Lawrence Berkeley National Laboratory
LBL Publications

Title

Engineered bacterial microcompartments: apps for programming metabolism

Permalink
https://escholarship.org/uc/item/8r59w8v4

Authors
Kerfeld, Cheryl A
Sutter, Markus

Publication Date
2020-10-01

DOI
10.1016/j.copbio.2020.05.001

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Engineered Bacterial Microcompartments: Apps for Programming Metabolism

Cheryl A. Kerfeld[a,b,*] and Markus Sutter[a,b]

[a]MSU-DOE Plant Research Laboratory and Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, Michigan, USA
[b]Environmental Genomics and Systems Biology and Molecular Biophysics and Integrated Bioimaging Divisions, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA

Short title: Engineered Bacterial Microcompartments

Keywords: bacterial microcompartment; carboxysome; metabolosome; bioengineering; metabolism

*Corresponding author: ckerfeld@lbl.gov, Lawrence Berkeley National Lab, Building 64, 1 Cyclotron Road, Berkeley CA 94702. 510-486-6078.

Abstract
Bacterial Microcompartments (BMCs) are used by diverse bacteria to compartmentalize enzymatic reactions, functioning analogously to the organelles of eukaryotes. The bounding membrane and encapsulated components are composed entirely of protein, which makes them ideal targets for modification by genetic engineering. In contrast to viruses, in which generally only one protein forms the capsid, the shells of BMCs consist of a variety of shell proteins, each a potential unit of selection. Despite their differences in permeability, the shell proteins are surprisingly interchangeable. Recent developments have shown that they are also highly amenable to engineered modifications which poise them for a variety of biotechnological applications. Given their modular structure, with a module defined as a semi-autonomous functional unit, BMCs can be considered apps for programming metabolism, that can be de-bugged by adaptive evolution.

Bacterial Microcompartments are Metabolic Organelles
Metabolic diversification is an essential determinant of bacterial fitness. The ability to metabolize a newly encountered substrate enables bacteria to adapt to changing environments, outcompete other organisms, and exploit new niches. It is now well-established that prokaryotic metabolic networks evolve by acquisition of peripheral reactions to confer adaptive metabolic flexibility. The ability to use and integrate the new functions conferred by horizontal gene transfer (HGT) leads to their retention. Bioengineers likewise introduce new pathways into cells to develop production platforms; this sets up similar retain-or-discard, life-or-death challenges for the bacterium. Frequently, metabolic engineering efforts are hampered by unintended consequences resulting from even the most rationalized engineering, such as incompatibility of reactions, sensitivity of co-factors to cytosolic conditions, or the draining of intermediates to diffusion or competing pathways. The hallmark feature of eukaryotic cells—the compartments that insulate otherwise incompatible functions—have recently been shown to be found in bacteria as well. Among specialized subcellular compartments, those known as Bacterial Microcompartments (BMCs) function as organelles by sequestering metabolic pathways from the rest of the cytosol [1]. Several types have been experimentally characterized, including carboxysomes for CO_2 fixation (reviewed in [2-4]) and catabolic BMCs (also known as metabolosomes) for the breakdown of ethanolamine (EUT)[5], 1,2-propanediol (PDU) [6] and GRM3 [7,8]) , choline (GRM2)[9,10], small saccharides (GRM2, PVM)[11,12], xanthine (Xau; formerly known as BMC-of-unknown function 1 (BUF1 [13])) [14] and amino alcohols (RMM) [15-17].

Although the compartmentalized chemistries differ, all BMCs share a common membrane architecture, a polyhedral shell that consists entirely of protein. The shell facets are composed of homologous proteins that form hexamers (BMC-H)[18] or trimers (BMC-T)[19]; the BMC-H proteins are all members of the Pfam00936 domain family, the BMC-T proteins consist of tandem fusion of two Pfam00936 domains (BMC-T^S) that are sometimes stacked on top of each other (BMC-T^D) (Figure 1A). The cyclically symmetric hexamers and trimers have a pronounced hexagonal shape with a central depression that renders one surface convex and the other concave (Figure 1A). The vertices of the shells are capped by a pentameric Pfam03319 domain protein (BMC-P)[20]. Shell protein oligomers are typically perforated by pores that selectively allow passage of metabolites across the shell. Genes encoding BMC-H, BMC-T and BMC-P proteins are readily identifiable in genome sequences and there are typically multiple paralogs of BMC-H and BMC-T; using homology modeling, it is apparent that one of the most prominent differences among paralogs are the residues that surround the pores, that form conduits for different metabolites. Shell protein genes are typically clustered with genes for the encapsulated enzymes as well as ancillary genes encoding proteins that integrate the BMC with the rest of the cell's metabolism, such as transmembrane proteins that are used at the cell membrane for uptake of the BMC substrate [1,13] (Figure 1D). This compact genetic organization also suggests a strategy for engineering of compartmentalized metabolism. Here we review principles and progress in designing and constructing synthetic BMCs using a "bottom-up" approach, the encapsulation of enzymes of choice within a BMC-derived protein shell. We focus here the recent advances, particularly since 2017, in constructing catalytic compartments that are analogs of natural BMCs.

Principles of Natural BMC assembly

The beta carboxysome, conserved in all beta cyanobacteria, was the first BMC discovered [21] and it provides a model system to study principles of BMC assembly, as many of the assembly features appear to be shared by catabolic BMCs [1,3,10]. All beta carboxysomes contain Form I Rubisco, which is an assembly of 8 small and 8 large subunits, a gamma carbonic anhydrase (CcmM) that is unique in its class because it contains a C-terminal extension composed of multiple copies of a domain that is homologous to the small subunit of Rubisco (small subunit-like domain, SSLD). A third protein, CcmN is structurally homologous to the beta-helical carbonic anhydrase domain of CcmM and it contains a C-terminal extension of a poorly conserved linker region that terminates in a conserved segment of about 25 amino acids that was predicted [22] (and later confirmed [23]) to form an amphipathic alpha helix. These peptide extensions are now known as Encapsulation Peptides (EPs) [24] and are found on a wide range of BMC-encapsulated enzymes. Key steps in the sequence of events in beta carboxysome assembly involve nucleation of Rubisco by the SSLDs, the interaction of the gamma domain of CcmM and the helical repeats of CcmN and encapsulation of this core by EP-mediated recruitment of the shell. Both the gamma domain of the CA and the predicted C-terminal amphipathic EP of CcmN interact with the shell. Deletion of just the EP segment precludes carboxysome formation, highlighting its critical importance [22].

