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Protein stoichiometry, structural plasticity and regulation of bacterial microcompartments

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Bacterial microcompartments (BMCs) are self-assembling prokaryotic organelles consisting of a polyhedral proteinaceous shell and encapsulated enzymes that are involved in CO_2 fixation or carbon catabolism. Addressing how the hundreds of building components self-assemble to form the metabolically functional organelles and how their structures and functions are modulated in the extremely dynamic bacterial cytoplasm is of importance for basic understanding of protein organelle formation and synthetic engineering of metabolic modules for biotechnological applications. Here, we highlight recent advances in understanding the protein composition and stoichiometry of BMCs, with a particular focus on carboxysomes and propanediol utilization microcompartments. We also discuss relevant research on the structural plasticity of native and engineered BMCs, and the physiological regulation of BMC assembly, function and positioning in native hosts.

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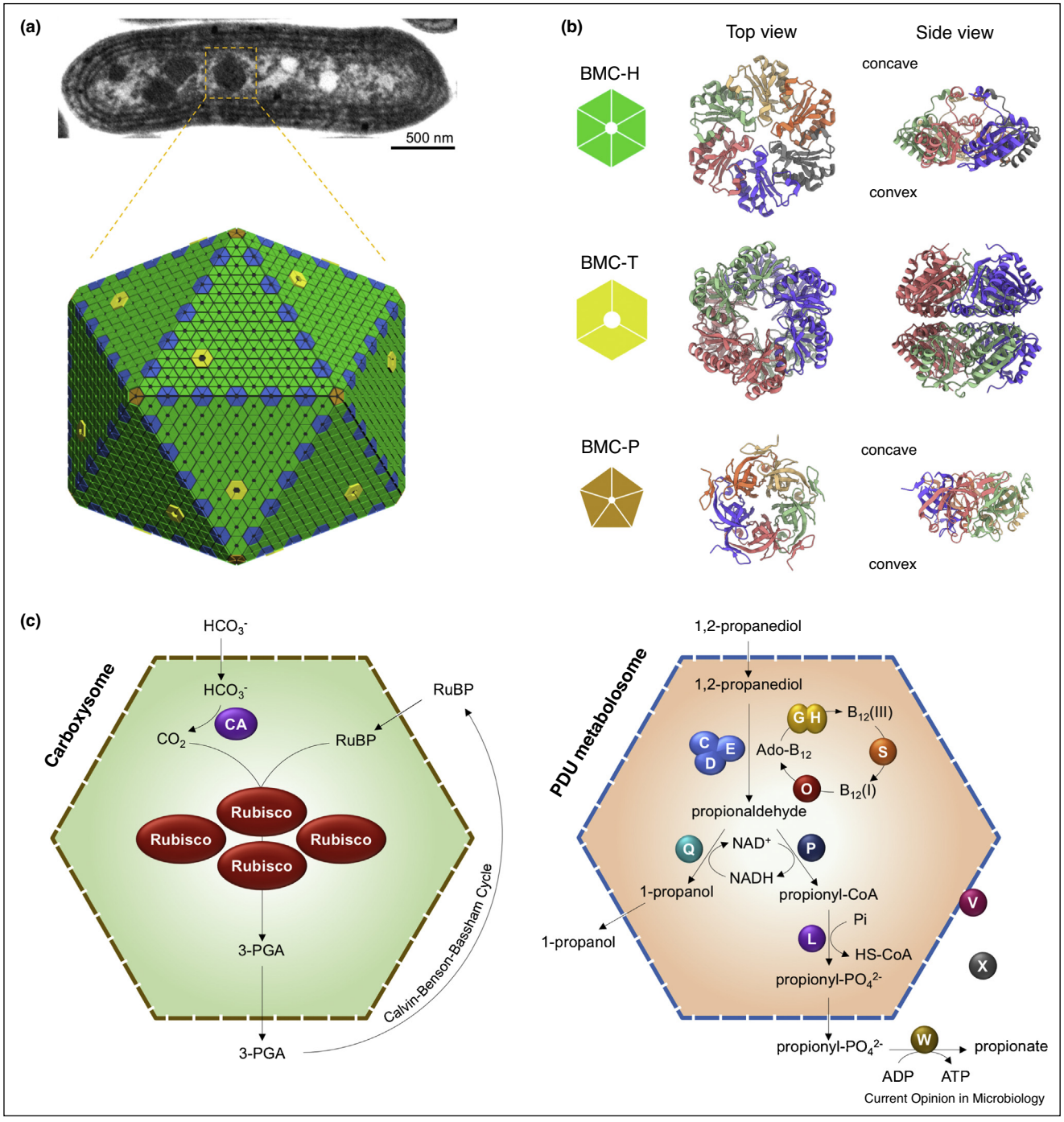
Introduction

