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Dynamic structural determinants in bacterial microcompartment shells

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Bacterial microcompartments (BMCs) are polyhedral structures that segregate enzymatic cargo from the cytosol via encapsulation within a protein shell. Unlike other biological polyhedra, such as viral capsids and encapsulins, BMC shells can exhibit a highly advantageous structural and functional plasticity, conforming to a variety of anabolic (CO_2 fixation in carboxysomes) and catabolic (nutrient assimilation in metabolosomes) roles. Consequently, understanding the subunit properties and associated protein–protein interaction processes that guide shell assembly and function is a necessary step to fully harness BMCs as modular, biotechnological nanomachines. Here, we describe the recent insights into the dynamics of structural features of the key BMC domain (Pfam00936)-containing proteins, which serve as a structural template for BMC-H and BMC-T shell building blocks.

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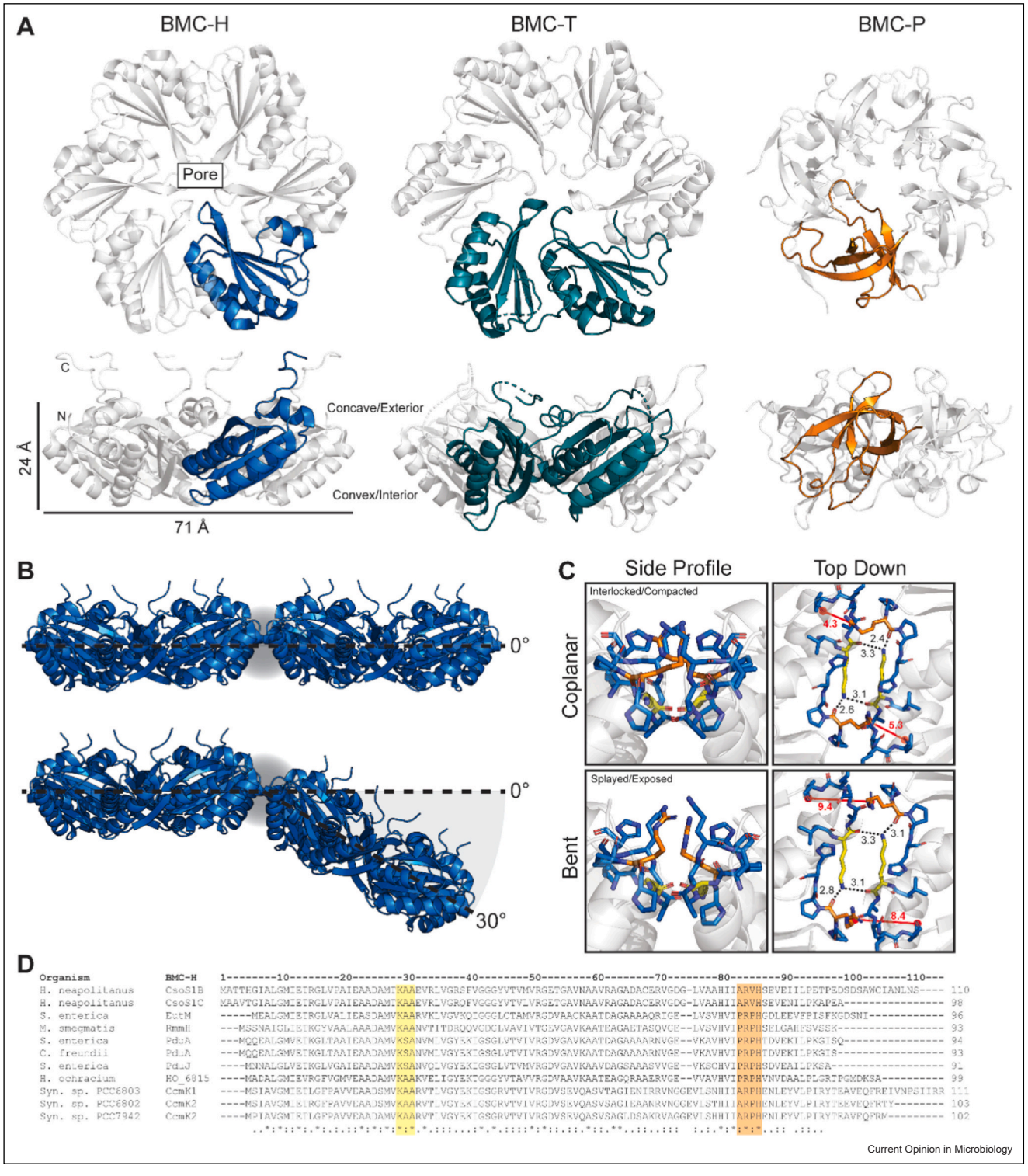
Introduction

