Supplementary Information

Exploring targeting peptide-shell interactions in encapsulin nanocompartments

Wiggert J. Altenburg[1,2], Nathan Rollins[1,2], Pamela A. Silver[1,2] and Tobias W. Giessen[1,2,3,4*]

[1] Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA

[2] Wyss Institute for Biologically Inspired Engineering at Harvard, Boston, MA 02115, USA

[3] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

[4] Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109, USA

[*] Corresponding author: tgiessen@umich.edu

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Additional modeling data

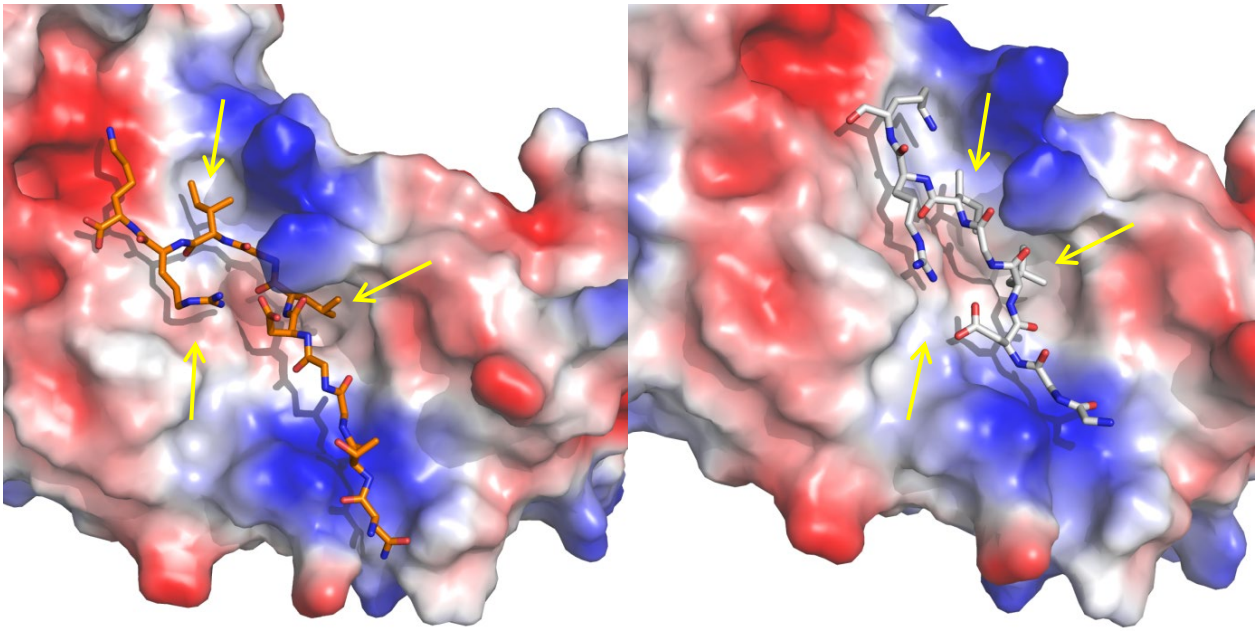

Figure S1. Comparison of best calculated binding interaction with x-ray structure of the T1 *T. maritima* encapsulin. Left: Modeled structure. Right: X-ray structure (3DKT). Yellow arrows indicate key interactions. The computed binding mode does recapitulate the intra-peptide interaction between the penultimate arginine and the glutamate residue. PyMOL Molecular Graphics System, Version 1.8.2.0. was used to create the surface representations and atomic structures shown (http://www.pymol.org).

Table S1. Metrics from Rosetta FlexPepDock for all the best scoring models.

T1	**minusRama score**	**Total Score**	**Rama**	**Omega**	**Peptide_score**	**Reweighted Score**
Native	-392.478	-351.805	49.777	-9.104	1.163	-376.499
Consensus	-390.189	-350.946	49.720	-10.477	1.076	-374.954
Control	-385.955	-350.310	46.990	-11.345	-5.238	-371.539
T3	**minusRama score**	**Total Score**	**Rama**	**Omega**	**Peptide_score**	**Reweighted Score**
Native	453.953	847.084	350.944	42.187	-2.876	814.846
Consensus	474.573	867.827	351.049	42.205	3.733	856.684
Control	472.279	860.525	347.959	40.287	-8.078	846.304

Table S2. Raw data of the refinement runs for all point mutations in the native T3 targeting peptide. This data is the foundation for the heat map shown in Figure 5. Energy contribution per residue is shown. The native sequence is highlighted in green.