Intracellular compartmentalization and self-assembly of proteins into large supercomplex structures underpin most biological activities in living organisms. Bacterial microcompartments (BMCs) are a paradigm of proteinaceous compartmentalizing organelles widespread in prokaryotes [1] (Figure 1). These nanoscale organelles (typically 100–400 nm in size) sequester key metabolic pathways in the cytoplasm to enhance metabolic performance. Bioinformatic analysis suggested that 23 types of BMC genetic operons or loci have been identified in up to 80% of the bacterial phyla [2]. An increasing number of new types of BMCs have been predicted in 45 phyla across diverse bacterial species [3]. All BMCs exhibit some common building principles: self-assembly, encapsulation, modularity, shell permeability, and structural plasticity.

(1) Self-assembly: BMCs consist of thousands of protein peptides, which are highly efficient in recognizing and interacting with each other to form the megadalton-sized organelles.
(2) Encapsulation: BMCs sequester multiple cargo enzymes that catalyze a series of biochemical reactions and toxic or volatile metabolic intermediates within an outer shell (Figure 1). This facilitates generation of a catalytically favorable microenvironment for the enclosed enzymes and pathways to enhance metabolism, enzyme stability and cooperation, and prevent unnecessary side reactions.
(3) Modularity: The BMC loci comprise contiguous or dispersed clusters of genes required for BMC formation, function and regulation (Figure 2). These genes encode BMC shell components and cargo enzymes, as well as ancillary proteins for protein/complex assembly, metabolite transporters, regulatory proteins, and cytoskeletal proteins likely required for intracellular partitioning. The shell structure is generally conserved among distinct BMCs and is constructed of a series of homologous shell proteins (Figure 1). Shell proteins exist mainly in three forms: hexamers (BMC-H, containing one Pfam00936 domain) and pseudohexameric trimers (BMC-T, with two Pfam00936 domains) that tile the shell facets, and pentamers (BMC-P, with one Pfam03319 domain) that cap the vertices of the polyhedral shell [1,4]. A Bacterial Microcompartment Database, MCPdb (https://mcpdb.mbi.ucla.edu/), has recently been developed to facilitate searching the structures of BMC proteins and assemblies [5].
(4) Shell permeability: The shell proteins are perforated by a central pore that varies in size, permitting selective passage of metabolites in and out of the BMC [6•,7•]. The concave side of shell proteins faces the cytoplasm and the convex side faces the BMC lumen [8] (Figure 1). These features are crucial for the shell semi-permeability to control the metabolic activities within the BMC.
(5) Structural plasticity: The structural variations of BMCs and flexible protein–protein interactions