Bacterial microcompartments (BMCs) are self-assembling protein-based analogs to eukaryotic organelles [1]. In BMCs, a defined fraction of cellular metabolism is organized into discrete particles and segregated from the cytosol. BMCs achieve this by building a selectively permeable protein shell that surrounds an enzymatic 'cargo'. The function of a BMC is defined by the content and biochemistry of the inner cargo and specifically its 'signature enzyme'; the two widespread classes are called carboxysomes and metabolosomes [2,3]. Carboxysomes are anabolic BMCs with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) as its signature enzyme, and they serve the role of carbon fixation in cyanobacteria and some chemoautotrophic and phototrophic bacteria [4,5]. Metabolosomes are catabolic BMCs with functionally diverse signature enzymes, and they support the assimilation of various niche metabolites in a range of bacterial phyla [3]. Regardless of their function, all BMCs rely on the formation of a structurally conserved, multimeric protein shell that has evolutionary adapted to support the function of the enzymatic core. Together, shell and core components constitute a 'metabolic module' supported by accessory proteins encoded in the same genetic module (operon) [6], which can be deployed and integrated into heterologous hosts' metabolism for biotechnological purposes [7–16].

The shells of BMCs are polyhedral, composed of facets made from the tessellation of self-assembling hexameric (BMC-H) and trimeric (BMC-T) oligomers (one or two Pfam00936 domains, respectively), and capped at the vertices by the pentameric (BMC-P) proteins (containing the Pfam03319 domain) [17–20] (Figure 1a). Conserved assembly principles dictate that shell subunits associate uniformly in their facet 'sidedness' (i.e. concave versus convex), which directly impacts the permeability properties of the shell [21]. Additionally, interaction with the shell interior is mediated by specific protein regions — encapsulation peptides or scaffold proteins [22–24]. The BMC architecture may invoke analogies to viral capsids, but they are evolutionarily and structurally distinct [25]. BMCs are also unique from other protein polyhedra, like encapsulins [26–28], in that they regularly demonstrate size and composition heterogeneity. In fully formed shells, the structure forms a barrier between the cytosol and interior cargo that can range from 20 to 400 nm in diameter. Alternative configurations have been reported as well, particularly for shell proteins expressed outside of their native context, such as sheets, nanotubes, nanocones, rosettes, and 'wiffle balls' [29–33]. Overall, BMC shells demonstrate a modular, structurally conserved, and tractable chassis that can be engineered to support an array of

Figure 1



(caption on next page)

The key proteins and interactions that guide shell assembly. **(a)** The shells of BMCs are composed of three classes of proteins: BMC-H (left), BMC-T (center), and BMC-P (right). Both BMC-H and BMC-T make up BMC facets and utilize the Pfam00936 domain (colored blue), with BMC-T containing two decoupled copies (colored teal). Meanwhile, BMC-P (Pfam03319, orange) occupy the vertex positions. All proteins are oriented with their concave sides facing the viewer. Protein Data Bank (PDB) structures 5DJB [31], 5DIH [69], and 5V76 [17] (left to right) were used to generate this panel. **(b)** BMC facets are composed of interlocking proteins that can be coplanar or bent relative to each other. The gray circled interfaces in **(b)** are visualized in **(c)**; these conformations maintain a specific hydrogen bonding network among their KAA motifs (K25, yellow) but differ in the position of a conserved arginine (R78, orange) in the PRPH motif. Hydrogen bond distances among the KAA motif (dashed black lines) are maintained in both coplanar and bent conformations. However, R78 can bridge the interface and dock into the adjacent hexamer, forming an interlock when coplanar, but it is flipped out of position when bent. This displacement is visualized as the distance (red solid line) from the central guanidinium carbon of R78 to the backbone oxygen of V29. The PDB structure 6N06 [35] was used to generate panels **(b)** and **(c)**. All panels were visualized using PyMOL. **(d)** The KAA (yellow highlight) and PRPH (orange highlight) motifs are broadly conserved across shell proteins from disparate organisms.

