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Title: Biotechnological advances in bacterial microcompartment technology

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Abstract

Bacterial microcompartments (BMCs) represent proteinaceous macromolecular nano-bioreactors that are found in a broad range of bacteria, and which are associated with either anabolic or catabolic processes. They consist of a semi-permeable outer shell that packages a central metabolic enzyme or pathway, providing both enhanced flux and protection against toxic intermediates. Recombinant production of BMCs has led to their repurposing with the incorporation of altogether new pathways. Deconstruction of BMCs into their component parts has shown that some individual shell proteins self-associate into filaments that can be further modified into a cytoplasmic scaffold to which enzymes/proteins can be targeted. BMCs therefore represent a modular system that is highly suited for the engineering of biological systems for useful purposes.

Bacterial microcompartment structure and function.

One of the defining differences between prokaryotes and eukaryotes is the presence of specific organelles and subcellular structures in the cytoplasm of the latter [1]. The advantages of compartmentalisation are multiple and include an ability to provide a physical barrier for part of the cell allowing separation from the cytoplasmic milieu, the generation of a microenvironment to suit specific biochemical processes and an intracellular location to which material can be addressed. It is therefore often quite a surprise for many to learn that a broad range of bacteria also have specialised cytoplasmic structures or organelles (Figure 1), the most common of which are called **bacterial microcompartments** (BMCs) (see Glossary) [2-4]. Although BMCs are not lipid-bound they are, however, surrounded by a semi-permeable proteinaceous shell to generate polygonal structures that encase a specific metabolic process [5]. The genetic software encoding these large macromolecular structures is often found arranged in a modular fashion within operons. This not only assists in their identification through data mining but this information can also be used to help develop and adapt these bioreactors in a plug and play fashion for useful purposes.

There are two basic types of BMCs reflecting their participation in anabolic or catabolic processes, which are termed **carboxysomes** and **metabolosomes** respectively (Figure 1) [2]. The anabolic carboxysomes are involved in carbon fixation, utilising an encapsulated carbonic anhydrase and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Figure 1) [6, 7]. Carboxysomes are divided into two distinct classes that are named according to the form of encapsulated RuBisCO, with α-carboxysomes containing 1A RuBisCO and β-carboxysomes containing 1B RuBisCO. Whilst these subtypes share functionality, they vary in their structural components and mode of assembly (discussed below). The catabolic metabolosomes are often associated with the breakdown of mucosal-derived metabolites such as fucose, propanediol, choline and ethanolamine and contain multistep pathways that involve the conversion of the metabolite into an aldehyde by a **signature enzyme** and its subsequent disproportionation into an alcohol and acid (Figure 1) [2]. In this respect metabolosomes display much greater diversity comprising 27 of the 30 recently discovered BMC loci [8]. The best studied metabolosome is the propanediol utilisation (Pdu) system [9], although a number of other BMCs have also been characterised including those associated with

ethanolamine, ethanol and choline utilisation as well as rhamnose and fucose degradation [10-13]. Many metabolosomes appear to have the capability to acquire and recycle cofactors internally, including ATP, NAD^+, CoA, adenosylcobalamin and S-adenosylmethionine [8, 14]. The most recent bioinformatics approach undertaken on BMCs has revealed the presence of 23 distinct BMC types with 30 distinct loci across 23 bacterial phyla, with both anabolic and catabolic functionality [8]. These can be separated into 8 main subtypes many of which remain experimentally uncharacterised.

The best way to visualise BMCs is by transmission electron microscopy (TEM), where they are generally observed to have a size of between 100-200 nm in diameter, although smaller and larger versions have also been observed. Carboxysomes are more often reported to have an icosahedral appearance whereas metabolosomes are generally seen with a less regular shape. There are two main trains of thought as to why bacteria require these elaborate structures. The first centres around the idea that the compartment, with its high internal concentration of enzymes, enhances metabolic flux utilising proximity and channelling effects [15], thereby providing a competitive growth advantage over other organisms [15-17]. The second theory revolves around the idea that the compartment, and specifically its outer shell, provides a diffusion barrier for toxic or volatile intermediates such as aldehydes or CO_2 [12, 18]. The likelihood is that it is a combination of both that has led to their successful integration across a broad range of bacterial species.