Figure 1



Overview of bacterial microcompartments (BMCs).
(a) Electron microscopy of a bacterial cell showing BMC polyhedrons (top) and a schematic model of the icosahedral BMC structure (bottom). **(b)** Models and structures of the BMC-H (CcmK2, PDB ID 2A1B), BMC-T (CcmP, PDB ID 5LSR), and BMC-P (CcmL, PDB ID 2QW7) proteins that are the building components of the BMC shell. **(c)** Schematic representation of the functions of two representative BMCs: the carboxysome (anabolic BMC) and PDU metabolosome (catabolic BMC). The BMC shell encases signature cargo enzymes and prevents the escape of CO_2 or toxic propionaldehyde. Abbreviations: CA, carbonic anhydrase; 3-PGA, 3-phosphoglycerae; RuBP, ribulose 1,5-bisphosphate; Ado-B12, coenzyme B12 or adenosylcobalamin; HS-CoA, coenzyme A; B_{12}(I), cob(I)alamin; B_{12}(III), cob(III)alamin; Pi, inorganic phosphate.

Figure 2

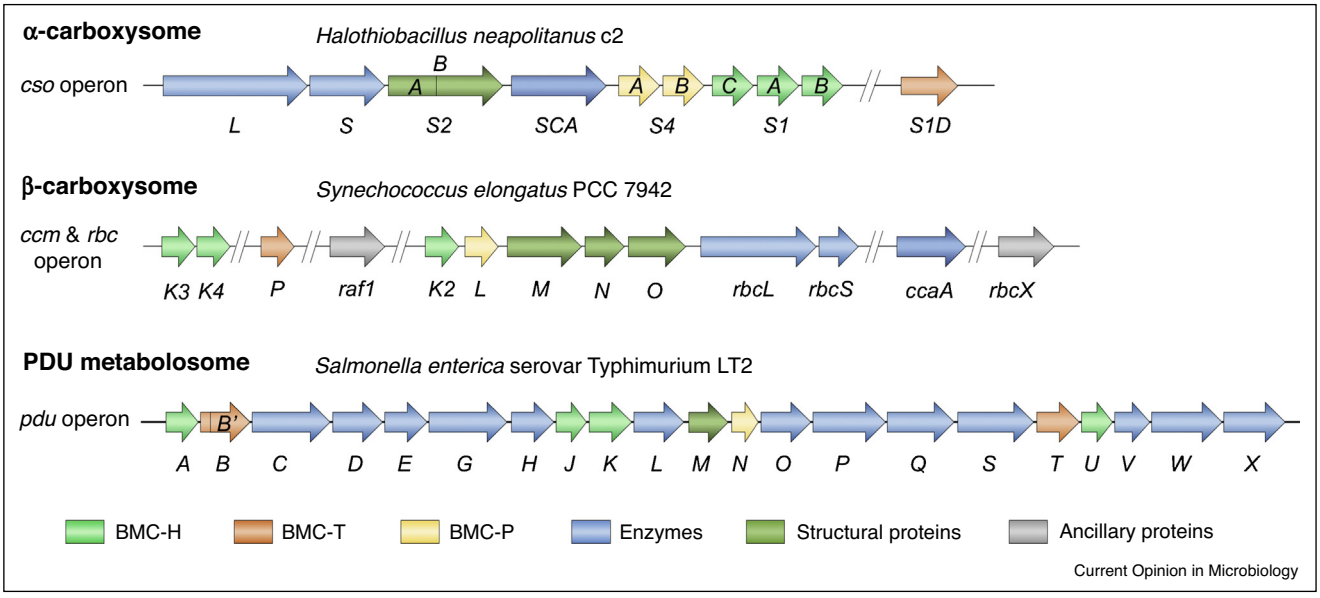


Diagram of the genomic organization of the α-carboxysome and β-carboxysome and PDU metabolosome operons in representative bacterial species.
Genes encoding structurally and/or functionally similar proteins are presented in the same colors. Double-slash lines represent gaps between separated BMC operons.

may enable fine tuning of BMC assembly and shell permeability in response to a varying environment (see details below).

These structural and regulatory features provide the framework for the metabolic factories to play pivotal roles in autotrophic CO_2 fixation and catabolic processes and promoting bacterial fitness in specific environmental niches [9]. Moreover, they hold the promise for rationally repurposing BMC structures in various applications in synthetic biology [10,11], such as biofuel production [12••,13].

