Figure S1

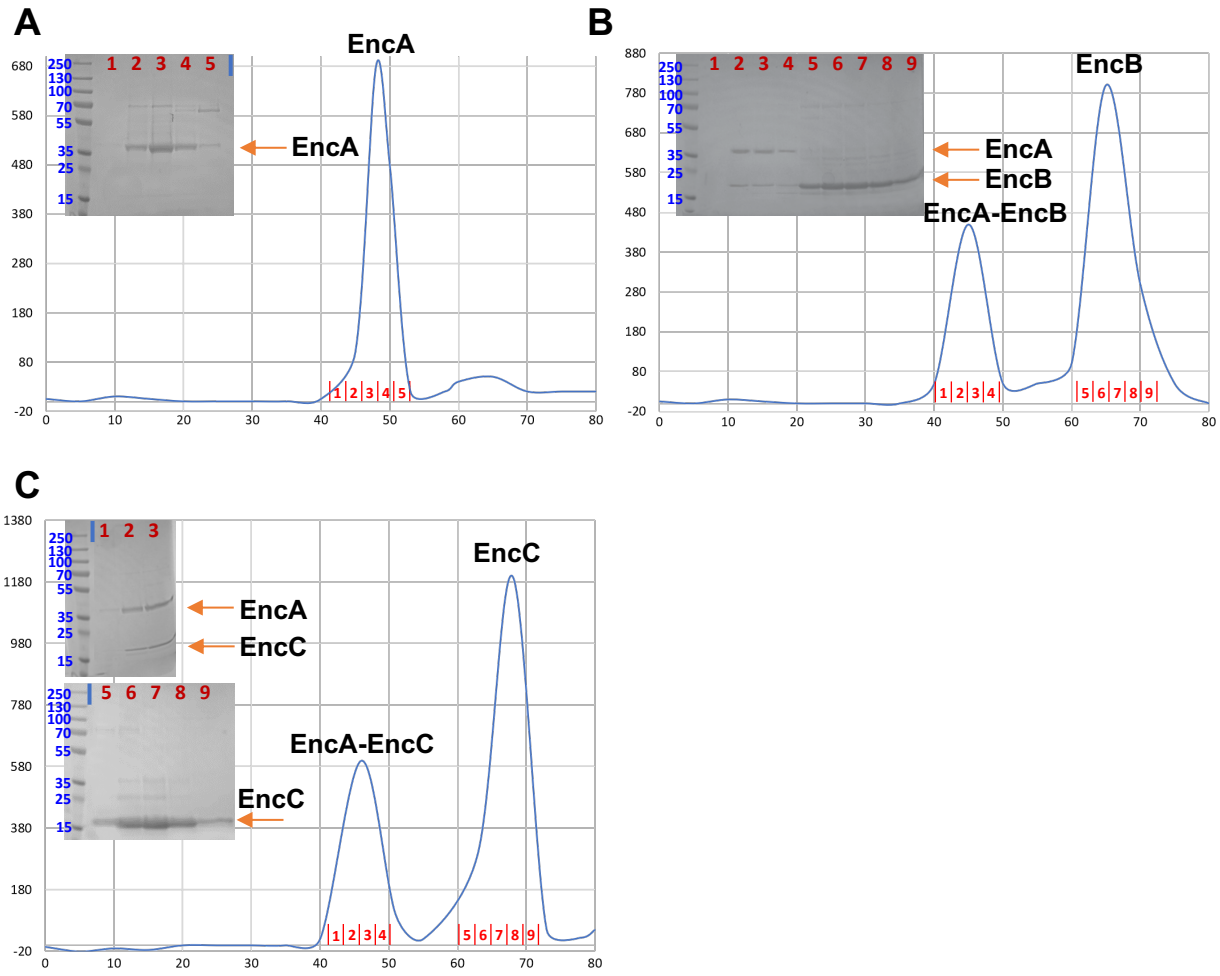

Figure S1. Purification of *M. xanthus* encapsulin and cargo proteins. Related to STAR Methods. Representative size exclusion chromatograms of (A) EncA (B) EncA co-expressed with EncB and (C) EncA co-expressed with EncC. Peaks corresponding to EncA, EncA encapsulating EncB (EncA-EncB) and EncA encapsulating EncC (EncA-EncC) are indicated. Peaks corresponding to EncB decamers and EncC decamers that are not encapsulated are also indicated. Size exclusion chromatography for each preparation was run after nickel column purification. SDS-PAGE gels corresponding to the fractions collected during size exclusion chromatography are shown as insets. The fractions run on the gels are indicated by numbers. In some cases, some lanes from the gels have been removed for simplicity (the location of removed lanes are indicated by blue lines). The sizes of the proteins in the protein marker are shown in blue.