Cyanobacterial carboxysomes provide an ideal model system to test principles of BMC structure and function. They are readily visible in micrographs (beta carboxysomes are typically 200-400 nm diameter [25]) and by fluorescent labeling. Cyanobacteria require a functioning carboxysome to grow on air, providing a sensitive read-out for function and there are several strains that are well-studied and genetically tractable. The deduced pathway for beta carboxysomes assembly, including the role of the EP and domain mimics such as the SSLDs was tested by designing a synthetic protein combining a subset of these domains, ordered to preserve critical interactions in carboxysome assembly [26]. This strategy reduces the amount of genetic information required to construct a functional carboxysome. Focusing on discrete protein domains and their roles in carboxysome assembly and function, a single composite

protein consisting the SSLDs of CcmM, an EP and a carbonic anhydrase was shown to replace four gene products in the assembly of functional beta carboxysomes, highlighting the role of both domain mimics and EPs in BMC assembly. Moreover, identification of assembly determinant, like the SSLDs can facilitate discovery of other BMC components encoded in satellite loci (Figure 1D) distal from the main BMC locus. Searches for other SSLD-containing proteins in cyanobacterial genomes led to the identification of a Rubisco activase homolog that was recently shown to be a component of the beta carboxysome [27].

The principle of hierarchical assembly as observed for beta carboxysomes [28] likely applies to the majority of BMC types [1,10]. By surveying all BMC loci found in bacterial genomes as a collection of Pfam domains, it is evident that both EPs, as terminal extensions or inter-domain insertions, and domain mimics can be identified across functionally diverse BMCs. Whether or not they are all critical for assembly, or in the case of domain mimics, serve additional regulatory or function roles as metabolite reservoirs awaits further confirmation. One of the assumptions made in defining EPs is that they likely interact with shell proteins, as EPs and shell proteins are the only absolutely conserved features for the majority of BMCs.

Design and Construction of Synthetic BMC Shells

The understanding of the principles of BMC assembly provides design strategies for constructing catalytic cores and encapsulating them with shells. Another breakthrough in establishing the potential for engineering bespoke BMCs was the observation that, in the absence of cargo, BMC shells can still assemble, albeit smaller than the cognate BMC from which the shell protein genes derive [29]. Pioneering efforts involved systematically expressing different combinations of the seven shell proteins (four BMC-H, two BMC-T and one BMC-P) encoded by the PDU locus [30]. Two other model shell systems have been developed, one derived from the metabolosome produced by the myxobacterium *Haliangium ochraceum* (HO) [31] that consists of one BMC-H, three BMC-T and one BMC-P and the other from beta carboxysomes [32,33], consisting of two BMC-H, one BMC-T and one BMC-P. The HO and carboxysome shell systems also led to the first structural descriptions of intact shells [34,35] (Figure 1B). Remarkably, given the functional and phylogenetic distance between the HO metabolosome and beta carboxysome shells, the overall architectural principles are strongly conserved, including amino acid motifs for intermolecular interactions among the different shell proteins [34]. Particularly important for the goal of constructing novel catalytic cores to be internalized in shells, was the elucidation of the sidedness of the shell proteins (Figure 1B): One of the shared architectural principles is that the concave sides face outward [34,35]. Knowledge of the sidedness also reveals that for the vast majority of shell proteins the N- and C-termini of hexamer protomers are on the external surface (Figure 1A,B). Our suggestion that the architectural principles of shell assembly are broadly shared across all BMC types has recently been substantiated by the structural characterization of the GRM2 shell [10].
From these examples it appears that shells with a diameter of about 250 Å form quite readily [10,33] when a BMC-H and a BMC-P are co-expressed. For larger shells it seems that additional BMC-H or BMC-T subunits are needed and expression levels might need to be carefully regulated either by genetic design [31] or by using a construct that aims to recapitulate the expression of the native system [29]. Smaller synthetic systems might have an advantage when used in metabolic engineering because fewer proteins will have to be engineered, e.g. for permeability. However, they might not have enough cargo capacity and larger shells consisting of different shell proteins will also offer more options to engineer permeability.

Engineering of Cargo Encapsulation into BMCs

The first attempts to engineer novel BMCs combined synthetic shell systems and their cognate EPs fused to selected cargo, with the EP presumably binding to the shell as it assembles and therefore promoting cargo encapsulation. The first proof-of-concept made use of the shell derived from a PDU BMC to encapsulate enzymes for ethanol production [23]. Other examples include the use of EPs to catalytically promote polyphosphate accumulation [36], to sequester the expression of a toxic protein [37] and to demonstrate the potential for installing BMCs in industrially important microbes [38]. Subsequent studies showed that EPs are interchangeable across functionally different shell types [39] which, given that the shell protein amino acid sequence homology is the only other known commonality, supports the early assertion that EPs interact with shell proteins [22]. However, in general, studies using EPs to target to synthetic shells reported relatively modest encapsulation efficiency [31,37,38,40]. A high affinity of the EP to the shell proteins would likely be detrimental for shell assembly so the interaction might be intricately tuned. Moreover, EPs are more than just a tag for shell interaction; EPs also promote enzyme aggregation in the absence of shell proteins [41,42], suggesting that EPs have a dual role in core nucleation and encapsulation by the shell. Notably, EP-mediated enzyme coalescence alone provides a catalytic advantage as compared to enzymes free in solution [43], and the interplay of this aggregation and encapsulation is not well understood.