biochemically diverse functions. Notably, researchers are interested in leveraging these qualities and developing BMC platforms for biomanufacturing or enhanced carbon fixation applications [34–38].

Here, we review the aspects of BMC shells where structural flexibility impacts assembly, function, morphology, and hence their eventual re-design efforts. We define structural flexibility as any plasticity in the position or interaction of BMC components relative to each other. Mastering these structure–function relationships that stem from structural flexibility is critical towards establishing BMCs of programmable size, shape, and cargo capacity. Within this scope, interaction mechanisms in BMC shells are reviewed and discussed in the context of chimeric BMC shells, noncanonical shell architectures, disordered termini, and other functional polymorphisms.

Areas of structural flexibility in microcompartment shells

The bacterial microcompartment domain is tuned to support an array of interactions

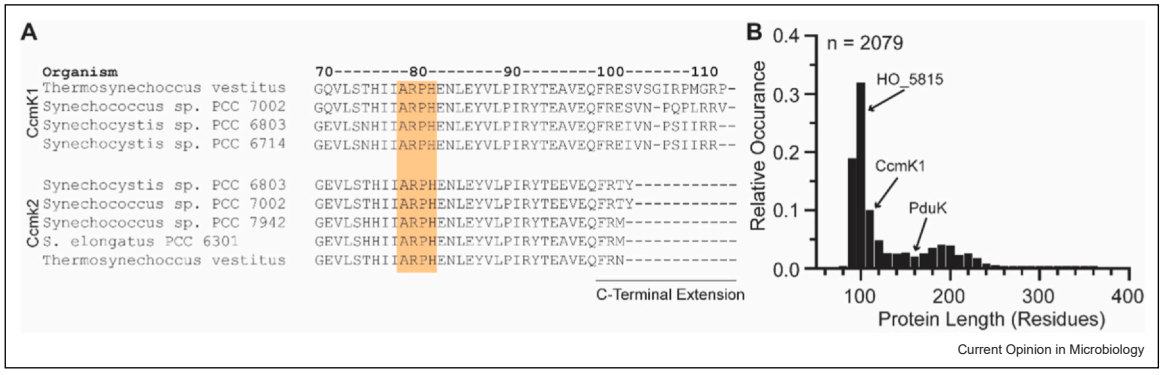
BMC shell assembly is supported by several key interactions that are conserved among all classes of shell proteins. Most critical among these are (1) shape complementarity and (2) a specific hydrogen bonding network supported by both the KAA and PRPH motifs along the outer edge of each subunit [17]. These motifs reside at the edge of the hexamer subunits, allowing interface formation between those adjacent subunits (Figure 1b). Utilization of an antiparallel hydrogen bonding network formed by the KAA motif (Figure 1c) is nearly universal between BMC-H and BMC-T and creates specific edge pairing [17]. In addition, the arginine in the PRPH motif has been observed physically bridging the edge interface and docking into the opposing hexamer in both BMC polyhedra [17] and in some crystal structures of BMC-H [39] depending on their crystal packing. This interlocking mechanism is, however, only observed when adjacent hexamers are coplanar (Figure 1b and c). Both coplanar and bent interactions demonstrate similar hydrogen bonding networks between their KAA motifs, forming a central hinge, and differ by the position of the PRPH arginine relative to a pocket on the adjacent hexamer formed largely by the backbone oxygen of surrounding residues (Figure 1c). The angles of non-coplanar BMC-H are likely confined by steric clashes along the concave/convex faces [40] as well as the combination of interactions by BMC domains locally. The KAA and PRPH motifs, which are central to these interactions, are broadly conserved across BMC-H (Figure 1d). Further structural diversity is conferred by BMC-T, which contain two genetically fused Pfam00936 domains and have been observed to host different interactions along alternating edges [41,42].