	1	2	3	4	5	6	7	8	9	10	11
A	9.00	-5.687	0.393	-8.615	8.634	2.104	-0.067	-2.201	3.648	4.026	2.251
C	1.03	-3.067	-1.510	-0.667	-1.732	7.448	-2.501	4.176	8.195	7.967	5.197
D	2.12	-2.307	-3.070	4.042	2.089	6.025	1.808	-0.864	5.901	13.349	4.608
E	4.92	-6.151	5.078	5.711	-1.074	3.821	1.299	11.709	7.545	0.526	10.921
F	4.70	-4.372	-2.383	-4.634	2.303	6.912	0.838	7.945	4.464	12.993	7.549
G	6.25	-7.751	-2.214	0.279	0.000	2.168	6.301	4.574	9.587	0.000	0.000
H	2.28	-1.153	-1.653	3.886	1.688	7.914	3.703	0.831	2.917	7.085	5.354
I	-3.07	-5.181	-1.324	1.449	14.795	9.626	-1.544	-2.580	13.152	4.411	5.753
K	6.04	-1.421	-3.641	18.086	1.855	4.781	0.776	13.067	3.342	7.677	12.966
L	9.87	0.000	-4.615	3.193	2.317	4.614	0.000	-2.736	2.670	0.938	12.804
M	2.95	1.248	-5.732	11.514	5.453	9.013	0.665	8.821	13.152	14.061	9.778
N	8.17	1.496	0.321	-2.467	6.212	14.943	2.012	5.703	17.454	4.549	8.845
P	0.00	-0.438	-6.061	6.233	1.138	-2.478	1.418	2.011	9.698	4.570	6.653
Q	1.25	-0.865	-1.218	4.390	7.646	12.256	1.448	-1.886	6.102	10.228	1.968
R	3.07	-6.252	-0.412	7.060	6.573	11.221	5.422	0.000	0.000	12.170	8.665
S	7.77	-4.507	-1.528	-2.801	4.094	0.000	2.947	-4.878	0.717	7.585	7.652
T	0.77	-10.787	0.000	1.307	-0.535	8.625	1.123	2.271	1.943	7.520	9.431
V	-0.37	-7.070	-2.549	0.000	1.566	-0.509	-0.349	-3.682	10.030	11.862	11.276
W	8.76	2.547	-2.870	3.513	9.463	3.904	1.276	1.716	5.007	7.415	2.353
Y	7.00	4.163	-6.568	-0.270	8.564	12.077	-0.067	4.952	0.423	7.613	8.251

Encapsulin purification

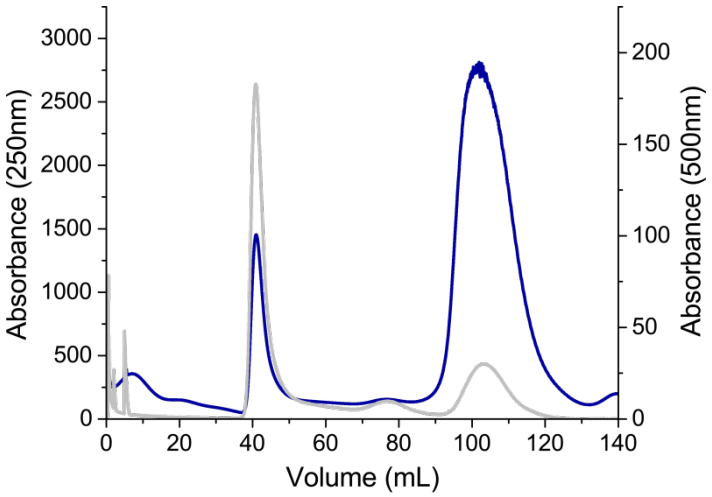

Figure S2. First encapsulin purification step. Sample chromatogram of a T1 sample + mNeonGreenTP (native) purification using a HiPrep 16/60 Sephacryl S-500 HR size exclusion column. Blue: Absorption at 280 nm. Gray: Absorption at 500 nm.