According to their distinct functions, BMCs can be categorized into anabolic BMCs (carboxysomes) and catabolic BMCs (metabolosomes). The carboxysome is the central CO_2-fixing organelle in all cyanobacteria and many chemoautotrophs. The metabolosomes degrade diverse carbon substrates in heterotrophs; the experimentally characterized metabolosomes include propanediol utilization (PDU), ethanolamine utilization (EUT), glycyl radical enzyme-associated microcompartments (GRM), choline utilization (GRM2), fucose and rhamnose utilization (GRM5 and PVM), and 1-amino-2-propanol utilization (RMM) metabolosomes [14,15].

Recent technological advances in structural biology, microscopy, synthetic biology, proteomics, bioinformatics and computational modeling provide an unprecedented opportunity to understand the assembly principles of BMCs [3,8,12••,13,16,17,18•,19•,20,21••,22••,23••,24••]. In this review, we will focus on the recent advances in elucidating the composition, stoichiometry, structural plasticity and physiological regulation of BMCs, in particular the carboxysomes and PDU metabolosomes.

Protein composition and stoichiometry of BMCs

β-carboxysome protein stoichiometry

The stoichiometric ratios of different building components and their interactions are key factors in driving the assembly and architecture of BMCs [17]. However, we still have limited knowledge about the actual protein composition and stoichiometry of BMCs. Cyanobacterial carboxysomes were the first discovered BMCs by electron microscopy (EM). Carboxysomes encapsulate carbonic anhydrase (CA) and the primary carboxylating enzymes, ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco). Bicarbonate (HCO_3^-) in the cytosol can diffuse across the shell through the central pores of shell proteins, and is then converted to CO_2 by CA; the shell can prevent unwanted entry of O_2 and diminish CO_2 leakage into the cytosol. These mechanisms ensure the development of a CO_2-rich and oxidizing microenvironment within the carboxysome to improve Rubisco carboxylation [25]. Recently, increasing efforts have focused on building carboxysomes in heterologous organisms to boost CO_2 fixation and cell growth [26,27,28••,29].

Based on the forms of enclosed Rubisco, carboxysomes can be divided into two different classes: α-carboxysomes and β-carboxysomes. The β-carboxysome of the rod-shaped cyanobacterium *Synechococcus elongatus* PCC7942 (Syn7942) has been extensively characterized. The Syn7942 β-carboxysome shell is formed by BMC-H proteins (CcmK2, CcmK3, CcmK4) that tile the shell facets, the BMC-P protein CcmL that occupies the vertices of the polyhedron, and the BMC-T proteins (CcmO, CcmP). The core enzymes involve β-type CA (CcaA) and the key CO_2-fixing enzyme Rubisco (comprising RbcL and RbcS, denoted as $RbcL_8S_8$) (Figure 1). Assembly of functional Rubisco and β-carboxysomes also requires ancillary proteins, such as Rubisco assembly factor 1 (Raf1) and RbcX [21••,30].

To determine the exact stoichiometry of building components in the β-carboxysome, Sun *et al.* tagged a collection of carboxysome proteins (CcmK3, CcmK4, CcmL, CcmM, CcmN, RbcL, CcaA, RbcX) with fluorescent proteins, and then counted the copy numbers of these proteins in single β-carboxysomes by quantifying the discrete bleaching steps of tagged fluorescent proteins using single-molecule fluorescence microscopy [22••]. The research revealed that the internal enzyme Rubisco is the most abundant component among all β-carboxysome proteins (853 copies, under moderate light, Table 1). The Rubisco content of the β-carboxysome is up to two folds greater than that of the α-carboxysome [31], consistent with the highly dense packing of Rubisco within the β-carboxysome [32]. The second most abundant protein is CcmM (∼700 per β-carboxysome), which serves as a linker protein binding Rubisco to the shell via the recruitment protein CcmN and induces phase separation into a liquid-like Rubisco matrix [19•]. Protein quantification also offered the unique opportunity to evaluate the specific stoichiometric ratios of different carboxysome proteins, such as CcmK4 and CcmK3, which may be functionally correlated at the physiological context. Recently, CcmK4 and CcmK3 have been indicated to form hetero-hexamers with a 1:2 stoichiometry in the β-carboxysome [33•].