BMCs are composed of an outer shell that is itself largely comprised of hexagonal-shaped tiles with a central pore (Figure 2). These hexagonal tiles represent the facets of the structure and are generated from a number of different shell proteins that all share a common protein topology. There are two broad types of hexagonal tiles, those that are composed of six identical subunits, and which are referred to as **BMC-H** shell proteins, and those that are composed of a trimeric (pseudohexameric) arrangement made from three identical subunits (**BMC-T** shell proteins) (Figure 2). BMC-H proteins contain a single BMC-domain (Pfam: PF00936), whereas BMC-T proteins contain two fused PF00936 domains [19-22]. To form a closed structure the vertices of the microcompartment are capped using a pentagonal-shaped tile

composed of a homo-pentameric shell protein (**BMC-P**), with each subunit containing a single PF03319 domain [23-25].

As the various proteins of BMCs are often encoded within operons it is possible to predict and prove experimentally which shell proteins are integrated into BMCs. For instance, with the α-carboxysome the compartment is composed of a number of different hexagonal tiles that are derived from 4 different BMC-H class of shell protein (CsoS1A-C) and a BMC-T shell protein (CsoS1D) with the BMC-P pentagonal tile provided by CsoS4. Similarly, for the Pdu metabolosome the shell is derived from 4 different BMC-H components (PduA, J, K, U), two different BMC-T components (PduB, T) and a BMC-P in the form of PduN.

BMC shell protein classes

However, within the BMC-H and BMC-T classes there is further variation, suggesting optimisation of different BMCs for their specific function (Figure 2). This increasing diversity has led us to expand the classification system to reflect the assortment of shell proteins that are observed. For the BMC-H class, we propose that they are sub-grouped into BMC-H_C, BMC-H_P, BMC-H_{Ex} and BMC-H_{Fe}. As highlighted in Figure 2, BMC-H_C refers to canonical BMC-H proteins such as PduA or CsoS1 that contain a charged pore at the symmetry axis, and can be found in the majority of BMC systems [8]. These pores range from ~4 to ~7 Å in diameter and vary in charge depending on function [26-28].

BMC-H_P includes BMC-H proteins where the BMC domain is circularly permuted, allowing for a similar tertiary structure using an alternative arrangement of secondary structure elements as exemplified with PduU (Figure 2) [29]. Such proteins sometimes have an N-terminal extension that forms a β-barrel structure [29, 30] that sits over the pore, presumably occluding the pore and preventing metabolite transport. BMC-H_{Ex} refers to another family of BMC-H proteins that contain C-terminal extensions of unknown function [8]. Finally, BMC-H_{Fe} covers a fourth family of specialised BMC-H proteins that contain an Fe-S cluster, which is thought to play a role in electron transfer, or transport of intact metal clusters across the shell (Figure 2) [31].

As with the BMC-H shell proteins the BMC-T class can be further divided into three families. Single layered BMC-T (BMC-T_S) proteins such as PduB and EtuB also contain pores similar to their hexameric counterparts, however, evidence suggests that these are gated with the complexes adopting alternative conformations allowing diffusion of substrates in/out of the BMC lumen (Figure 2) [11, 20, 22, 28]. The pores are larger than those found in BMC-H complexes (~12 Å) and are predicted to allow larger molecules to diffuse in/out of the lumen of the BMC [22, 28]. A second family of BMC-T proteins (BMC-T_D) contain two permuted BMC domains and form a stacked dimer of trimers with an enclosed central space which is likely accessed via gated pores [19, 20]. These larger pores (~14 Å) are suggested to provide access of substrates to the lumen without compromising the integrity of the shell by forming an airlock-like structure (Figure 2) [19, 20]. The third BMC-T family (BMC-T_{Fe}) contains an iron sulphur centre which occludes the pore, and has been suggested to be involved in processes such as electron transfer, redox sensing or regeneration of iron sulphur clusters of proteins within the lumen of the BMC (Figure 2) [21, 32].

