Cryo-EM Structural Characterizations of EncB, a Ferritin-like Encapsulin Cargo Protein

Bing Wang[1], Oliver W. Bayfield[2], Rick K. Huang[3], J. Bernard Heymann[1], Fred Antson[2], Alasdair C. Steven[1]

[1.] Laboratory of Structural Biology Research, National Institute of Arthritis, Musculoskeletal and Skin Diseases, Bethesda, MD, USA.
[2.] York Structural Biology Laboratory, Department of Chemistry, University of York, York, UK.
[3.] Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA.

Cells are compartmentalized to increase the metabolic efficiency and sequester redox-active substances accumulated under oxidative stress [1]. Unlike the membrane-bound organelles in eukaryotes, prokaryotes have protein-based compartments, such as encapsulin or nanocompartments [2, 3]. Encapsulins exist in many bacteria and archaea, including some medically important parasites, such as *Mycobacterium tuberculosis* and *Myxococcus xanthus* [4, 5]. However, the physiological roles of encapsulins are still not clearly understood. *M. xanthus* forms elaborate spore-filled fruiting bodies upon amino acid starvation, making this organism an ideal model to study cellular response to environmental changes [6]. These encapsulins contain four different proteins: the shell-forming protein EncA and three internal cargo proteins, EncB, EncC, and EncD. Sequence alignment suggests EncB and EncC are probably ferritin-like proteins [5]. *M. xanthus* encapsulins can store an order of magnitude more iron than ferritin suggesting the iron-binding role of EncB and EncC in the core [5].

We previously solved the structure of EncA shell to 4.6 Å resolution [5]. It is a hollow icosahedral particle of 180 subunits which have a fold first seen in the capsid of bacteriophage HK97. To further understand the role of the cargo proteins, we expressed and purified EncB. We are currently using cryo-electron micrograph (EM) to investigate the structure of EncB. The micrographs were collected on a Titan Krios (FEI company), operated at 300 kV. Image processing was done with the Bsoft package [7]. From the micrograph images, we found EncB forms ring-shaped particles ~7 nm in diameter (Figure 1). Asymmetric reconstructions were done to assess possible symmetries. We found the symmetry of recombinant EncB is predominantly C5 based on current data. The final map has a resolution of ~11 Å (Figure 2), and shows five globular subdomains in a ring of an outer diameter of ~68 Å . Each subdomain has a diameter of ~28 Å, which is consistent with one 17 kDa EncB monomer. We estimated the mass from the map density of the pentamer to be ~60kDa. The lower than expected mass may be due to unstructured parts or limited degradation. In the future we would like to identify where EncB binds to the inside of EncA shell (Figure 3).

References:

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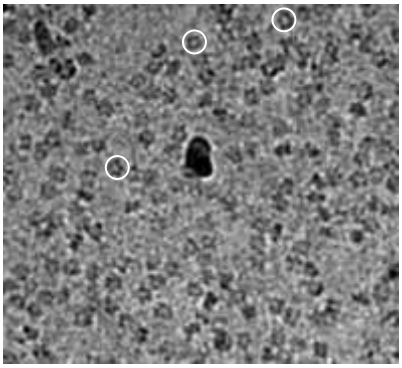

Figure 1. Cryo-electron micrograph of EncB collected on a Titan Krios operated at 300 kV. EncB forms ring-shaped particles ~7 nm in diameter (white circles in the figure).

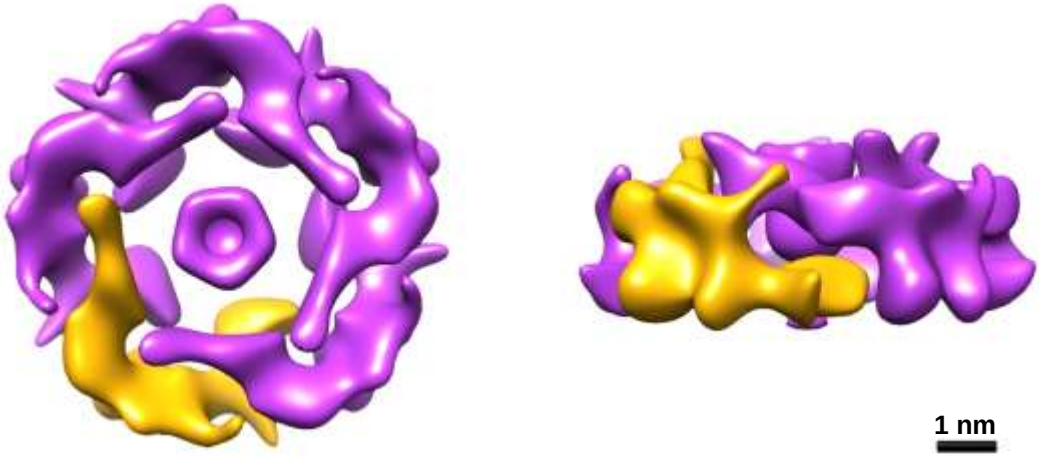

Figure 2. Top and side views of final reconstruction of EncB at 11 Å resolution. It has 5-fold symmetry. One subunit is shown in yellow.

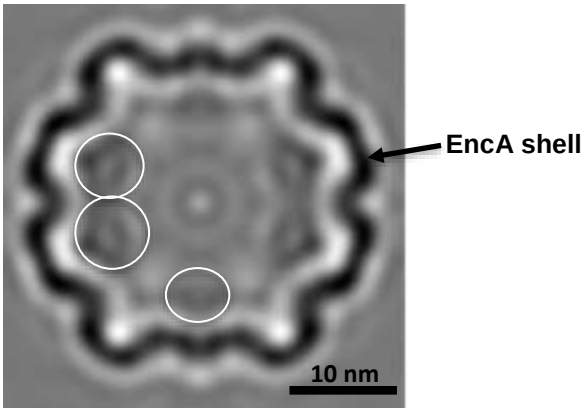

Figure 3. Central slice of subtomogram average of an encapsulin particle from *M. xanthus* [5]. EncA forms a $T = 3$ icosahedral shell (see arrow). White circles show density that may be EncB.