While there are some computational models, the molecular details of the interaction between EPs and shell proteins are still unknown, but it is likely a relatively passive immobilization in comparison to what can be achieved by designed associations. This has now been realized with the use of coiled-coil domain interactions [44,45] or covalent bonding of shell proteins and cargo through the insertion of the SpyTag-SpyCatcher system [46]. The successful development of these orthogonal adaptor domains to predictably direct known amounts of cargo to shells was predicated on the precise structural information about the nature and number of sites for fusions, information available from the structures of the phylogenetically and functionally disparate shells [33,34]. More broadly, given that the sidedness of the proteins in the shell is conserved, insertions and fusions to any homologous shell protein, from any BMC system, can be designed to specifically localize cargo to either the lumen or the external surface. For example, knowing the location of features such as loops within shell protein protomers guided the insertion of the SpyTag and SpyCatcher domains. Identifying interior surface accessible sidechains enabled design of an surface that interacts with cargo or metabolites through electrostatic interactions [47]. Fusions of enzymes to outward facing shell protein termini displays them on the exterior of the shell; this may be useful, for example, to catalyze a reaction to produce a high concentration of a substrate near shell protein pores specific for the metabolite. Alternatively, circular permutation has been used to relocate these termini to the interior of the shell for cargo internalization [48].

The ability to produce terminal fusions to individual shell proteins also has led to the development of a loading-and-purification protocol that takes advantage of the location of the C-terminus of the BMC-P protein. During the crystallization of the HO shell it was realized that increasing the amount of pentamer present increased the diffraction limit of the crystals; this suggested that the synthetic shells initially crystallized were likely heterogeneous with respect to the amount of pentamer present. This led to the inference that shells can form in the absence of pentamers. This knowledge was used to develop the ‘CAP’ (complementation-based affinity purification) strategy for loading, sealing and purifying shells [46]. Shells lacking pentamers (wiffle balls, Figure 1C) can be loaded by diffusion through the vertex vacancies (a gap of about 47 Å in diameter) and then subsequently capped by the addition of pentamers that are functionalized with an affinity tag for purification. This wiffle ball shell and the development of an in vitro system for shell assembly expands the repertoire of cargo that could be encapsulated to include the abiotic. The recent development of in vitro methods of shell assembly [47] should accelerate

prototyping of shell designs, direct measurements of permeability as a function of shell composition, as well as the construction of synthetic BMCs that can be structurally and functionally characterized.

From these examples it is notable that a wide variety of individual shell proteins have proven remarkably robust to manipulation without interfering with shell assembly. In addition to the insertions, fusions, electrostatic engineering and the permutation of secondary structure elements, shell proteins have proven amenable to manipulation of pore residues to alter permeability [32,49] and the recent functionalization of pores with metal centers to allow electron transfer across the shell [50,51].

From Modular Evolution to Modular Engineering of Metabolism

Compartmentalization is a fundamental attribute of the organization across all living systems, and the study of the diversity, structure and function of BMCs is likewise multiscale, drawing insights from disparate research areas such as microbial ecology, bacterial cell biology, biochemistry of multiprotein complexes, to the physics of biological self-assembly of a protein shell. The integration of these insights underlies the transformative potential of BMCs in biotechnology, where engineering can learn from and build on the results of evolution.

In general, bacterial metabolic networks evolve by uptake of peripheral reactions in response to changes in the environment [52]. The HGT of BMC loci provides a particularly evolutionarily successful example of how to introduce, integrate and insulate a desired biochemical pathway en bloc. The shell, by providing a potentially controllable interface with the new cellular context, can prevent the disruption to metabolite balances that results from introduction of enzymes into a new metabolic context. In natural BMCs, the ability to tune the shell permeability in response to environmental conditions is becoming apparent; for example in cyanobacteria, which obligately form carboxysomes for CO_2 fixation, organisms that inhabit dynamic environments tend to contain expanded sets of shell protein paralogs [53]. These include combinations that form heterohexamers from two BMC-H paralogs [54,55]; modelling indicates that an important difference in these structurally equivalent blocks is found at the pores, suggesting they are a response to changing permeability requirements. More broadly, for many functionally diverse BMCs, genes encoding additional shell protein paralogs are located distal to the main BMC locus, in satellite loci, suggesting that they are perhaps more recent innovations, and regulated separately from the main structural genes of the organelle. Accordingly, unlike a lipid-based boundary, the "membrane" of BMCs is modular, in that it is composed of multiple different types of shell proteins with different permeabilities. Each shell protein type is directly encoded by the genome thereby providing a building block poised for engineering and evolution.

BMC structure and function is inherently modular, from the protein domains that constitute the shell and the core to the organelle-within a cell in a community. Engineered BMC modules can be combined in the same cell (Figure 2A), as part of engineered communities (Figure 2B), or in cell free/in vitro contexts (Figure 2D, 2E, 2F). The concept of modular construction, the recursive composition of structural and functional units into increasingly more complex systems, has an informative parallel in classic evolutionary biology where it is the foundation of our understanding of evolution of plant and animal complexity. In macroform evolution, modularity increases evolvability [56,57]; modules, as semi-autonomous functional units, can undergo relatively independent evolution being optimized individually by natural selection. Whereas in this classic evolutionary sense modularity confers the ability to dissociate developmental processes within an organism, in metabolic engineering of bacteria compartmentalizing catalysis in selectively permeable membranes enables the dissociation metabolic processes—potentially controlling cross-talk—within cells, among cells in a designed community [58] or in cell-free bioengineering platforms [59]. BMC shells are generally quite stable so they seem to be well

suited for applications such as immobilized surfaces (Figure 2C) and the ability to assemble them from the components in vitro (Figure 2E) makes it possible to combine them in defined ratios and even with abiotic components.