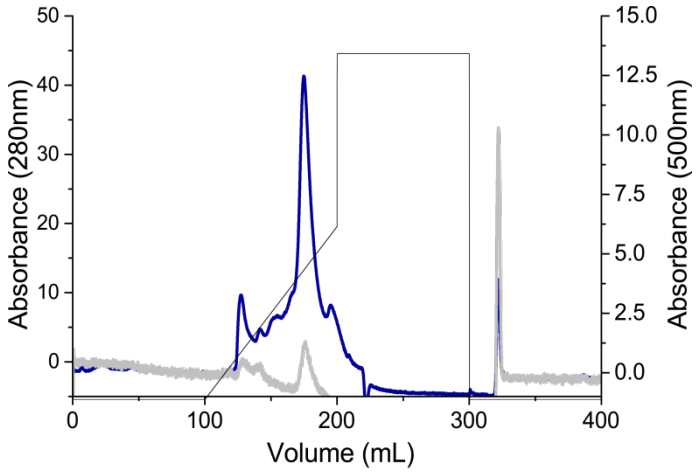

Figure S3. Second encapsulin purification step. Sample chromatogram of a T1 sample + mNeonGreenTP (native) purification using a HiPrep DEAE FF 16/10 Ion Exchange column. Blue: Absorption at 280 nm. Gray: Absorption at 500 nm.

Additional PAGE gels

T1:

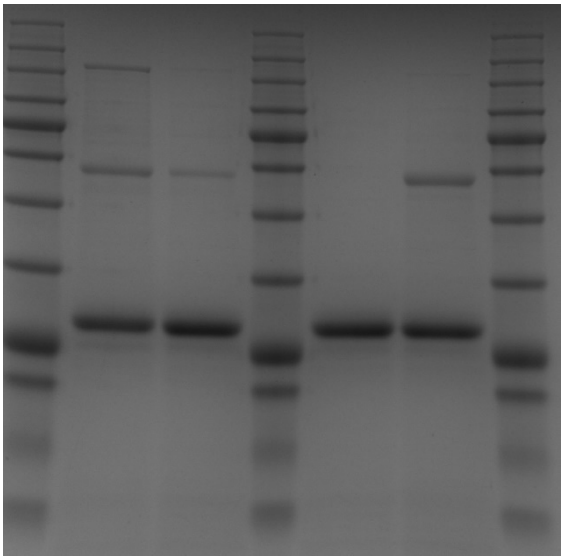

Figure S4. Sample SDS-PAGE gel of purified T1 encapsulins. Left to right: MW Marker, T1 Enc no cargo, native TP, MW Marker, consensus TP, control TP. Full gel image of the lanes shown in Figure 3 top row. As outlined in the main text, cargo and the T1 capsid protein could not be separated on the gel but were confirmed via in-gel tryptic digest followed by mass spectrometry analysis of the resulting peptide fragments.

T3:

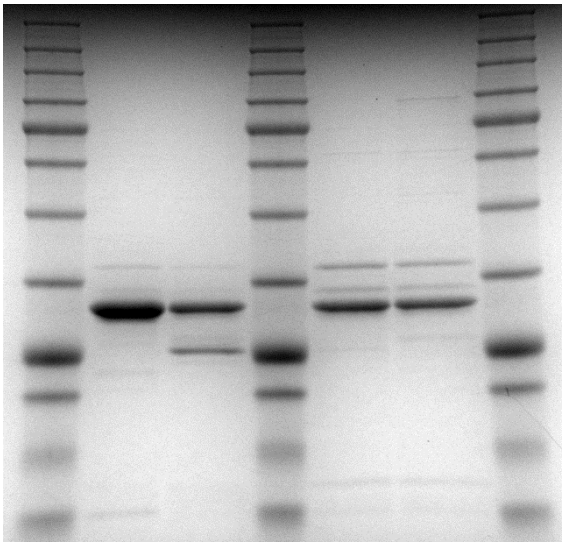

Figure S5. Sample SDS-PAGE gel of purified T3 encapsulins. Left to right: MW Marker, T3 Enc no cargo, native TP, MW Marker, consensus TP, control TP. Full gel image of the lanes shown in Figure 3 bottom row.