Figure S2

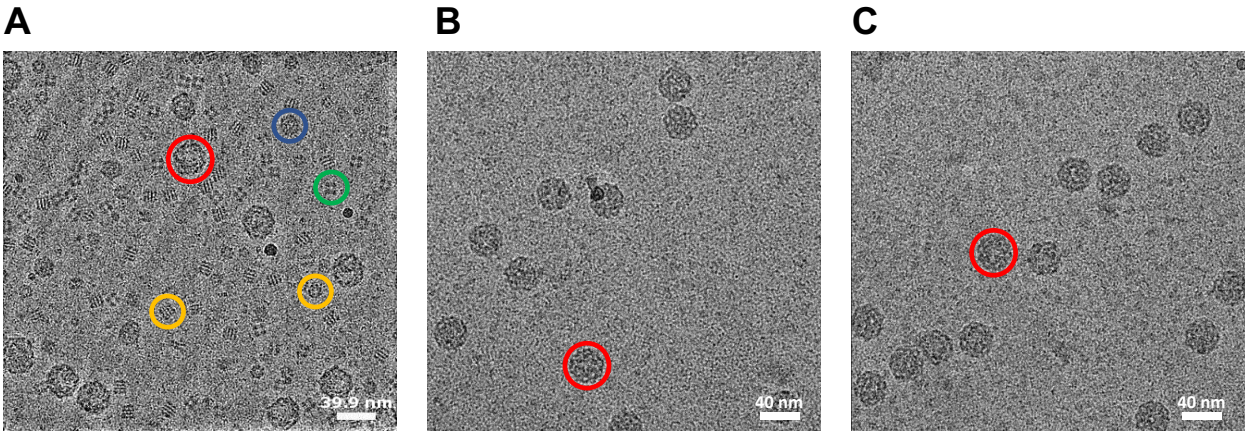

Figure S2. Representative micrographs of *M. xanthus* encapsulins expressed in *E. coli*. Related to STAR Methods and Figure 1. (A) EncA (B) EncA-EncB and (C) EncA-EncC. Representative particles observed in each micrograph are indicated with circles. Red: *T*=3 encapsulin, blue: *T*=1 encapsulin, yellow: groEL and green: RuBisCO. Scale bars (~40 nm) are shown at the bottom left corner for the micrographs.