Moreover, the analogy can be extended to protein domains, the modules that constitute the shell and core of a BMCs. Catalytic cores of fully and directionally coupled enzymes can be rationally fabricated, and encapsulated in a shell designed with the requisite permeability. As genetically encoded catalytic devices, optimization can be carried out by laboratory evolution under selective pressure for module function, which simultaneously operates on the shell, the enzymatic core and the cellular context. Adaptive evolution provides a powerful means for debugging programmed metabolism; successful examples of laboratory evolution in metabolic engineering demonstrate the unexpected changes leading to successful pathway integration [60]. The resulting optimization would have defied any rational, step by step approach. As metabolic modules composed of modules, BMCs, as apps for programming metabolism provide a biological system of subsumption architecture in which higher levels of complexity are composed of lower level functions, each potentially subject to refinement by non-linear debugging by evolution.

Figure legends

Figure 1. Overview of BMC shell proteins, shells and BMC loci. A) Structural representation of the BMC shell building blocks: the pentameric Pfam03319 BMC-P and different variants of the Pfam00936 domain BMC-H, BMC-T^S and BMC-T^D. View onto the symmetry axis (top) with secondary structure elements and transparent surface and sideview (bottom) of the surface representation. B) Shell proteins in the context of a complete shell as seen in the model HO shell. Red shapes indicate different pore functionalities. C) Model of a "wiffle ball" type HO shell that is missing BMC-P. D) Overview of the components of a typical main BMC locus as well as a satellite locus. Red shapes represent the functional differences (permselectivity) of the pores as in (B).

Figure 2. Applications of engineered BMCs in cellular and cell-free metabolic engineering. A) BMCs with different encapsulated enzymatic pathways (represented by different colored icosahedra, substrates/products shown as various small shapes) in a single cell expand the metabolic repertoire. B) BMCs can be used in cellular communities (each with a different BMC, suited to its host metabolism). C) Immobilization of BMCs could enable in vitro reactions where substrate/products are continuously added/removed. D) Lattices of BMCs facilitate exchange between different types of BMCs. E) In vitro mixing of BMCs, soluble enzymes and substrate/cofactors allow for fine-tuning of reaction conditions.

Acknowledgements

This work was supported by the National Institutes of Health, National Institute of Allergy and Infectious Diseases (NIAID) grant 1R01AI114975-01 and the U.S. Department of Energy, Basic Energy Sciences, Contract DE-FG02-91ER20021.

Figure 1

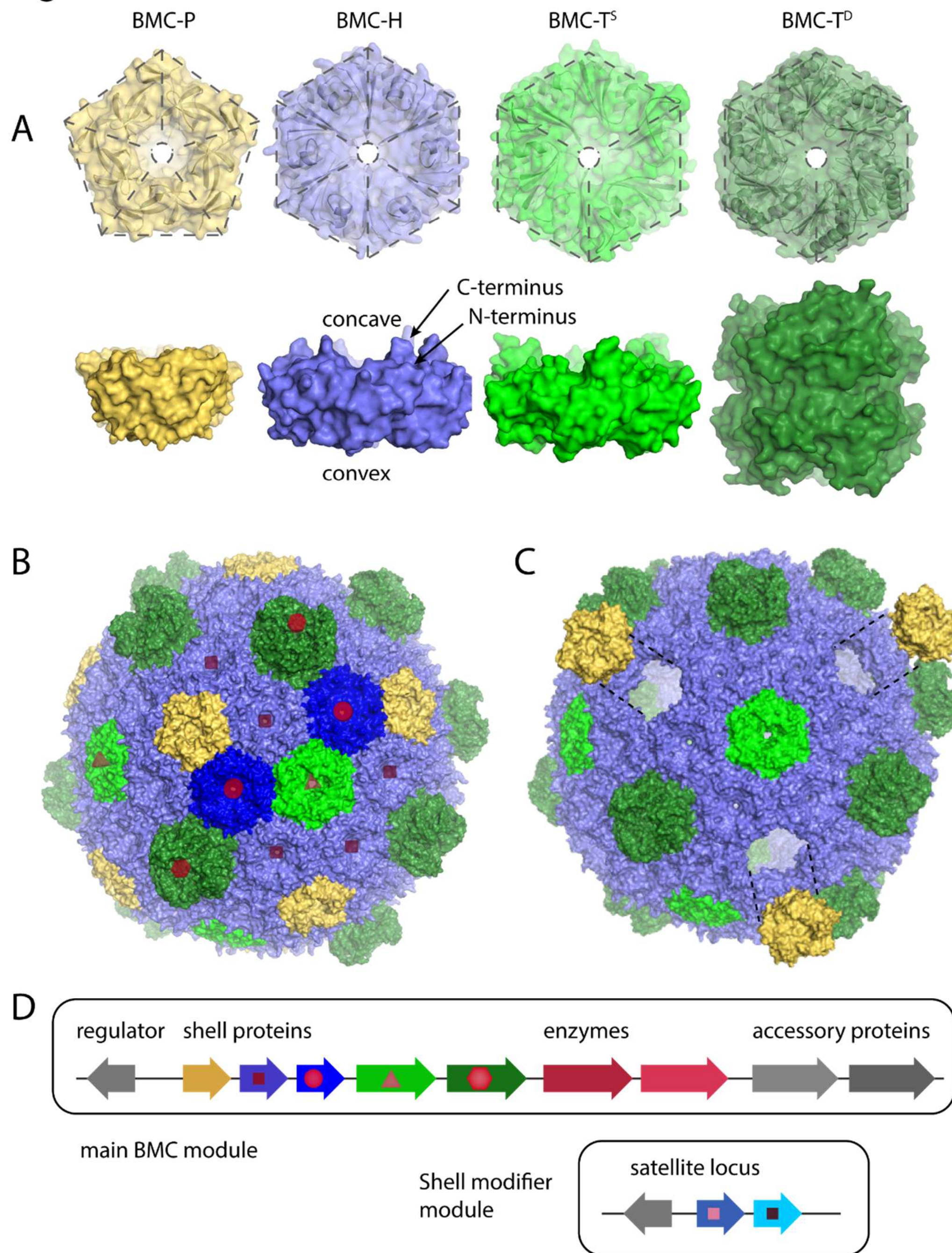
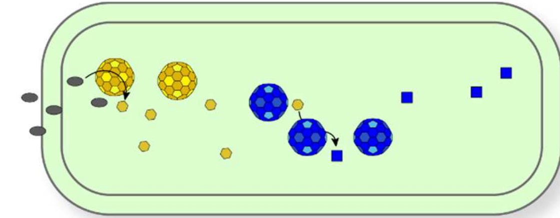
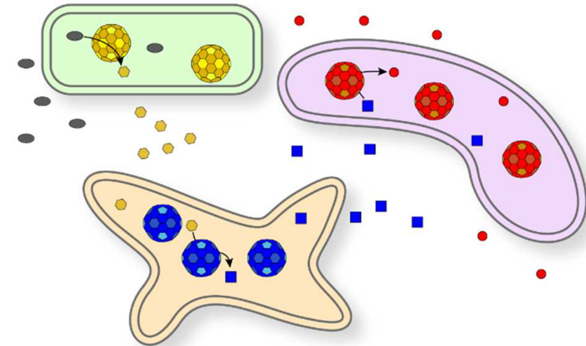