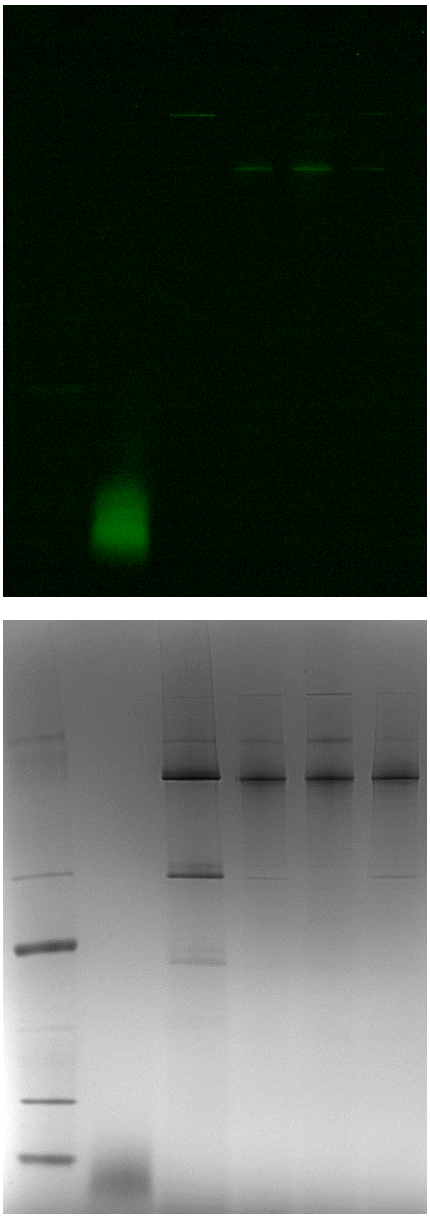

Figure S6. Sample Native PAGE gel for T1 encapsulins. Top: Fluorescence image of the gel at 500 nm, Bottom: Coomassie stained gel. Samples Left to right: Ladder (NativeMark Unstained Protein Standard, Life Technologies), mNeonGreen, no cargo, consensus TP, native TP, control TP.

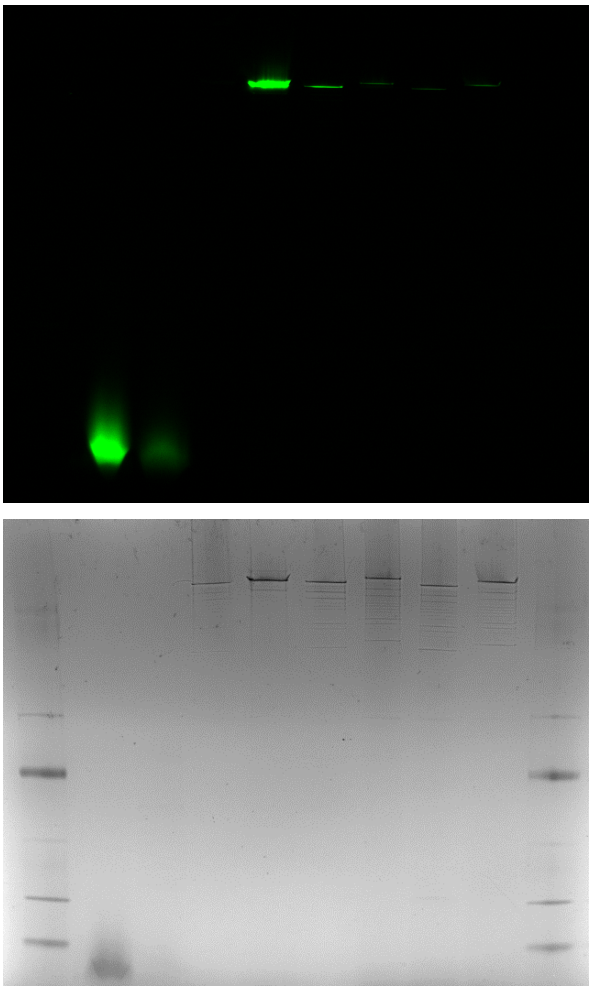

Figure S7. Sample Native PAGE gel for T3 encapsulins. Fluorescence image of the gel at 500 nm, Bottom: Coomassie stained gel. Samples Left to right: Ladder (NativeMark Unstained Protein Standard, Life Technologies), mNeonGreen, 1:2 dilution of mNeonGreen, no cargo, native TP, consensus TP, 1:2 dilution of consensus TP, 1:3 dilution of consensus TP, control TP, ladder.