The shell proteins themselves can adjust to a range of edge interactions and other physical stresses by adopting polymorphisms distinct from the classic 'flat disk' representation. A subclass of naturally occurring BMC-H that are circularly permuted (BMC-H^P) has served as a model for studying these phenomena. For instance, wild-type EutS from *Salmonella enterica* has been shown to crystallize in a bent conformation [43], and CutR from *Streptococcus intermedius* has likewise been crystallized in several different screw conformations [44], though these might not occur in its solution states. Rationally permuted BMC-H can also change its oligomeric state from homohexameric to pentameric [45]. Similarly, the BMC-P EutN has been shown to crystallize as a homohexamer [46]. Despite their questionable physiological relevance, these instances spotlight the flexibility in quaternary structure that shell proteins can exhibit to adapt to their local environments; BMC-H^P proteins seem specifically poised for these roles. BMC-H^P abundancies are sparingly low both in stoichiometry (typically 1 hexamer per facet) [47] and within BMC loci (with loci having 0 or 1 copies) [3]. These observations, in combination with their polymorphic diversity, suggest that BMC-H^P play a unique role in supporting shell formation.

Observations of structural flexibility in bacterial microcompartment shells

The examples above account for the well-understood assortment of interactions that are mainly hardcoded into the Pfam00936 domain, common to all BMC-H and BMC-T proteins. However, beyond the Pfam00936 domain (~80 amino acids), we find known instances where flexibility prevails, such as prevalent C-terminal extensions of BMC-H proteins. These termini are predicted to be flexible [41,48] and display variety in length,

Figure 2



Carboxysome BMC-H proteins encode diverse C-terminal extensions. **(a)** The C-termini of CcmK1 and CcmK2 are compared across several cyanobacterial species. The C-termini of CcmK1 is longer than that of CcmK2 and conserved, reflecting an unknown physiological role. The PRPH motif is highlighted in orange. The alignment was performed in ClustalX. **(b)** While the single occurrence of a Pfam00936 domain that defines BMC-H proteins is ~80 residues, BMC-H display a wide range of sequence lengths. Three BMC-H are shown (HO_5815, CcmK1 from *Synechocystis* sp. PCC 6803, and PduK from *Salmonella enterica*). BMC-H sequences were acquired from Sutter et al. [3].

sequence composition, and, therefore, physiochemical properties (Figure 2b). Some termini have been connected to roles in cargo [49,50] and shell interactions [41], or metal binding [51], and it is tempting to speculate further about the potential for cytosolic interactions as well, as the C-termini of BMC-H proteins face the cytosol. In the case of BMC-H nanotubes assembled *in vitro*, it is suggested that diameter differences (from 20 to 70 nm) can be due to subtle sequence differences, including its disordered C-termini [40,52].

Similarly, while strictly a type of polymorphism, several BMC-T variants have been captured in conformations with central pores in open, closed, or mixed states for stacked BMC-T^D [43,53–55]. These pore conformations are correlated with global structure changes in synthetic BMC shells [42] presumably in response to ligand occupancy and/or environmental conditions. The physiological role of this gating has yet to be determined. It could simply be another, more controlled, mechanism for specific substrate permeation. However, the global structure changes that result from ligand binding suggest the ability to globally tense or relax the shell in response to environmental conditions.

