

Unraveling the Assembly Principles of Bacterial Microcompartments

Bacterial microcompartments (BMCs) are large, specialized subcellular compartments for colocalizing enzymes to enhance reaction rates, protect sensitive proteins, and sequester toxic intermediates. The prominent examples include the carboxysome for CO₂ fixation¹ and catabolic microcompartments found in many pathogenic microbes. The shell sequesters enzymatic reactions from the cytosol, analogous to the lipid-based membrane of eukaryotic organelles. The major components of BMC shells are cyclic hexamers with a pronounced concave-versus-convex sidedness². These proteins, referred to as BMC-H, contain a single BMC (pfam00936) domain (Fig. 1, blue). A derivative of BMC-H proteins, BMC-T, is a fusion of two BMC domains forming trimers or pseudo-hexamers (Fig. 1, green). BMC-P proteins belong to pfam03319; they are structurally unrelated to the BMC/pfam00936 domain and form pentamers shaped like a truncated pyramid (Fig. 1, yellow). Metabolites and cofactors must be allowed to cross the shell in order for BMCs to function.

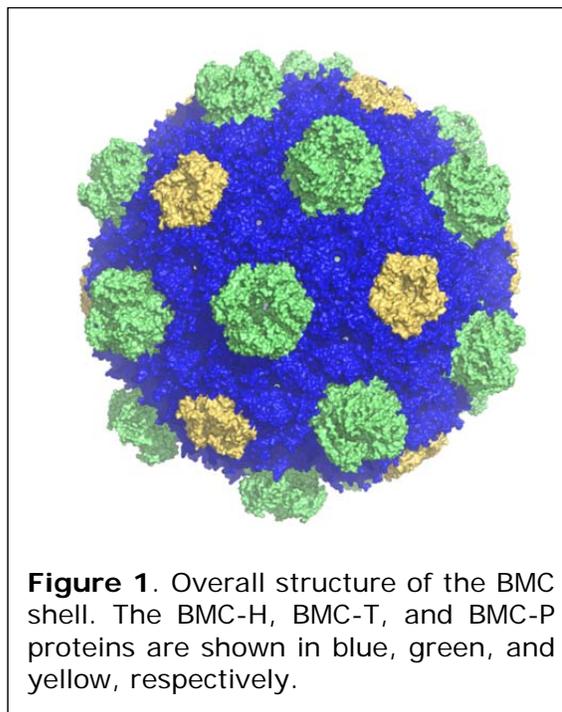


Figure 1. Overall structure of the BMC shell. The BMC-H, BMC-T, and BMC-P proteins are shown in blue, green, and yellow, respectively.

Despite detailed structural knowledge of the individual shell components, the architectural principles governing shell self-assembly are unknown.

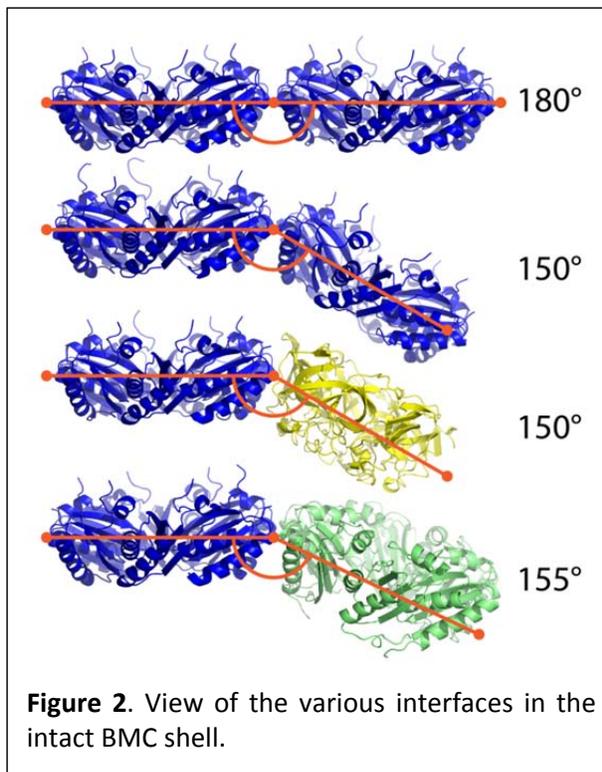
Understanding how the microcompartment membrane is assembled, as well as how it lets some compounds pass through while impeding others, could contribute to research in enhancing carbon fixation and, more broadly, bioenergy. This class of organelles also helps many types of pathogenic bacteria metabolize compounds that are not available to normal, non-pathogenic microbes, giving the pathogens a competitive advantage. The contents within these organelles determine their specific function, but the overall architecture of the protein membranes of BMCs are fundamentally the same. The microcompartment shell provides a selectively permeable barrier which separates the reactions in its interior from the rest of the cell.

A group of researchers led by Cheryl Kerfeld have determined the atomic-resolution structure of a complete 6.5-megadalton bacterial microcompartment shell. The data for the *H. ochraceum* shell was collected at SSRL Beam Line 12-2. A low resolution (8.7 Å) cryo-electron microscopy density map of the whole particle allowed constructing a model by placing the known structures of the hexameric and pentameric subunits which was of suitable quality for molecular replacement. The shell is composed of hundreds of copies of five distinct proteins that form hexamers, pentamers, and three types of trimers.

There are four distinct interfaces in the intact shell: two different hexamer-hexamer interactions, the hexamer-pentamer interaction, and the hexamer-pseudo-hexamer interaction (Figure 2). The hexamers connecting pentamers between two vertices of the intact shell are in a side-by-side, planar orientation, whereas the hexamers surrounding the pentamers are tilted by 30°. Considering the high structural conservation among all

hexamer and pentamer proteins, these orientations are likely universal among BMCs. The surface view of the intact shell shows that it is tightly packed; the only conduits to the interior of the shell are the pores formed at the cyclic symmetry axes of the hexamers and pseudohexamers. The largest channel to the interior is formed by the BMC-T proteins; the pore across the trimer within the facet is at least 5 Å wide with the potential to be larger owing to the flexibility of the loops surrounding the pore.

This model of the basic architecture of the bacterial microcompartment shell likely applies to functionally diverse organelles found across the bacterial kingdom. Due to high structural conservation, the discovered assembly principles can be applied to all bacterial microcompartments and serves as a prototype blueprint for the rational design of tailor-made nanofactories and the development of therapeutics to disrupt the shells of pathogens.



References

1. J. M. Shively, F. Ball, D. H. Brown and R. E. Saunders, "Functional Organelles in Prokaryotes: Polyhedral Inclusions (Carboxysomes) of *Thiobacillus neapolitanus*", *Science*, **182**, 584 (1973), doi: 10.1126/science.182.4112.584.
2. C. A. Kerfeld, M. R. Sawaya, S. Tanaka, C. V. Nguyen, M. Phillips, M. Beeby and T. O. Yeates, "Protein Structures Forming the Shell of Primitive Bacterial Organelles", *Science* **309**, 936 (2005), doi: 10.1126/science.1113397.

Primary Citation

M. Sutter, B. Greber, C. Aussignargues and C. A. Kerfeld, "[Assembly Principles and Structure of a 6.5-MDa Bacterial Microcompartment Shell](#)", *Science* **356**, 1293 (2017) doi: 10.1126/science.aan3289.

Contact

Cheryl Kerfeld, Lawrence Berkeley National Laboratory

SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences.