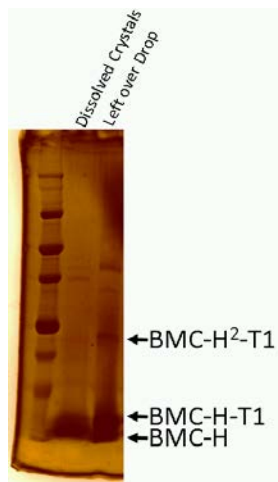

Figure S2. Silver staining of crystals and crystallization drop solution.

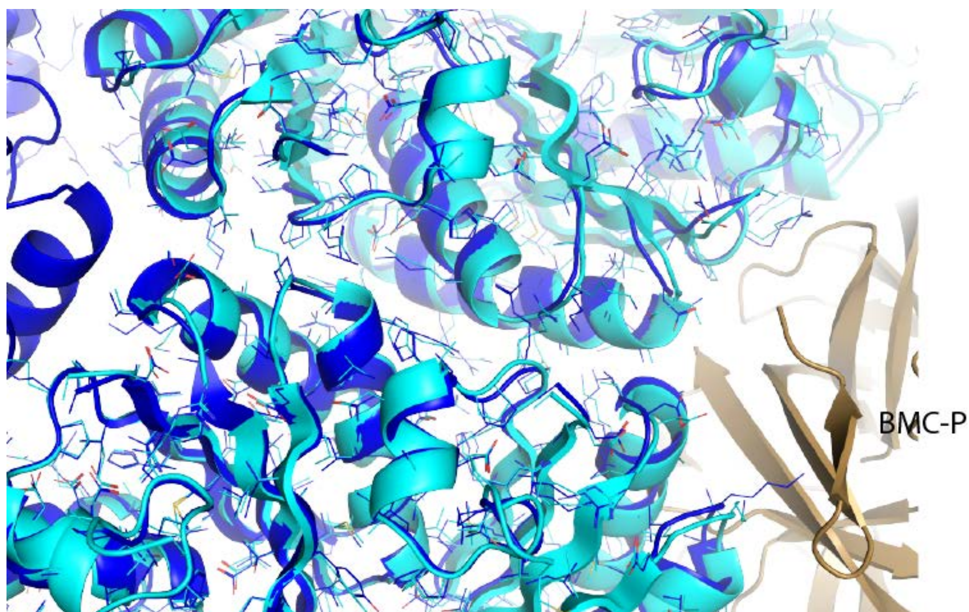

Figure S3. Alignment of BMC-H^2 shell with full HO-Shell. BMC-H^2 shell in dark blue and HO-shell in cyan, BMC-P of the HO shell in sand.

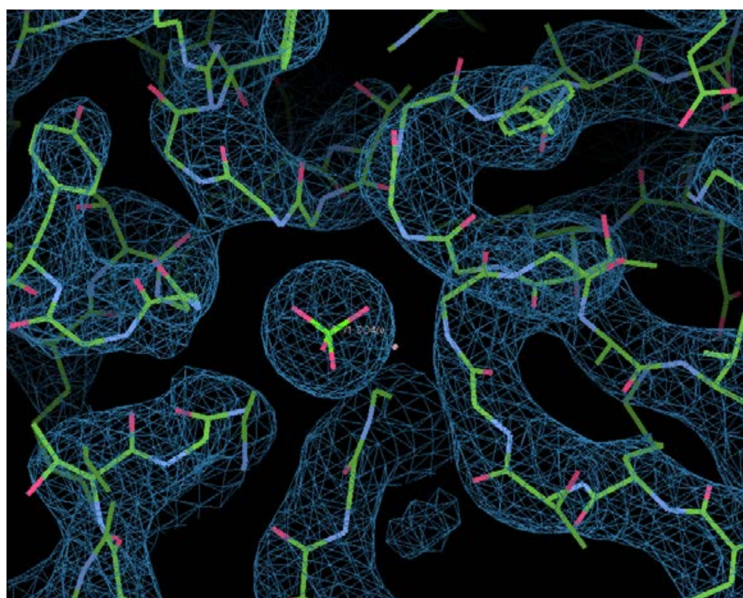

Figure S4. 2Fo-Fc density of the central pore of BMC-H^2 trimer contoured at 0.31 e/A^3.

