SUPPORTING INFORMATION

Figure S1  The genetic sequences of A) EETI-II; B) mEETI-II and C) Thermotoga Maritima (TM) encapsulin

**A**  EETI-II plasmid and knottin sequence

**B**  mEETI-II plasmid and knottin sequence

**Bold and underlined** triplets indicate the genetic modification from lysine (EETI-II) to serine (mEETI-II). In **blue** we show the inserted SGSGS linker, in **red** the added tryptophan.
The plasmids were made using A plasmid Editor (ApE) software, version 2.0.48.

In S1 the genetic sequences are shown that we used to produce the knottins (A and B) and the TM encapsulin (C). For the knottins the difference between the EETI-II and mEETI-II knottins is the alteration of a lysine to a serine, as marked with the **bold and underlined** codon. These sequences were also used to calculate the theoretical molecular weight of the proteins for MALDI TOF mass spectrometry and SDS-PAGE analyses.
The FPLC and DLS data (S2 A, B) confirm that particles are formed with the expected size (diameter ~20 nm). Figure S2 C shows the FPLC analysis of the knottin-TFP (Teal Fluorescent Protein) complex that we collected and used to prepare the knottins from. To increase solubility before using the knottins and to be able to detect them and calculate the concentrations, the knottins are created and extracted from the bacteria attached to a TFP protein in a 1:1 ratio. This complex is cleaved prior to binding the knottins to the TM encapsulin at an enterokinase cleavage site.
Figure S3 Visualization of Crystal Structure of TM Encapsulin

Figure S3. This space filled image shows two pentamer subunits that comprise the TM encapsulin. The colors correspond to different atoms: Carbon (white), Nitrogen (blue), Oxygen (red) and Sulfur (Yellow). The sulfur groups that are visible are from the Cys123. The cysteines reside in pockets but are exposed to the exterior. This image was made using the RCSB Protein Data Bank protein 3DKT, which is based on the crystal structure as derived by Sutter et al.22
Figure S4 MALDI of knottins

A

B

EETI-II

mEETI-II

Figure S4 MALDI-TOF analysis of EETI-II (A) and mEETI-II (B) knottins.
There is the clear difference in sizes between EETI-II and mEETI-II caused by the change from lysine to serine, respectively.

Figure S5 shows the absorbance at $\lambda = 405$ nm, which emerges when l-BApNA is cleaved by trypsin. The x-axis shows different the different concentrations of inhibitor. All measurements were performed after 1 hour. The error bars indicate the standard deviation ($N = 3$).
Figure S6 shows the SDS-PAGE of TM (lane 1), ladder (lane 2) and TM-mEETI-II (lane 3). The TM monomers have a mass of ~30 kDa (determined using ExPASy ProtParam). The TM encapsulin monomers and the native TM cargo (ferritin-like protein) are highlighted by the arrows. We did not observe a significant difference between TM encapsulin monomers that did and did not have an mEETI-II knottin attached to it, although a faint band observed above the main TM band in lane 3. This is probably due to the small size of the knottin compared to the TM encapsulin monomer (30 kDa and 33 kDa, respectively, for TM and TM-mEETI-II) and the presence of a large fraction of unmodified TM protein in the sample. Other assays were not sufficient to quantify the amount of knottins bound to TM encapsulin.