PDU metabolosome protein stoichiometry

The majority of BMCs are the metabolosomes that are found in a variety of bacteria and archaea including human gut microbes. The functionally distinct metabolosomes share universal biochemical reactions catalyzed by a signature enzyme, an aldehyde dehydrogenase, an alcohol dehydrogenase, and a phosphotransacylase. In the model pathogen *Salmonella enterica* serovar Typhimurium LT2 (*S.* Typhimurium LT2), the PDU metabolosome is constructed by 22 different types of proteins that are encoded by genes clustered in a single *pdu* operon (Figure 2). The core enzymes include diol dehydratase (PduCDE), phosphotransacylase (PduL), aldehyde dehydrogenase (PduP), alcohol dehydrogenase (PduQ), and propionate kinase (PduW). The signature enzyme PduCDE catalyzes the conversion of 1,2-propanediol (1,2-PD) to propionaldehyde, which is then converted to propionyl coenzyme A (propionyl-CoA) or 1-propanol by PduP or PduQ, respectively. PduL catalyzes the conversion of propionyl-CoA to propionyl-phosphate, which is then converted into propionate by PduW to generate ATP. There are also other enzymes involved in the 1,2-PD metabolism, such as cobalamin reductase (PduS), adenosyltransferase (PduO), diol dehydratase reactivase (PduGH), and L-threonine kinase (PduX) for the reactivation of diol dehydratase and vitamin B_{12} recycling.

To evaluate the accurate protein composition and stoichiometry of PDU metabolosomes, Yang *et al.* used mass spectrometry-based absolute quantification and a QconCAT (concatamer of standard peptides for absolute quantification) strategy to characterize the isolated PDU metabolosomes from *S.* Typhimurium LT2 [24••]. Unlike the β-carboxysome in which the cargo enzyme Rubisco is the predominant component, the most abundant PDU element is the BMC-H shell protein PduJ, which accounts for over 44% of all PDU proteins (Table 1). This suggested a higher ratio of shell/cargo proteins and a relatively less crowded internal environment of PDU metabolosomes than those of the β-carboxysome [22••]. As a comparison, in the PDU metabolosomes from *Citrobacter freundii*, PduB' appeared as the most abundant protein (31%) and PduJ only accounted for 18% of all PDU proteins [34]. This discrepancy probably implied the species-dependent variation of the PDU metabolosome protein stoichiometry. Protein quantification analysis also indicated the stoichiometric links of shell and cargo proteins, implicating their physiological coordination (Table 1). For example, the ratio of the trimeric shell protein PduB to the PduCDE dimer was roughly 1:1, and the ratio of the PduT trimer to the cargo PduS was 2:1. The physical associations of the minor proteins PduV, PduW and PduX with the PDU metabolosome were characterized using live-cell confocal imaging [24••].

Structural plasticity of BMCs

Unlike the robust and regular BMC structures that we used to think, more experimental results have shed light on the plasticity of natural BMC architectures. The biosynthesis and structures of β-carboxysomes in Syn7942 are highly regulated in response to environmental growth conditions. EM and fluorescence imaging showed that the size of β-carboxysomes and the abundance of individual proteins in the β-carboxysome could be adaptively modulated in response to changes in light intensities and CO_2 availabilities [22••] (Figure 3, Table 1).

The BMC architectures are morphologically heterogeneous and vary in size and shape in their native hosts.