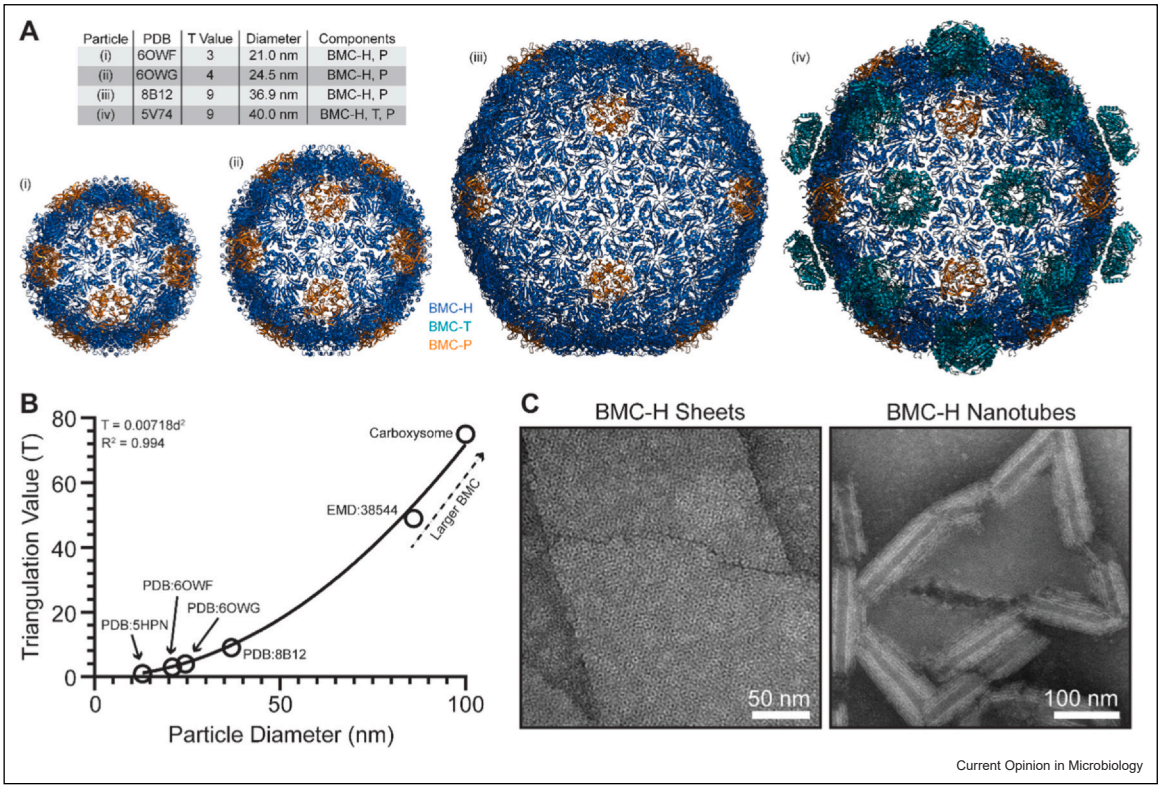
Functional consequences of shell flexibility

Diversity in size and composition of bacterial microcompartment shells

BMC shells have been observed occupying triangulation values from T = 3 (synthetic systems) [20,56] to well over 75 (natural systems) [57] (Figure 3a). Occasionally, the existence of multiple distinct BMC shell species within the same sample is described [18,20,33,56] indicating adaptable particle formation over a range of triangulation values (Figure 3b). The coexistence of distinct shell polymorphs perhaps owes to kinetic traps that may exist during the shell assembly process and less influenced by equilibrium [33,58]. It is also proposed that the distribution of pentamer packing influences stiffness of their local environment, thereby altering the allowable angle landscape in different assemblies [33]. This may explain why BMCs, while strictly requiring just one type of BMC-P for formation, sometimes occur as multiple paralogs [3,59]. Cargo-related factors can likewise influence shell assembly. For example, the C-terminus of the intrinsically disordered interior scaffold protein CsoS2 can promote the formation of larger α-carboxysome shells [60]. Modifying the copy number of the various domain repeats within the CsoS2 architecture is also an accessible route to alter particle morphology [61]. Cargo-filled shells, generally, have also been appreciated to exhibit larger sizes than when empty [62,63] regardless of loading methodology [64].