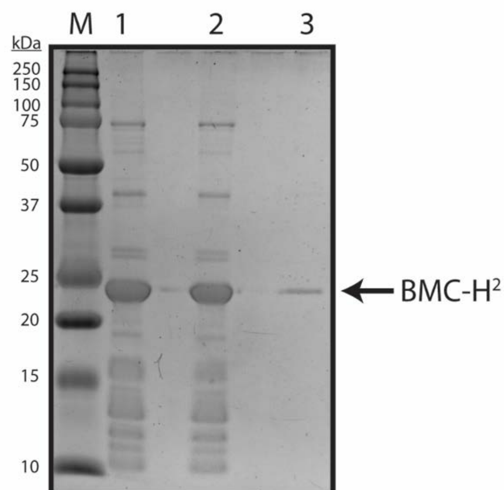

Figure S5. BMC-H^2 alone does not appreciably bind Ni-resin. Free BMC-H^2 in 50 mM Tris pH 7.8 and 300 mM NaCl following removal of SUMO domain (lane 1) was loaded onto a HisTrap column equilibrated in equilibration buffer (50 mM Tris pH 7.8, 300 mM NaCl). Eight column volumes (CV) of equilibration buffer followed by eight CV of elution buffer (equilibration buffer with 500 mM imidazole) were passed over the column and the flow through and elution fractions were pooled separately and concentrated over 30 kDa MWCO filters to final volumes of 300 uL each. Equal volumes of the retentate from the flow through fractions (lane 2) and the elution fractions (lane 3) were then run on SDS-PAGE with a molecular weight marker (M).

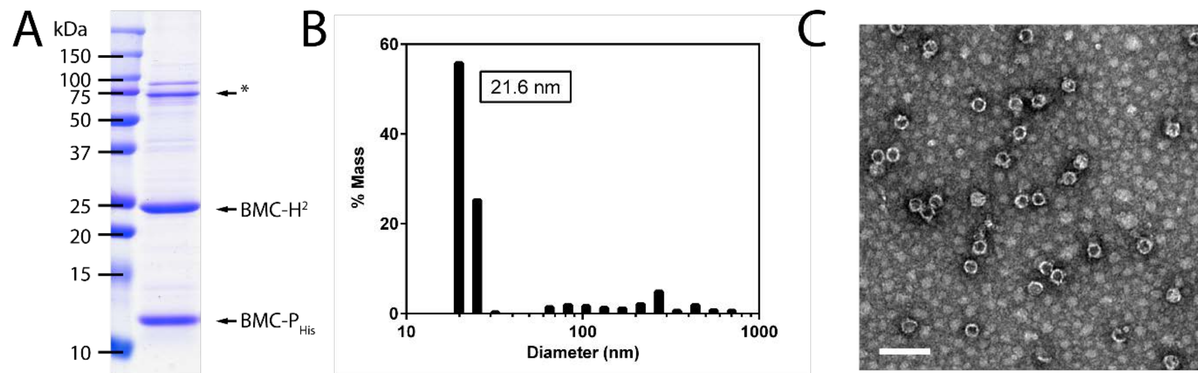

Figure S6: **Characterization of in vivo assembled BMC-H^2 shells in the presence of BMC-T^S and BMC-P_{His}.** A: SDS-PAGE analysis of concentrated HisTrap elution fractions. A common contaminant from the HisTrap is indicated by an asterisk. B: Size distribution of concentrated HisTrap elution fractions from (A) using dynamic light scattering. C: TEM micrograph of the same sample in (A) and (B). Diameter = 26 ± 1.8 nm (n=326). Scale bar = 100 nm.