Table 1

Protein composition and stoichiometry of the Syn7942 β-carboxysome [22••] and *S.* Typhimurium LT2 PDU metabolosme [24••]

β-carboxysome

Protein	Description & function	Structure	Number of oligomers/monomers per BMC			
			Air/ML	CO_2/ML	LL	HL
CcmK3	Minor shell proteins likely forming CcmK3/4 heterohexamers and CcmK4 homohexamer to tune shell permeability	BMC-H	15 ± 25	29 ± 14	14 ± 5	14 ± 9
CcmK4		BMC-H	52 ± 32	94 ± 44	52 ± 20	51 ± 16
CcmL	Minor shell vertex protein, required for proper carboxysome assembly	BMC-P	7.4 ± 3.4	13.2 ± 4.8	6.8 ± 3.0	13.8 ± 4.8
CcmM	Linker protein that induces Rubisco condensation, interacts with CcmN to the shell	Monomer	719 ± 1433	468 ± 425	483 ± 366	1176 ± 691
CcmN	Structural protein, interact with CcmM and the shell proteins CcmK	Monomer	74 ± 51	52 ± 28	51 ± 20	82 ± 34
Rubisco	Key CO_2-fixation enzyme	$RbcL_8S_8$	853 ± 1150	550 ± 832	367 ± 687	1507 ± 648
CcaA	Carbonic anhydrase, encapsulated enzyme in carboxysomes for the conversion of HCO_3^- to CO_2	Hexamer	14 ± 14	21 ± 14	11 ± 4	20 ± 10
RbcX	Rubisco chaperone, required for proper carboxysome functions	Dimer	20 ± 16	19 ± 5	20 ± 5	20 ± 5