Figure S3

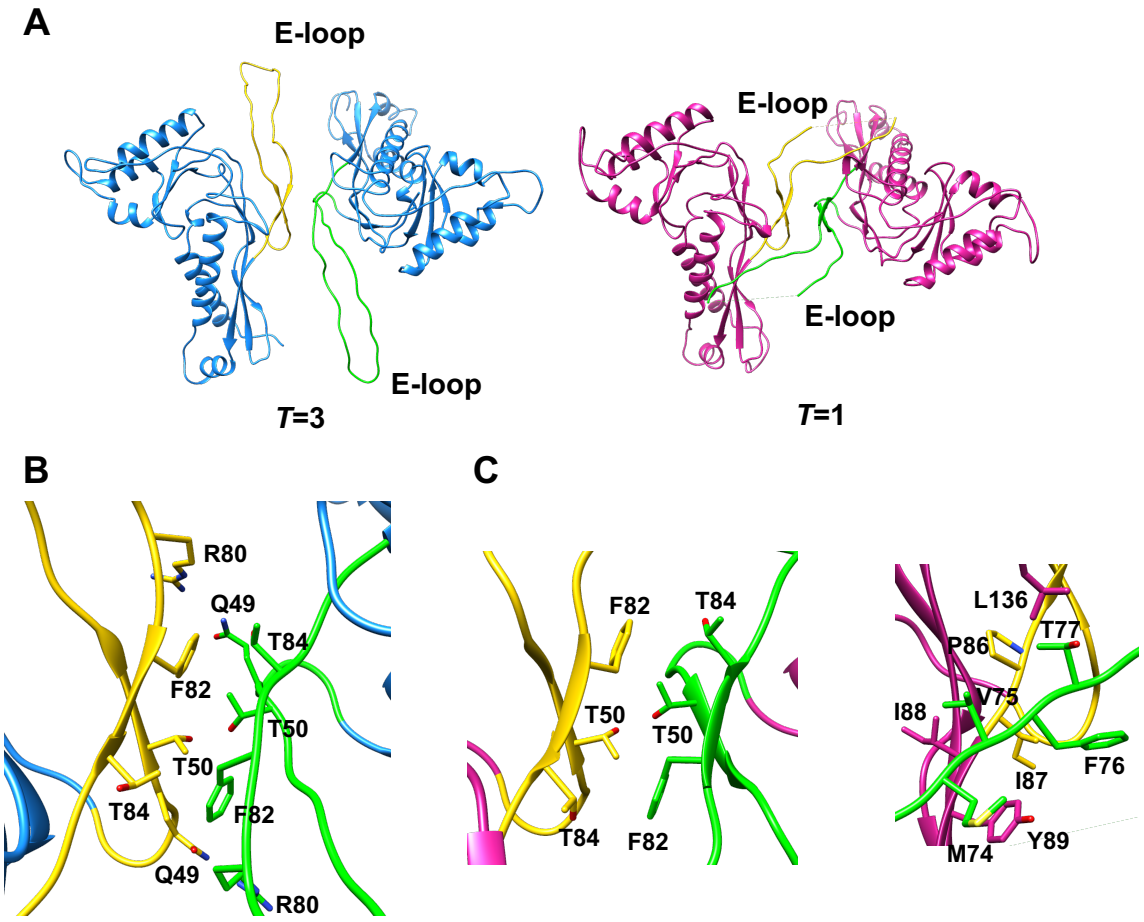

Figure S3. Structural comparison of EncA protomers arranged in *T*=1 and *T*=3 symmetry. Related to Figure 1. (A) Arrangement of E-loops between EncA dimers as viewed from the 2-fold-axis. EncA is shown in blue and pink for *T*=3 and *T*=1, respectively. E-loops from different monomers are colored in yellow and green. (B) Close-up view of interactions between two E-loops in *T*=3 symmetry. (C) Close-up view of interactions between two E-loops in *T*=1 symmetry (left) and additional β-sheet interactions between the E-loop and the P-loop of neighboring protomer (right).