Figure 2

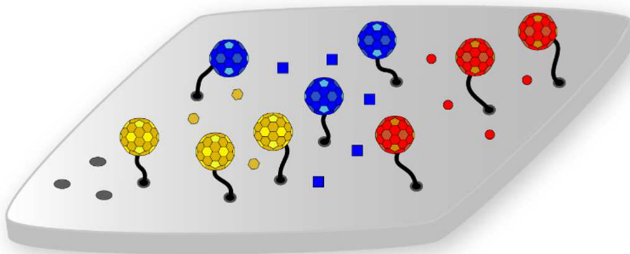
A Combinations of BMCs in a cell



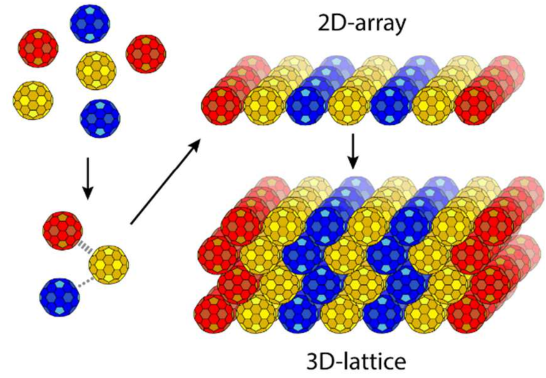
B Communities with different BMCs



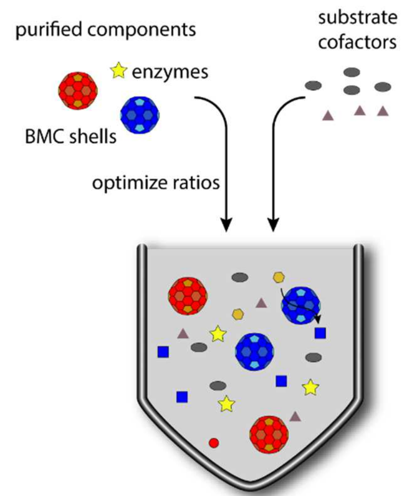
C BMC immobilization



D BMC lattice engineering



E Cell-free in vitro reactions



References

*special interest
**outstanding interest

1. Kerfeld CA, Aussignargues C, Zarzycki J, Cai F, Sutter M: **Bacterial microcompartments**. *Nat Rev Microbiol* 2018, **16**:277-290.
2. Rae BD, Long BM, Badger MR, Price GD: **Functions, compositions, and evolution of the two types of carboxysomes: polyhedral microcompartments that facilitate CO_2 fixation in cyanobacteria and some proteobacteria**. *Microbiol Mol Biol Rev* 2013, **77**:357-379.
3. Kerfeld CA, Melnicki MR: **Assembly, function and evolution of cyanobacterial carboxysomes**. *Curr Opin Plant Biol* 2016, **31**:66-75.
4. Turmo A, Gonzalez-Esquer CR, Kerfeld CA: **Carboxysomes: metabolic modules for CO_2 fixation**. *FEMS Microbiol Lett* 2017, **364**.
5. Kofoid E, Rappleye C, Stojiljkovic I, Roth J: **The 17-gene ethanolamine (*eut*) operon of *Salmonella typhimurium* encodes five homologues of carboxysome shell proteins**. *J Bacteriol* 1999, **181**:5317-5329.
6. Bobik TA, Havemann GD, Busch RJ, Williams DS, Aldrich HC: **The propanediol utilization (pdu) operon of *Salmonella enterica* serovar Typhimurium LT2 includes genes necessary for formation of polyhedral organelles involved in coenzyme B12-dependent 1,2-propanediol degradation**. *J Bacteriol* 1999, **181** 5967-5975.
7. Zarzycki J, Sutter M, Cortina NS, Erb TJ, Kerfeld CA: ***In Vitro* Characterization and Concerted Function of Three Core Enzymes of a Glycyl Radical Enzyme - Associated Bacterial Microcompartment**. *Sci Rep* 2017, **7**:42757.
8. Schindel HS KJ, McKinlay JB, Bauer CE.: **Characterization of a Glycyl Radical Enzyme Bacterial Microcompartment Pathway in Rhodobacter capsulatus.** *J Bacteriol* 2019, **201**:e00343-00318.
9. Jameson E, Fu T, Brown IR, Paszkiewicz K, Purdy KJ, Frank S, Chen Y: **Anaerobic choline metabolism in microcompartments promotes growth and swarming of Proteus mirabilis**. *Environ Microbiol* 2016, **18**:2886-2898.
10. Kalnins G, Cesle EE, Jansons J, Liepins J, Filimonenko A, Tars K: **Encapsulation mechanisms and structural studies of GRM2 bacterial microcompartment particles**. *Nat Commun* 2020, **11**:388.
11. Petit E, LaTouf WG, Coppi MV, Warnick TA, Currie D, Romashko I, Deshpande S, Haas K, Alvelo-Maurosa JG, Wardman C, et al.: **Involvement of a bacterial microcompartment in the metabolism of fucose and rhamnose by *Clostridium phytofermentans***. *PLoS One* 2013, **8**:e54337.
12. Erbilgin O, McDonald KL, Kerfeld CA: **Characterization of a planctomycetal organelle: a novel bacterial microcompartment for the aerobic degradation of plant saccharides**. *Appl Environ Microbiol* 2014, **80**:2193-2205.
13. Axen SD, Erbilgin O, Kerfeld CA: **A taxonomy of bacterial microcompartment loci constructed by a novel scoring method**. *PLoS Comput Biol* 2014, **10**:e1003898.
14. Ravcheev DA, Moussu L, Smajic S, Thiele I: **Comparative Genomic Analysis Reveals Novel Microcompartment-Associated Metabolic Pathways in the Human Gut Microbiome**. *Front Genet* 2019, **10**:636.
15. Urano N, Kataoka M, Ishige T, Kita S, Sakamoto K, Shimizu S: **Genetic analysis around aminoalcohol dehydrogenase gene of *Rhodococcus erythropolis* MAK154: a putative GntR transcription factor in transcriptional regulation**. *Appl Microbiol Biotechnol* 2011, **89**:739-746.