Standard curve for mNeonGreen fluorescence

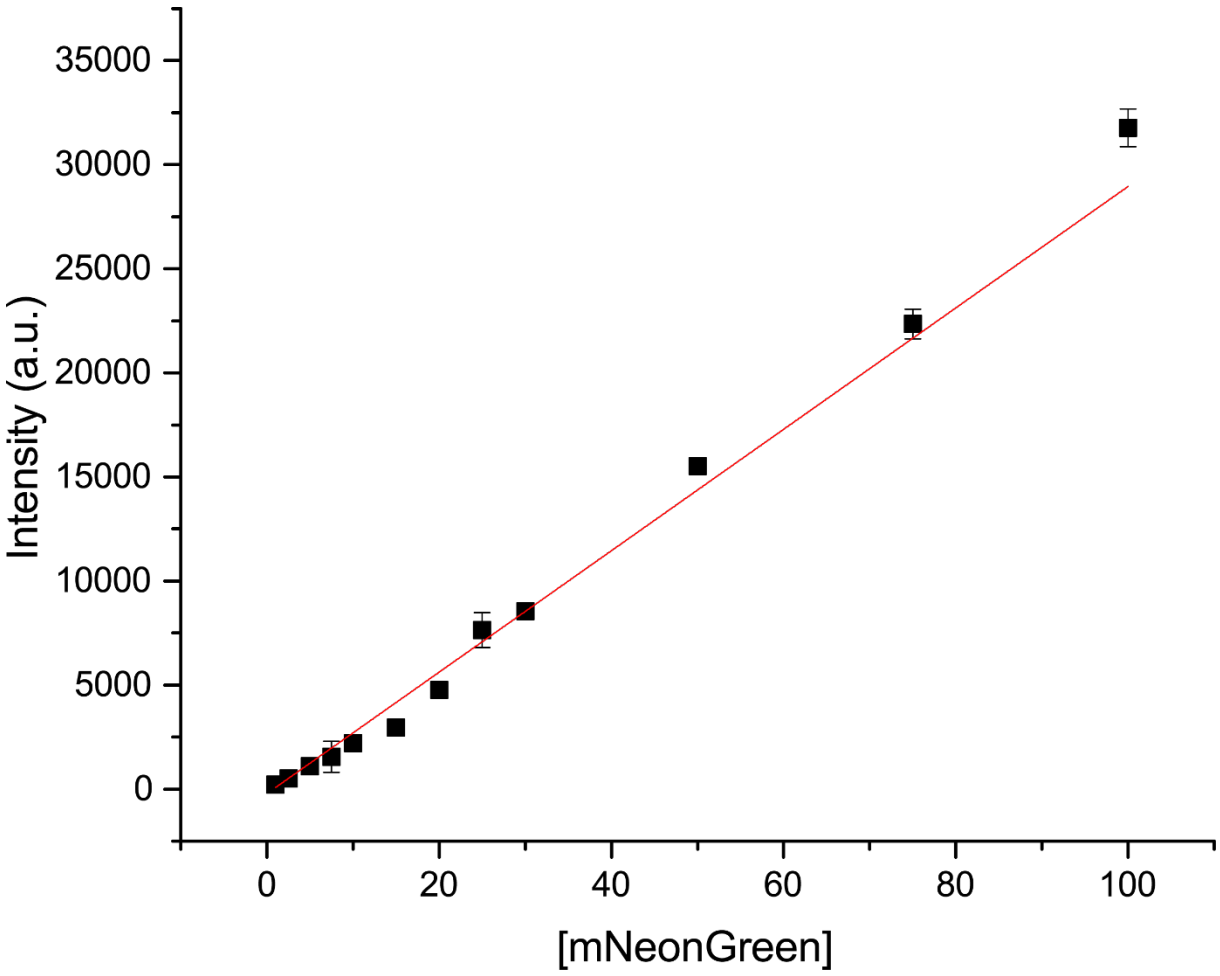

Figure S8. mNeonGreen fluorescence standard curve. Measured intensity vs mNeonGreen concentration in nM. Fit: x = (y+215.1)/291.7.