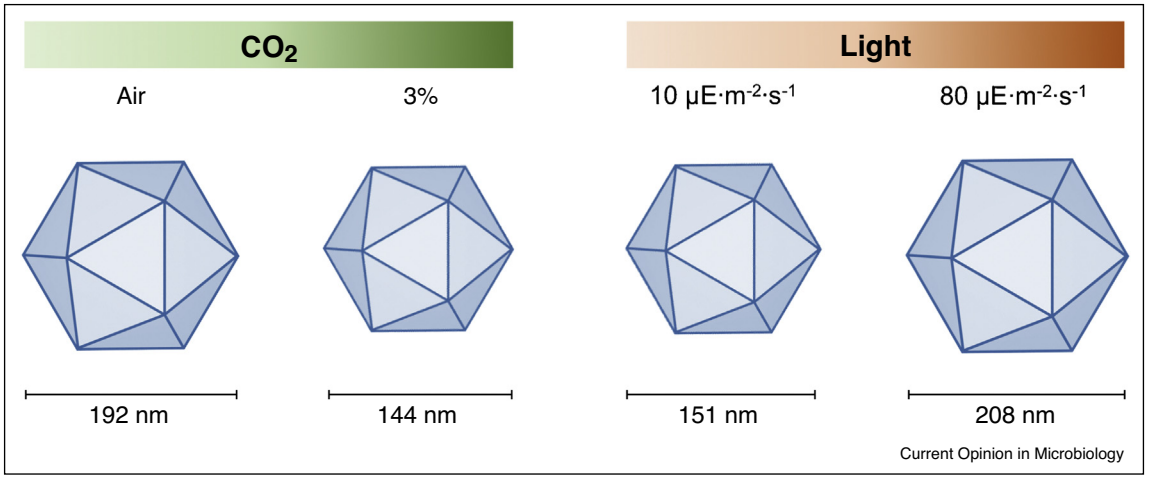
PDU metabolosome

Protein	Description & function	Structure	Number of oligomers/monomers per BMC	
			WT	Δ*pduA*
PduA	Major shell protein, involved in selective molecular transport and interaction with PduP	BMC-H	307 ± 17	2 ± 0
PduB	Major shell protein, involved in shell and cargo binding	BMC-T	224 ± 14	52 ± 2
PduB'	major shell protein, not essential for the assembly of PDU metabolosomes	BMC-T	278 ± 17	52 ± 4
PduJ	major shell protein, essential for the assembly and function of PDU metabolosomes, interact with PduP	BMC-H	869 ± 72	1200 ± 148
PduK	minor shell protein, involved in spatial organization of PDU metabolosomes	BMC-H	86 ± 7	97 ± 7
PduM	Structural protein, essential for the assembly and function of PDU metabolosomes	unknown	56 ± 9	50 ± 7
PduN	Minor shell protein, occupy the vertex of shell	BMC-P	12 ± 1	12 ± 2
PduT	Minor shell protein, interact with PduS for electron transport	BMC-T	96 ± 6	92 ± 9
PduU	Minor shell protein, not essential for the assembly and function of PDU metabolosomes	BMC-H	22 ± 3	18 ± 2
PduC	Subunits of diol dehydratase, the N-terminus of PduD acts as an encapsulation peptide	Dimer $(\alpha\beta\gamma)_2$	272 ± 40	126 ± 11
PduD			212 ± 23	104 ± 12
PduE			188 ± 28	102 ± 4
PduG	Subunits of diol dehydratase reactivase	unknown	76 ± 9	81 ± 10
PduH			38 ± 4	43 ± 4
PduL	Phosphotransacylase, the N-terminal region acts as an encapsulation peptide	Dimer	16 ± 1	16 ± 2
PduO	Adenosyltransferase	unknown	146 ± 15	118 ± 7
PduP	Aldehyde dehydrogenase, the N-terminal region acts as an encapsulation peptide	unknown	214 ± 31	255 ± 29
PduQ	Alcohol dehydrogenase	unknown	145 ± 12	114 ± 14
PduS	Cobalamin reductase	unknown	49 ± 4	48 ± 6
PduV	Sequence similar to Ras-like GTPase superfamily, connecting with filament-associated PDU metabolosome movement	unknown	7 ± 2	6 ± 2

ML, moderate light; LL, low light; HL, high light [22••]. The proteins with unknown structures are considered as monomers to show the stoichiometry.

Nanoindentation based on atomic force microscopy (AFM) demonstrated that the β-carboxysome architecture is mechanically softer than virus capsids, representing a mechanical signature of the BMC shells [32]. The absence of specific building components could also result in BMC structural remodeling, such as the elongated BMCs when lacking BMC-P at the vertices [35,36]. In the PDU metabolosome, deleting the major shell protein PduA resulted in the altered abundance of shell proteins (such as the rising content of the shell protein PduJ) and internal enzymes, and thus the modified metabolic activities [24••]. The results indicated the redundant roles of

Figure 3



Structural variations of β-carboxysomes in Syn7942 in response to changes in the CO_2 levels and light intensities during cell growth [22••].

PduA and PduJ in retaining the assembly and overall architecture of PDU metabolosomes [37•].

The structural plasticity of BMCs also occurred in specific protein–protein interactions. Experiments have suggested that disordered scaffolding proteins (CcmM35 in the β-carboxysome and CsoS2 in the α-carboxysome) drive Rubisco coalescence in the cytoplasm via weak and transient multivalent interactions [18•,19•], promoting carboxysome assembly via liquid–liquid phase separation. Additionally, BMCs contain several paralogs of shell proteins that are structurally and functionally correlated to each other. It was proposed that CcmK3 and CcmK4 of Syn7942 could form heterohexamers in a pH-dependent manner, with a 2:4 stoichiometry [33•]. Similar CcmK3–CcmK4 heterohexamers were suggested to exist in the *Synechocystis* sp. PCC 6803 β-carboxysomes [38], implying a general principle that may alter the β-carboxysome shell structure and permeability. Another fashion to tune the molecule passage across the shell has been proposed by the dynamic 'capping' of BMC-P and BMC-H shell proteins in the BMC shells [33•,39]. Consistently, high-speed AFM has visualized the dynamic self-assembly and protein–protein interactions of BMC shell proteins [40], and has revealed that the self-assembly dynamics of shell facets is sensitive to environmental changes [41]. Although we still do not fully understand the underlying molecular mechanisms, these assembly and modular properties may play roles in the intrinsic regulations of shell assembly and permeability and the structural remodeling of BMCs at multiple levels.