Figure S4

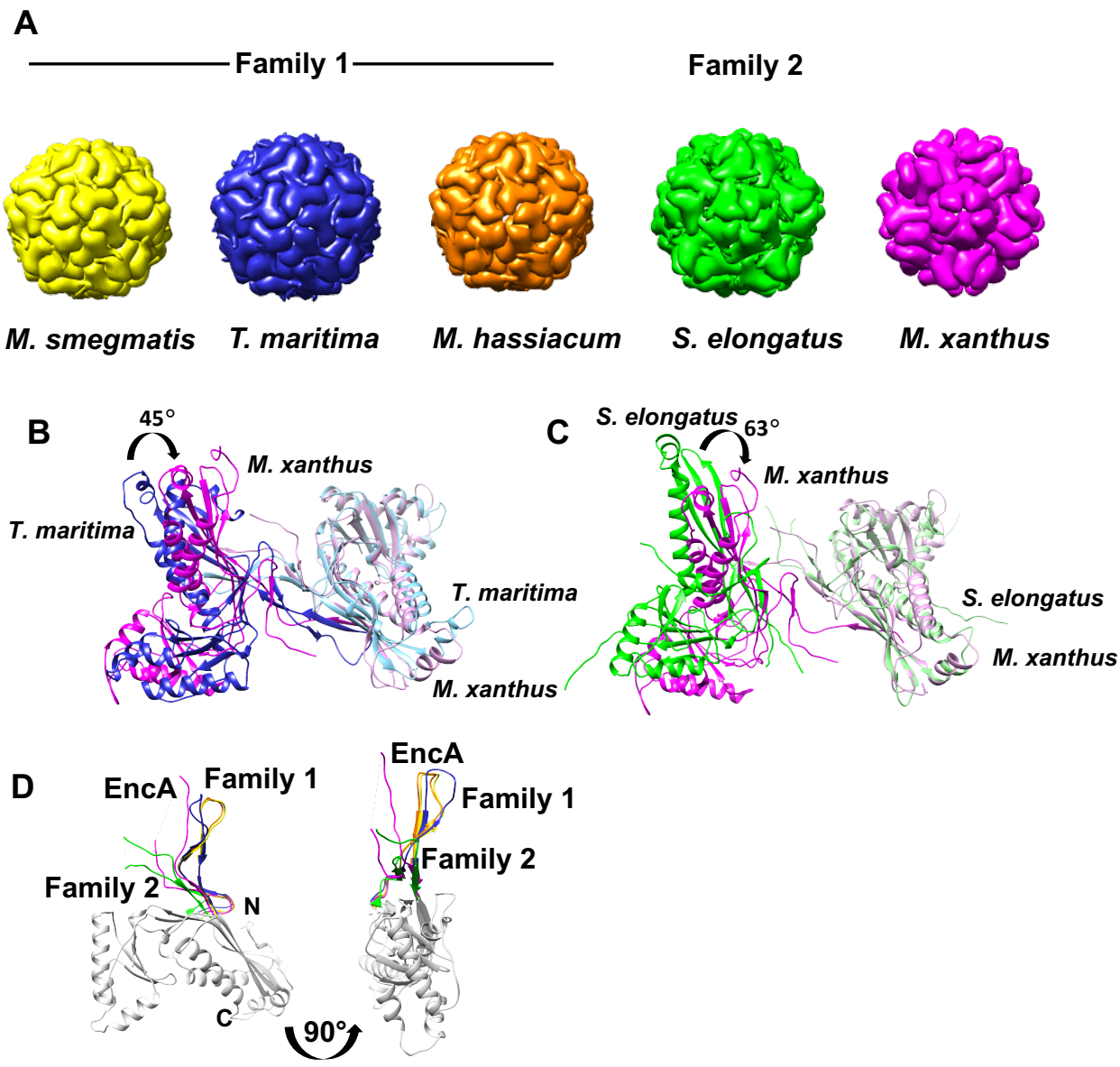

Figure S4. Comparison of *T*=1 encapsulins from different organisms. Related to Figure 1. (A) Surface representations of *M. smegmatis* (PDB ID: 7BOJ), yellow; *T. maritima* (PDB ID: 7K5W), blue; *M. hassiacum* (PDB ID: 6I9G), orange; *S. elongatus* (PDB ID: 6X8M), green; and *M. xanthus* (PDB ID: 7S21), pink. (B) Structural alignment of *M. xanthus* EncA (magenta) and *T. maritima* protomers (blue) as viewed from the 2–fold axis. (C) Structural alignment of *M. xanthus* (magenta) and *S. elongatus* protomers (green) as viewed from the 2–fold axis. Different shades of color (dark and light) are used for individual monomers. (D) Structural alignment of E-loops from Family 1 and Family 2 *T*=1 encapsulins and EncA. The loops are color coded according to Figure A. For simplicity, the rest of the shell protein is only shown for *T. maritima.*

Figure S5

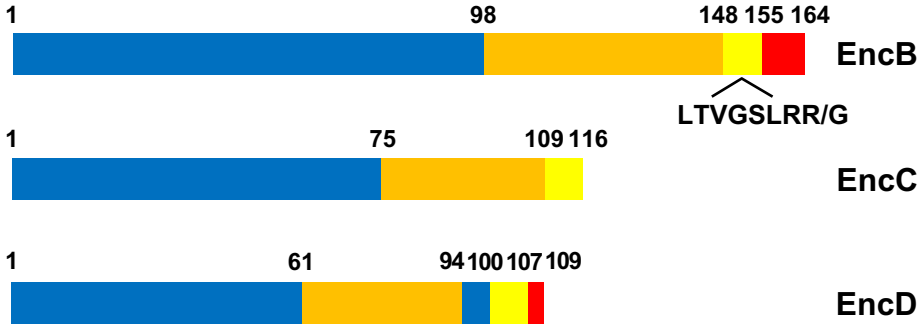

Figure S5. Predicted structural organization of *M. xanthus* cargo proteins. Related to STAR methods, Figure 2 and Figure 3. Regions with secondary structures are shown in blue, disordered regions are shown in orange, conserved TP regions are shown in yellow and additional C-terminus residues are shown in red. The consensus TP sequence LTVGSLRR/G is indicated.