16. Mallette E, Kimber MS: **Structural and kinetic characterization of (S)-1-amino-2-propanol kinase from the aminoacetone utilization microcompartment of Mycobacterium smegmatis**. *J Biol Chem* 2018, **293**:19909-19918.
17. Kataoka M, Nakamura Y, Urano N, Ishige T, Shi G, Kita S, Sakamoto K, Shimizu S: **A novel NADP+-dependent L-1-amino-2-propanol dehydrogenase from *Rhodococcus erythropolis* MAK154: a promising enzyme for the production of double chiral aminoalcohols**. *Lett Appl Microbiol* 2006, **43**:430-435.
18. Kerfeld CA, Sawaya MR, Tanaka S, Nguyen CV, Phillips M, Beeby M, Yeates TO: **Protein structures forming the shell of primitive bacterial organelles**. *Science* 2005, **309**:936-938.
19. Klein MG, Zwart P, Bagby SC, Cai F, Chisholm SW, Heinhorst S, Cannon GC, Kerfeld CA: **Identification and structural analysis of a novel carboxysome shell protein with implications for metabolite transport**. *J Mol Biol* 2009, **392**:319-333.
20. Tanaka S, Kerfeld CA, Sawaya MR, Cai F, Heinhorst S, Cannon GC, Yeates TO: **Atomic-level models of the bacterial carboxysome shell**. *Science* 2008, **319**:1083-1086.
21. Drews G, Niklowitz W: **Beiträge zur Cytologie der Blaualgen. II. Zentroplasma und granuläre Einschlüsse von Phormidium uncinatum**. *Arch Mikrobiol* 1956, **24**:147-162.
22. Kinney JN, Salmeen A, Cai F, Kerfeld CA: **Elucidating essential role of conserved carboxysomal protein CcmN reveals common feature of bacterial microcompartment assembly**. *J Biol Chem* 2012, **287**:17729-17736.
23. Lawrence AD, Frank S, Newnham S, Lee MJ, Brown IR, Xue WF, Rowe ML, Mulvihill DP, Prentice MB, Howard MJ, et al.: **Solution structure of a bacterial microcompartment targeting peptide and its application in the construction of an ethanol bioreactor**. *ACS Synth Biol* 2014, **3**:454-465.
24. Aussignargues C, Paasch BC, Gonzalez-Esquer R, Erbilgin O, Kerfeld CA: **Bacterial microcompartment assembly: The key role of encapsulation peptides**. *Commun Integr Biol* 2015, **8**:e1039755.
25. Gonzalez-Esquer CR, Smarda J, Rippka R, Axen SD, Guglielmi G, Gugger M, Kerfeld CA: **Cyanobacterial ultrastructure in light of genomic sequence data**. *Photosynth Res* 2016, **129**:147-157.
26. Gonzalez-Esquer CR, Shubitowski TB, Kerfeld CA: **Streamlined Construction of the Cyanobacterial CO_2-Fixing Organelle via Protein Domain Fusions for Use in Plant Synthetic Biology**. *Plant Cell* 2015, **27**:2637-2644.
27. Lechno-Yossef S, Rohnke BA, Belza ACO, Melnicki MR, Montgomery BL, Kerfeld CA: **Cyanobacterial carboxysomes contain an unique rubisco-activase-like protein**. *New Phytol* 2020, **225**:793-806.
28. Cameron JC, Wilson SC, Bernstein SL, Kerfeld CA: **Biogenesis of a bacterial organelle: the carboxysome assembly pathway**. *Cell* 2013, **155**:1131-1140.
29. Parsons JB, Frank S, Bhella D, Liang M, Prentice MB, Mulvihill DP, Warren MJ: **Synthesis of empty bacterial microcompartments, directed organelle protein incorporation, and evidence of filament-associated organelle movement**. *Mol Cell* 2010, **38**:305-315.
30. Parsons JB, Dinesh SD, Deery E, Leech HK, Brindley AA, Heldt D, Frank S, Smales CM, Lunsdorf H, Rambach A, et al.: **Biochemical and structural insights into bacterial organelle form and biogenesis**. *J Biol Chem* 2008, **283**:14366-14375.
31. Lassila JK, Bernstein SL, Kinney JN, Axen SD, Kerfeld CA: **Assembly of robust bacterial microcompartment shells using building blocks from an organelle of unknown function**. *J Mol Biol* 2014, **426**:2217-2228.
32. Cai F, Bernstein SL, Wilson SC, Kerfeld CA: **Production and Characterization of Synthetic Carboxysome Shells with Incorporated Luminal Proteins**. *Plant Physiol* 2016, **170**:1868-1877.
33. Sutter M, Laughlin TG, Sloan NB, Serwas D, Davies KM, Kerfeld CA: **Structure of a Synthetic beta-Carboxysome Shell**. *Plant Physiol* 2019, **181**:1050-1058.