In the context of reconstituted shells, both large α-carboxysome shells (~100 nm in diameter) [12••] and *Klebsiella pneumoniae* GRM2 BMC minishells expressed in *Escherichia coli* exhibited marked structural variations [42,43••]. Characterization of the reconstituted *Haliangium ochraceum* BMC shells of ~40 nm in diameter identified the structural plasticity of protein–protein interactions, which are subject to the local and global structural variations of the synthetic shells [44••]. The structural flexibility of shell structures has important implications on the variations of native BMC structures and the tuning mechanism of shell permeability.

Regulation of BMC biosynthesis and intracellular positioning

BMC biosynthesis, structure and function are physiologically integrated into the metabolic and regulatory networks of native host cells. Live-cell fluorescent imaging revealed that the increase in light intensity during cell growth could stimulate the biosynthesis of carboxysomes, represented by the increased numbers of carboxysomes per cell, and the enhanced carboxysomes CO_2-fixing activities [45]. This regulation is closely correlated with the redox states of the photosynthetic electron transport chain. The intracellular localization and CO_2-fixing activities of β-carboxysomes in Syn7942 cells were further demonstrated to be actively modulated under diurnal light-dark cycles that mimicked the natural growth conditions of cyanobacterial cells [46]. Deletion of the circadian clock protein KaiA altered the number of carboxysomes per cell and carboxysome localization, highlighting the role of the circadian clock in governing carboxysome biosynthesis and positioning in cyanobacteria [46].

The intracellular spatial positioning and regulation of carboxysomes are crucial for cell metabolism and growth. It was proposed that the cell poles play important roles in β-carboxysome and degradation of inactive or damaged β-carboxysomes in cyanobacteria [23••,47]. Moreover, equal segregation of β-carboxysomes between daughter

cells is required to retain carboxysome inheritance during cell division [48]. The specific localization of carboxysomes within cyanobacterial cells was suggested to be mediated by interactions with cytoskeleton components such as ParA [48] (also termed McdA [49]). Recently, a McdAB system was identified to determine β-carboxysome partitioning in Syn7942, through the interactions of McdB with both carboxysomes and McdA [49]. It was further indicated that the McdAB systems exist among β-cyanobacteria that possess β-carboxysomes [50]. Recently, the McdAB-like system has also been identified in α-carboxysome-containing proteobacteria, suggesting a common mechanism underlying the *in vivo* positioning of both α-carboxysomes and β-carboxysomes [51]. This mechanism might be extendable to the subcellular positioning of other BMCs across the bacterial kingdom.

Conclusions

The natural self-assembling features of BMCs and their significance in metabolic enhancement have attracted increasing interest in fundamental understanding of protein self-assembly and repurposing BMC structures for diverse biotechnological purposes. Advanced understanding of BMC protein stoichiometry, structural plasticity and regulation spotlighted the variations and tunability of native BMC structure and function, and the prospects for rational design and reprograming of BMCs for specific functions in a controllable manner. It would be interesting to explore the diverse mechanisms that govern the functional stoichiometry and assembly of different types of BMCs. In addition, understanding the protein composition and the roles of individual components of BMCs has fostered synthetic engineering of BMCs and shell structures with the 'minimal' composition [8,27,43••,44••,52•]. Future efforts can focus on how to select the minimal required building components and encapsulation strategies and how to adjust the stoichiometric ratios of distinct components and protein–protein interactions, to obtain specific BMC structures, efficient cargo encapsulation and programable shell permeability. To manipulate the functional performance of engineered BMC structures, we also need to consider how to ensure their functional integrity in the metabolic and regulatory networks of the heterologous hosts. This may involve genetic engineering and modulation of necessary auxiliary proteins, regulatory factors and other cellular components.

Conflict of interest statement

Nothing declared.

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