Figure S6

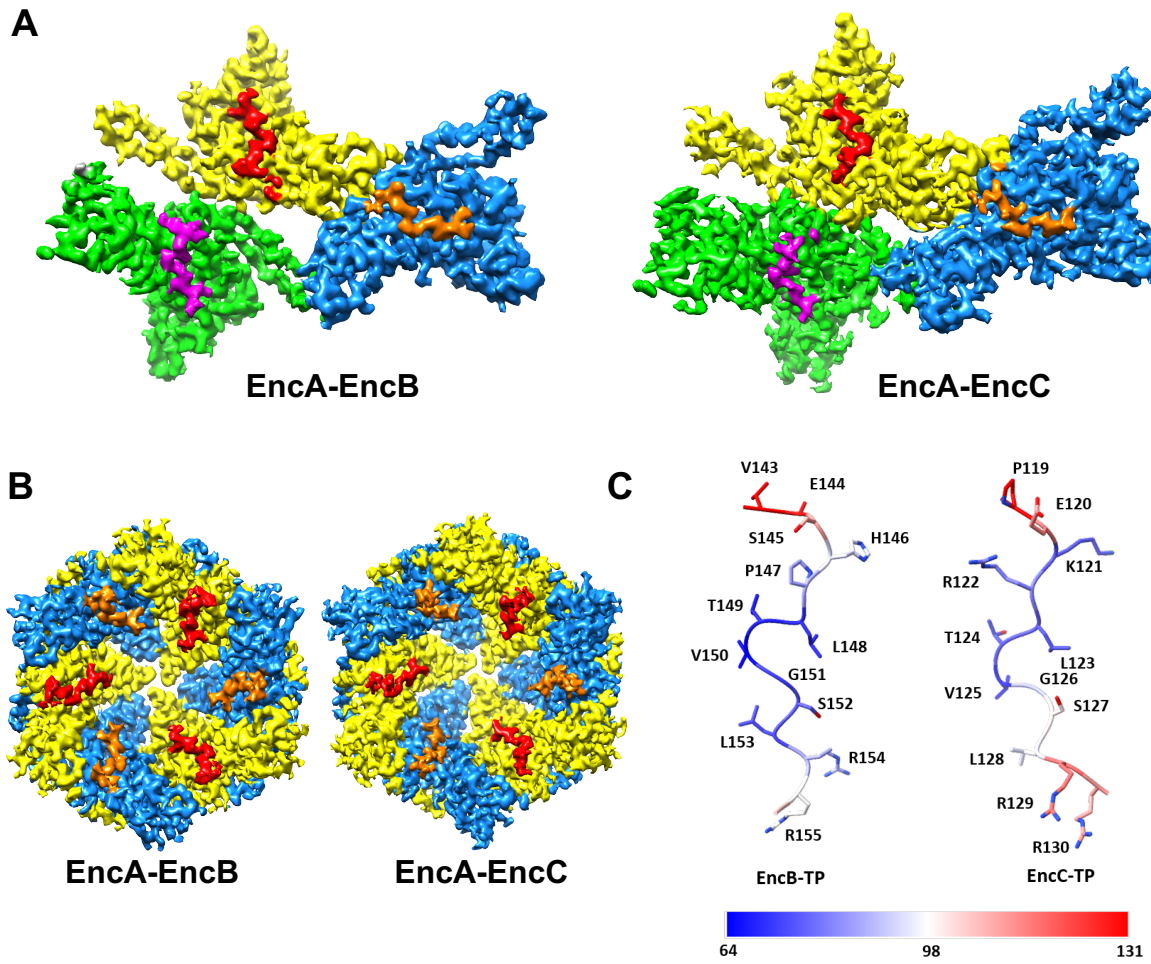

Figure S6. Asymmetric units showing TP densities. Related to Figure 2 and Figure 3. (A) EncA-EncB (left) and EncA-EncC (right). Individual EncA monomer densities are show in blue, yellow and green. Corresponding TP densities are shown in orange, red and magenta. (B) TP densities observed at the hexameric facets for EncA-EncB (left) and EncA-EncC (right). (C) EncB and EncC TPs colored by B-factor. A color key from blue to red is included. Blue shows regions with lowest B-factors while red shows the highest B-factors.