34. Sutter M, Greber B, Aussignargues C, Kerfeld CA: **Assembly principles and structure of a 6.5-MDa bacterial microcompartment shell**. *Science* 2017, **356**:1293-1297.
35. Greber BJ, Sutter M, Kerfeld CA: **The Plasticity of Molecular Interactions Governs Bacterial Microcompartment Shell Assembly**. *Structure* 2019, **27**:749-763 e744.
36. Liang M, Frank S, Lunsdorf H, Warren MJ, Prentice MB: **Bacterial microcompartment-directed polyphosphate kinase promotes stable polyphosphate accumulation in *E. coli***. *Biotechnol J* 2017, **12**:1600415.
37. Yung MC BF, Carpenter TS, Coleman MA.: **Re-directing bacterial microcompartment systems to enhance recombinant expression of lysis protein E from bacteriophage φX174 in Escherichia coli.** *Microb Cell Fact* 2017, **16**:71.
38. Huber I, Palmer DJ, Ludwig KN, Brown IR, Warren MJ, Frunzke J: **Construction of Recombinant Pdu Metabolosome Shells for Small Molecule Production in Corynebacterium glutamicum**. *ACS Synth Biol* 2017, **6**:2145-2156.
39. Jakobson CM, Kim EY, Slininger MF, Chien A, Tullman-Ercek D: **Localization of proteins to the 1,2-propanediol utilization microcompartment by non-native signal sequences is mediated by a common hydrophobic motif**. *J Biol Chem* 2015, **290**:24519-24533.
40. Wagner HJ, Capitain CC, Richter K, Nessling M, Mampel J: **Engineering bacterial microcompartments with heterologous enzyme cargos**. *Eng Life Sci* 2017, **17**:36-46.
41. Erbilgin O, Sutter M, Kerfeld CA: **The Structural Basis of Coenzyme A Recycling in a Bacterial Organelle**. *PLoS Biol* 2016, **14**:e1002399.
42. Juodeikis R, Lee MJ, Mayer M, Mantell J, Brown IR, Verkade P, Woolfson DN, Prentice MB, Frank S, Warren MJ: **Effect of metabolosome encapsulation peptides on enzyme activity, coaggregation, incorporation, and bacterial microcompartment formation**. *Microbiologyopen* 2020:e1010.
43. Lee MJ, Brown IR, Juodeikis R, Frank S, Warren MJ: **Employing bacterial microcompartment technology to engineer a shell-free enzyme-aggregate for enhanced 1,2-propanediol production in *Escherichia coli***. *Metab Eng* 2016, **36**:48-56.
44. Young EJ, Sakkos JK, Huang J, Wright JK, Kachel B, Fuentes-Cabrera M, Kerfeld CA, Ducat DC: **Visualizing in Vivo Dynamics of Designer Nanoscaffolds**. *Nano Lett* 2020, **20**:208-217.
45. Lee MJ, Mantell J, Brown IR, Fletcher JM, Verkade P, Pickersgill RW, Woolfson DN, Frank S, Warren MJ: **De novo targeting to the cytoplasmic and luminal side of bacterial microcompartments**. *Nat Commun* 2018, **9**:3413.
46. Hagen A, Sutter M, Sloan N, Kerfeld CA: **Programmed loading and rapid purification of engineered bacterial microcompartment shells**. *Nat Commun* 2018, **9**:2881.
47. Hagen AR, Plegaria JS, Sloan N, Ferlez B, Aussignargues C, Burton R, Kerfeld CA: **In Vitro Assembly of Diverse Bacterial Microcompartment Shell Architectures**. *Nano Letters* 2018.
48. Ferlez B SM, Kerfeld CA: **A designed bacterial microcompartment shell with tunable composition and precision cargo loading.** *Metab Eng* 2019, **54**:286-291.
49. Slininger Lee MF, Jakobson CM, Tullman-Ercek D: **Evidence for Improved Encapsulated Pathway Behavior in a Bacterial Microcompartment through Shell Protein Engineering**. *ACS Synth Biol* 2017.
50. Aussignargues C, Pandelia ME, Sutter M, Plegaria JS, Zarzycki J, Turmo A, Huang J, Ducat DC, Hegg EL, Gibney BR, et al.: **Structure and Function of a Bacterial Microcompartment Shell Protein Engineered to Bind a [4Fe-4S] Cluster**. *J Am Chem Soc* 2016, **138**:5262-5270.
51. Plegaria JS, Yates, M.D., Glaven, S.M. and Kerfeld, C.A: **Redox Characterization of electrode-immobilized Bacterial Microcompartment Shell Proteins Engineered to Bind Metal Centers.** *ACS Appl Bio Mater* 2020, **3**:685-692.