The conserved nature of shell protein interactions plays a role in the formation of numerous noncanonical structures. Specifically, BMC-H proteins have been observed to form sheets, nanotubes, nanocones, and rosettes both *in vivo* or *in vitro* at various scales [30,40,52,65–67] (Figure 3c). These structures can be amenable to rational manipulation. For example, introducing BMC-P to BMC-H can induce the formation of polyhedra over BMC-H nanotubes [68]. To permit electron transfer across shells, a [4Fe-4S] cluster [69] or a copper atom [70] has been installed in a BMC-T protein by leveraging the size and accessibility of the pore at the cyclic axis of symmetry. Buffer compositions can also have an effect, as pH and various ions have been observed to affect both size and assembly rate of BMC-H sheets [66], nanotubes [30], and micron-sized compartments formed when phase-separated droplets are used to template purified hexamers [71]. We expect

Figure 3



The shell components of BMC can form an array of structures. **(a)** Combinations of BMC shell proteins can lead to polyhedra of different sizes, defined by their triangulation number (T). BMC-H (blue), BMC-T (teal), and BMC-P (orange) in several cryogenic electron microscopy (cryo-EM) reconstructions are shown on a 1:1 scale. **(b)** While BMC shells can form a variety of sizes, these sizes are defined by the triangulation value (T) of the particle. T is proportional to the square of the particle diameter for icosahedra, and the values for known BMC can be used to anticipate the landscape of accessible sizes. **(c)** BMC-H can form noncanonical structures when overexpressed and purified. Transmission electron micrographs are shown demonstrating sheets and nanotubes formed by the BMC-H PduA.
These micrographs are adapted from Trettel and Winkler [52]. PDB: 6OWF and 6OWG from Ref. [20], 8B12 from Ref. [60], 5V74 from Ref. [17], 5HPN from Ref. [45], EMD:38544 from Ref. [84], Carboxysome from [85].

continued developments in shell manipulation, which will enable precise control over the rich structural variability offered by the BMC shell.

Intermixing of shell proteins can yield chimeric structures

As mentioned, all BMC shell proteins assemble largely through shape complementary and conserved hydrogen bonding networks provided by their KAA and PRPH motifs. Researchers have leveraged these principles to intermix or 'swap' hexamers to create BMC shell chimera with concomitantly altered substrate specificity [72,73]. Even different BMC-T^D can occupy degenerate positions in BMC shells [42]. While these occupancies are plastic, it is currently not clear during which stages of BMC assembly shell protein intermixing can take place. Atomic force microscopy of purified shell proteins shows that BMC-H sheets can dynamically remodel [66,74], and BMC-H nanotubes can likewise intermix when observed with confocal microscopy approaches [52]. Native BMCs have traditionally been accepted as largely static, but data from *in vivo* carboxysomes show that they too can dynamically remodel in response to environmental conditions [75,76], similar to *in vitro* BMC-H sheets. These lines of evidence suggest that BMC shell intermixing may occur at all stages of the BMC lifecycle and serve to dynamically regulate aspects such as size, composition, and perhaps even their positioning [77,78]. Along these lines, bacteria encoding for more than one BMC are known to heavily regulate these to avoid intermixing [79].

Heterohexamers as specialized modules to influence permeability

The plasticity in quaternary structure extends not only to homohexamers but also to heterohexameric subunits. These have been recently reported for carboxysome BMC-H proteins CcmK1/K2 (various ratios) and CcmK3/4 (1:2 ratio) [80,81]. These mixed oligomers have been speculated to regulate shell composition and permeation when incorporated into or stacked onto a

BMC shell, in response to osmolytes, salt, and pH. Regardless of their natural role, these naturally occurring shell building blocks reflect the ability to integrate asymmetric pore designs into synthetic BMC systems. Indeed, researchers have synthetically mimicked this by copying the organization of the BMC domain in BMC-T to make BMC-H fusions of two [82] or even three pfam00936 repeats [83] that integrate into BMC shells.

Conclusions and future outlook

BMC shell proteins demonstrate conserved and modular assembly principles that facilitate their analysis and redesign via plug-and-play approaches. The variety of structures demonstrated by BMC shell proteins may be leveraged for roles in biomanufacturing, bioelectronics, passivation, and therapeutics, as well as feature in cytoskeletal roles for scaffolding cellular metabolism. Computational advances, such as machine-learning and artificial intelligence pipelines, may begin to play an important role in identifying and predicting sequence features and physiological characteristics that lead to precise structural and functional control of native and synthetic BMCs. The manipulation of BMCs as Nature-inspired nanomachines designed to optimize reaction efficiency, increase final product/biomass yields, and decrease/sequester toxicity of molecules is a promising path to develop our burgeoning biotechnological industry.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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