Figure S7

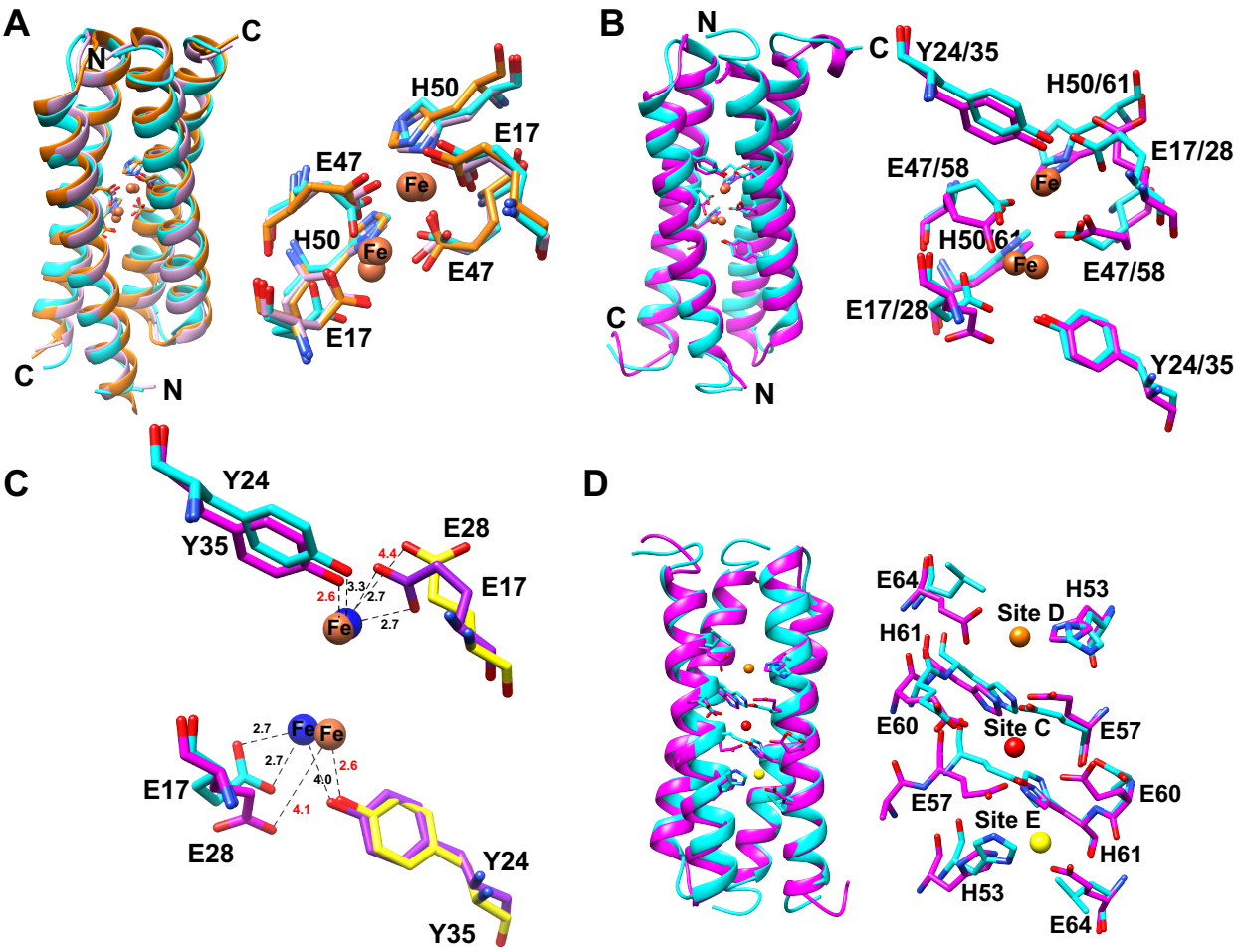

Figure S7. Comparisons of Fe binding sites in bacterial encapsulated ferritins. Related to Figure 6. (A) Structural alignment of dimers forming FOC of EncC (cyan), Rru_A0973 (PDB ID: 5DA5, orange) and PFC_05175 (PDB ID: 5N5E, pink) as observed from the central channel. Fe atoms are shown as orange spheres. A close-up view of the FOCs is shown on the right. The residues are numbered according to EncC. (B) Structural alignment of EncB and EncC dimers forming FOCs as observed from the central channel. A close-up view of the FOCs is shown on the right. (C) Structural alignment of EncB and EncC FOCs showing the conformational changes of the conserved tyrosine and glutamic acid residues. Fe atoms are colored in orange and blue for EncB and EncC structures, respectively. Distances between the coordinating residues and the Fe atoms are shown as black dashes and labeled in red for EncB and in black for EncC. (D) Structural alignment of EncB (magenta) and EncC (cyan) showing the glutamic acid-histidine ladder as observed from outside the channel. The Fe atom binding to site D is shown in orange, site C is shown in red and site E is shown in yellow. Residues are numbered according to EncB.