Protein	Amino acid (aa) sequence	Length	Predicted molecular weight (kDa)*	Notes
BMC-H	MADALGMIEVRGFVG MVEAADAMVKAAKVE LIGYEKTGGGYVTAVVR GDVAAVKAATEAGQR AAERVGEVVAVHVIPR PHVNVDAALPLGRTPG MDKSA	99 aa	10.1	
BMC-H^2	MADALGMIEVRGFVG MVEAADAMVKAAKVE LIGYEKTGGGYVTAVVR GDVAAVKAATEAGQR AAERVGEVVAVHVIPR PHVNVDAALPLGRTPG MDKSALDAPVVADAW EEDTESADALGMIEVR GFVGMVEAADAMVK AAKVELIGYEKTGGGYV TAVVRGDVAAVKAATE AGQRAAERVGEVVAV HVIPRPHVNVDAALPL GRTPGMDKSA	213 aa	21.8	BMC-H^2 is a linear fusion of two BMC-H (Hoch_5815) proteins using the 16 amino acid linker from the BMC-T^S protein encoded by Hoch_5816 (highlighted in yellow).
SUMO-BMC-H^2	MGSSHHHHHHGSGLV PRGSASMSDSEVNQE AKPEVKPEVKPETHINL KVSDGSSEIFFKIKKTTP LRRLMEAFAKRQGKE MDSLRFLYDGIRIQAD QTPEDLDMEDNDIIEA HREQIGG-(BMC-H^2)	332 aa	23.5	The SUMO domain carries a N-terminal 6xHis tag used for purification. This 6xHis tag is removed, along with the SUMO domain, following treatment with ULP protease resulting in isolation of BMC-H^2.
BMC-P$_{his}$	MVLGKVVGTVVASRKE PRIEGLSLLLVRACDPD GTPTGGAVVCADAVG AGVGEVVLYASGSSAR QTEVTNNRPVDATIM AIVDLVEMGGDVRFRK DGSSHHHHHH	105 aa	10.9	BMC-P (Hoch_5814) appended with an insulating C-terminal GSS linker and 6xHis tag (underlined) for purification.
BMC-T^S	MDHAPERFDATPPAG EPDRPALGVLELTSIAR GITVADAALKRAPSLLL MSRPVSSGKHLLMMR GQVAEVEESMIAAREI AGAGSGALLDELELPYA HEQLWRFLDAPVVAD	205	21.9	The 16 amino acid linker region separating the two Pfam00936 domains that was used to construct the synthetic BMC-H^2 construct is highlighted in yellow.

	AWEEDTESVIIVETATV CAAIDSADAALKTAPV VLRDMRLAIGIAGKAFF TLTGELADVEAAAEVV RERCGARLLELACIARP VDELRGRLFF			

*Calculated using ExPASy web server (https://web.expasy.org/compute_pi)

Table S1: Amino acid sequences used in this study.

ID	Name	Sequence 5' - 3'	Purpose
oBF22	Hoch_5815_SUMO_gibson_fwd	GGCTCACAGAGAACAGATTGG TGGTATGGCGGACGCACTGGG	Amplify BMC-H^2 for insertion into SUMO-pBbE2K vector
oCA189	SUMO_BMC-H^2_rev	CCTTACTCGAGTTTGGATCCT CAGGCTGACTTATCCATGCCAG	Amplify BMC-H^2 for insertion into SUMO-pBbE2K vector
AH106	SUMO_vector_fwd	TAAGGATCCAAACTCGA GTAAGGATCTCCAGGC	Amplify SUMO-pBbE2K vector
AH107	SUMO_vector_rev	CATACCACCAATCT GTTCTCTGTGAGCC	Amplify SUMO-pBbE2K vector
oSM5	Delete Hoch_5815_pARH329_fwd	TTTAGAGATTAAA GAGGAGAAATAC	Delete Hoch_5815 from pARH329
oSM6	Delete Hoch_5815_pARH329_rev	CTAGTATTTCTCCTCTTTCTC	Delete Hoch_5815 from pARH329
oSM7	Amplify BMC-H^2 in pARH329_fwd	GAAAGAGGAGAAATACTA GATGGCGGACGCACTGGGTAT	Amplify BMC-H^2 with Gibson Assembly for annealing to pARH329
oSM8	Amplify BMC-H^2 in pARH329_rev	TCTCCTCTTTAATCTCTAA ATCAGGCTGACTTATCCATGC	Amplify BMC-H^2 with Gibson Assembly for annealing to pARH329

Table S2: Primers used in this study.