52. Pal C, Papp B, Lercher MJ: **Adaptive evolution of bacterial metabolic networks by horizontal gene transfer**. *Nat Genet* 2005, **37**:1372-1375.
53. Sommer M, Cai F, Melnicki M, Kerfeld CA: **beta-Carboxysome bioinformatics: identification and evolution of new bacterial microcompartment protein gene classes and core locus constraints**. *J Exp Bot* 2017, **68**:3841-3855.
54. Sommer M, Sutter M, Gupta S, Kirst H, Turmo A, Lechno-Yossef S, Burton RL, Saechao C, Sloan NB, Cheng X, et al.: **Heterohexamers Formed by CcmK3 and CcmK4 Increase the Complexity of Beta Carboxysome Shells**. *Plant Physiol* 2019, **179**:156.
55. Garcia-Alles LF RK, Maveyraud L, Aubry N, Lesniewska E, Mourey L, Zenobi R, Truan G.: **Occurrence and stability of hetero-hexamer associations formed by β-carboxysome CcmK shell components.** *PLoS One* 2019, **14**:e0223877.
56. Hartwell LH, Hopfield JJ, Leibler S, Murray AW: **From molecular to modular cell biology**. *Nature* 1999, **402**:C47-52.
57. Carroll SB: **Chance and necessity: the evolution of morphological complexity and diversity**. *Nature* 2001, **409**:1102-1109.
58. Lawson CE, Harcombe WR, Hatzenpichler R, Lindemann SR, Loffler FE, O'Malley MA, Garcia Martin H, Pfleger BF, Raskin L, Venturelli OS, et al.: **Common principles and best practices for engineering microbiomes**. *Nat Rev Microbiol* 2019, **17**:725-741.
59. Silverman AD, Karim AS, Jewett MC: **Cell-free gene expression: an expanded repertoire of applications**. *Nat Rev Genet* 2019.
60. Antonovsky N, Gleizer S, Noor E, Zohar Y, Herz E, Barenholz U, Zelcbuch L, Amram S, Wides A, Tepper N, et al.: **Sugar Synthesis from CO2 in Escherichia coli**. *Cell* 2016, **166**:115-125.

Annotated References

Kalnins, G., Cesle, E., Jansons, J. *et al.* Encapsulation mechanisms and structural studies of GRM2 bacterial microcompartment particles. *Nat Commun* **11, 388 (2020).
A combined structure and assembly study of the GRM2 metabolosome, a representative of the functionally diverse GRM family, that are found in pathogens and in environmentally important microbes.

Parsons JB, Frank S, Bhella D, Liang M, Prentice MB, Mulvihill DP, Warren MJ: **Synthesis of empty bacterial microcompartments, directed organelle protein incorporation, and evidence of filament-associated organelle movement. *Mol Cell* 2010, **38**:305-315
Comprehensive study of shell protein architectures that can be formed by heterologous expression of the PDU BMC shell proteins; this led to the formation of empty shells and to which cargo could be targeted.

Sutter M, Greber B, Aussignargues C, Kerfeld CA: **Assembly principles and structure of a 6.5-MDa bacterial microcompartment shell. *Science* 2017, **356**:1293-1297.
Structural description of an intact metabolosome shell.

** Liang M, Frank S, Lunsdorf H, Warren MJ, Prentice MB: **Bacterial microcompartment-directed polyphosphate kinase promotes stable polyphosphate accumulation in *E. coli***. *Biotechnol J* 2017, **12**:1600415.
Pioneering example of construction of an enzymatically active synthetic BMC for bioremediation.

*Axen SD, Erbilgin O, Kerfeld CA: **A taxonomy of bacterial microcompartment loci constructed by a novel scoring method**. *PLoS Comput Biol* 2014, **10**:e1003898.
Comprehensive survey of all BMCs identified in microbial genomes and their classification into functional types from the perspective of protein domains as constituent modules.

*Kinney JN, Salmeen A, Cai F, Kerfeld CA: **Elucidating essential role of conserved carboxysomal protein CcmN reveals common feature of bacterial microcompartment assembly**. *J Biol Chem* 2012, **287**:17729-17736
First report that the extensions that they name EPs have common sequence features and are found across functionally diverse BMC types. This description provides the foundation for developing EPs as encapsulation tags.

* Lawrence AD, Frank S, Newnham S, Lee MJ, Brown IR, Xue WF, Rowe ML, Mulvihill DP, Prentice MB, Howard MJ, et al.: **Solution structure of a bacterial microcompartment targeting peptide and its application in the construction of an ethanol bioreactor**. *ACS Synth Biol* 2014, **3**:454-465.
First example of the use of EPs for targeting to synthetic BMCs and confirmation of EP structural features predicted by Kinney et al.

*Greber BJ, Sutter M, Kerfeld CA: **The Plasticity of Molecular Interactions Governs Bacterial Microcompartment Shell Assembly**. *Structure* 2019, **27**:749-763 e744
Structural characterization of a synthetic beta carboxysome shell that demonstrates the shared architectural prinicpcles with metabolosome shells and that provides evidence for global regualtion of permeability properties.

Hagen A, Sutter M, Sloan N, Kerfeld CA: **Programmed loading and rapid purification of engineered bacterial microcompartment shells. *Nat Commun* 2018, **9**:2881.
First example of the use of structural principles of shell formation for precise loading of synthetic shells through use of orthogonal adaptor domains

*Ferlez B SM, Kerfeld CA: **A designed bacterial microcompartment shell with tunable composition and precision cargo loading.** *Metab Eng* 2019, **54**:286-291.
Through secondary structure permutation, the major protein of a synthetic shell system was reconfigured for terminal fusions to load cargo to the shell interior, revealing a surprising of tolerance of shell proteins to modification, without negatively impacting assembly.

* Juodeikis, R., M. J. Lee, M. Mayer, J. Mantell, I. R. Brown, P. Verkade, D. N. Woolfson, M. B. Prentice, S. Frank and M. J. Warren (2020). **Effect of metabolosome encapsulation peptides on enzyme activity, coaggregation, incorporation, and bacterial microcompartment formation.** Microbiologyopen: e1010.
Analysis of the effect of encapsulation peptides on cargo in the PDU system. Shows that aggregation of cargo is a major role for encapsulation peptides and that the size of the aggregate might influence BMC size.