Figure S8

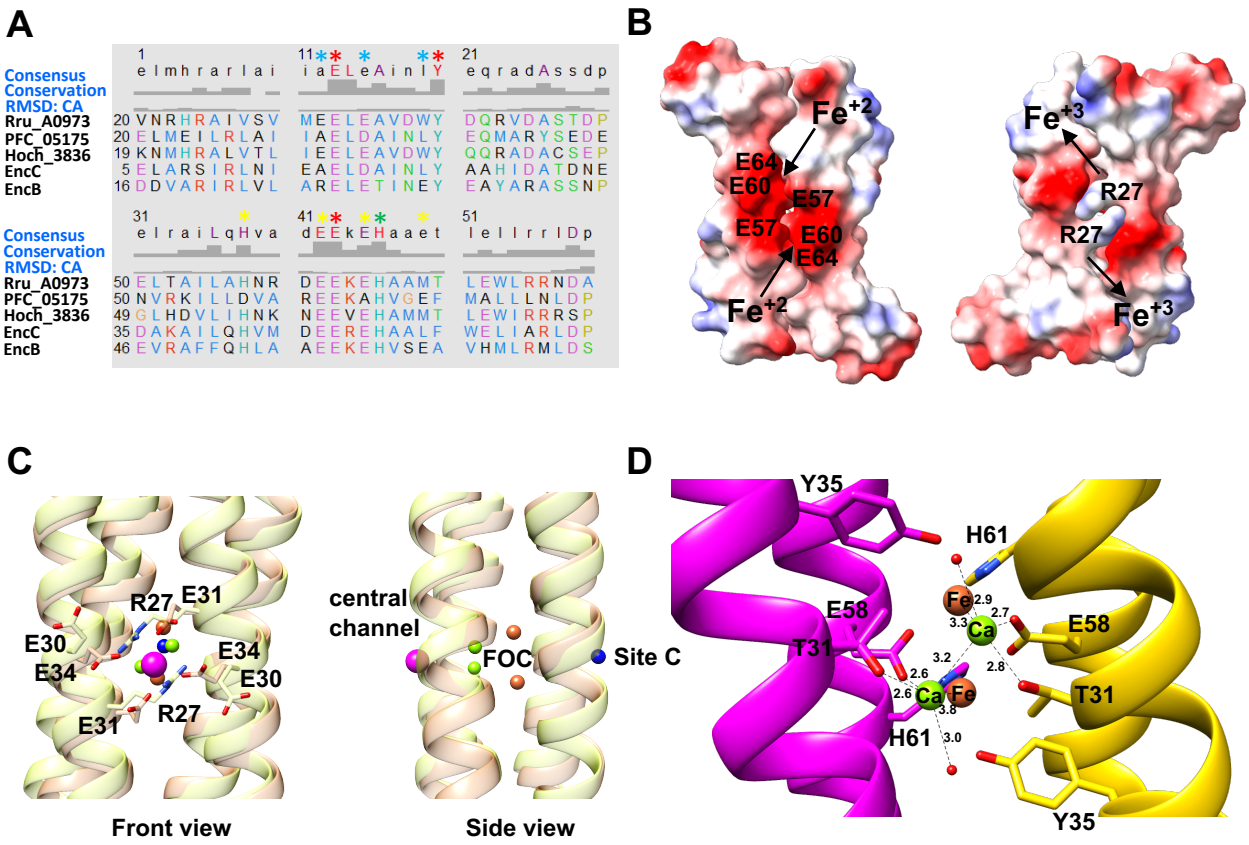

Figure S8. Analysis of metal binding sites in encapsulated ferritins. Related to Figure 6. (A) Sequence alignment of encapsulated ferritins for which a structure is available in PDB. Fe coordinating residues at the FOC are indicated with a red asterix, glutamic acid-histidine ladder residues are indicated with a yellow asterix, histidine residue that coordinates Fe both at site C and FOC is indicated with a green asterix. Rru_A0973 Fe "entry" residues are indicated with a blue asterix. (B) EncB dimers forming the FOC as viewed from outside (left) and from the central-channel (right). The dimers are colored according to electrostatic potential (blue, electropositive; red, electronegative; white, neutral). Proposed direction of Fe^{+2} and Fe^{+3} movement is shown with black arrows. (C) Structural alignment of EncB (yellow) and Rru_A0973 (orange) focusing on the central channel Fe entry site of Rru_A0973. Fe bound to the site as observed in the Rru_A0973 structure (PDB ID: 5DA5) is shown in magenta, FOC Fe atoms are shown in orange, site C Fe atom is shown in blue and Ca atoms observed in EncB structure are shown in green. (D) Calcium binding sites observed in EncB crystal structure as viewed from the central channel. Individual monomers forming the metal binding site are shown in magenta and yellow. FOC Fe atoms are shown in orange, Ca atoms are shown in green and water molecules are shown in red. Distances between Ca atoms and coordinating residues are shown as black dashes.

Figure S9

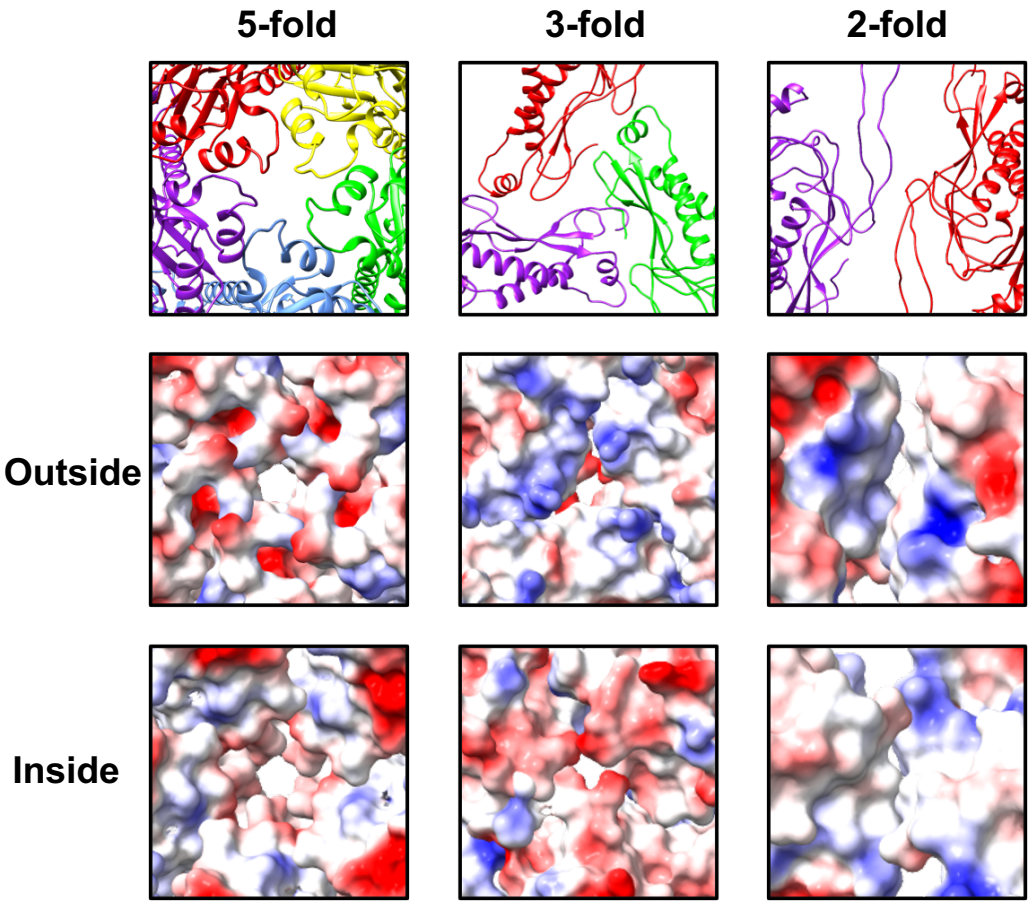

Figure S9. Analysis of EncA shell pores. Related to Figure 7. Cartoon representations of EncA protomers surrounding the 5-, 3- and 2-fold axes are shown on top. Electrostatic surface potential representations of EncA protomers surrounding the 5-, 3- and 2-fold axes pores as viewed from outside and inside are shown in the middle and bottom, respectively. Blue shows electropositive, red shows electronegative and white shows neutral areas.

Figure S10

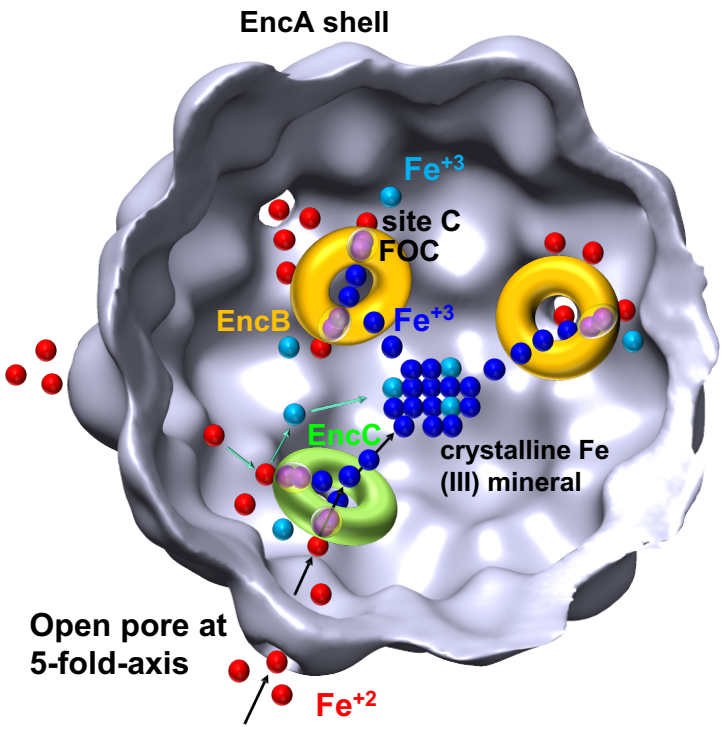

Figure S10. Schematic representation of proposed Fe storage model in the *M. xanthus* encapsulin lumen. Related to Figure 8. Fe^{+2} atoms (red spheres) enter the encapsulin shell from the 5-fold-axis pores in the "open" state and move towards the negatively charged sides of the asymmetrically located encapsulated ferritins (represented as toroids), where the glutamic acid-histidine ladder is located. Two Fe^{+2} atoms (purple spheres) then enter the FOC (represented as semi-transparent regions) while one Fe^{+2} binds to site C. At the FOC the Fe^{+2} atoms are then oxidized to Fe^{+3} atoms (dark blue spheres) which move towards the central channel of the encapsulated ferritin. Finally, Fe^{+3} atoms exit from the channel into the lumen of the encapsulin to be stored as a crystalline mineral. Some of the Fe^{+2} atoms are oxidized to Fe^{+3} (light blue spheres) at the "site C" of the ladder, as a part of the FOC redox mechanism, and move to the encapsulin lumen without entering the central-channel of the encapsulated ferritin. The direction of Fe^{+2}/Fe^{+3} movement from the pore to the encapsulin lumen *via* FOC is indicated with black arrows. The secondary direction of Fe^{+2}/Fe^{+3} movement to the lumen *via* site C is indicated with cyan arrows.