As mentioned previously, BMC-P proteins form pentamers that occupy the vertices of the BMC to mould a fully closed system (Figure 2) [24, 33, 34]. It has also been suggested that they aid with curvature of the shell [34]. Currently no specialised BMC-P shell proteins have been described, however, as some BMC systems contain multiple paralogues of BMC-P proteins within the same BMC operon, *e.g. Haliangium ochraceum* contains genes encoding two BMC-P proteins [8], there may be variations within this class that are yet to be discovered.

BMC encapsulation/targeting peptides

Whilst small molecules can enter the lumen of BMCs via the pores found within shell proteins, internalisation of proteins is thought to occur by a different process. The main method for incorporation of proteins into BMCs is through the use of **encapsulation peptides** (EPs). EPs have been found to be involved in all experimentally characterised BMCs, with the exception of the α-carboxysome family. Many BMCs contain multiple proteins with EPs [35-37]. EPs are located on the N- or C-terminus of encapsulated proteins. They are ~15-20 amino acids in length and form an amphipathic helix that interacts with shell proteins via a common

hydrophobic motif [37-39]. Some EPs interact with certain shell proteins whereas others may have multiple interactions within the BMC. For example, PduP contains an 18 amino acid EP and has been shown to interact with PduA, PduJ and PduK [36, 39]. Conversely, EPs on EutC and EutE from the ethanolamine utilising BMC have been shown to specifically interact only with the EutS shell protein [40]. EPs have been shown to interact with specific short helical regions on shell proteins. For example PduP18 binds to the C-terminal helix of PduA [36] and EutC19 binds to an equivalent helix in EutS [40]. EPs have also been shown to cause protein aggregation, suggesting they may be involved in forming a condensed protein core [14, 41].

However, not all proteins are localised to BMCs using EPs. For example, α-carboxysomes rely on alternative protein-protein interactions to scaffold RuBisCO to the shell proteins using the protein CsoS2 [42]. This protein contains regions of significant intrinsic disorder, allowing for entrapment of proteins through protein-protein interactions.

BMC formation

BMCs appear to be self-assembling entities. Insights into the construction of both α- and β-carboxysomes have been gained experimentally, however, formation of metabolosomes remains a largely unknown process. CsoS2 is an essential component of α-carboxysomes, with the full-length protein containing an N-terminal domain that recruits shell proteins, a middle domain (M-domain) that tethers RuBisCO units within the lumen of the carboxysome and a C-terminal domain that anchors the scaffold to the growing shell [42] . Utilising CsoS2 as a scaffold, the proteinaceous core and the shell assemble concomitantly. Despite their shared function, β-carboxysomes assemble through a different mechanism. Initially, an aggregate is formed between RuBisCO and several conserved β-carboxysome proteins called CcmM and CcmN. This complex then pulls in several other components as well as shell proteins to initiate the encapsulation process to allow a pre-carboxysome to bud off from the main aggregate [37, 43].

Formation of metabolosomes is suggested to occur in a similar manner to that of β-carboxysomes. However, since ‘empty’ Pdu BMCs can be formed recombinantly

from the shell proteins alone [44], there must be a high degree of self-assembly in some systems.

Engineering BMCs

Recombinant Engineering

The synthetic biology era has developed the idea of constructing internal factories inside bacteria and BMCs have long been thought to provide a platform for such an engineering challenge. The first example of the recombinant expression of BMCs was achieved in 2008 and involved the transfer of the entire *Pdu* operon from *C. freundii* and its expression in *E. coli* (Figure 3A) [32]. The resulting strains were able to utilize propanediol and when examined, after embedding and thin sectioning, by TEM BMC structures were observed. Similarly, the transfer of the α-carboxysome operon from *H. neapolitanus* to *E. coli* resulted in the appearance of icosahedral structures inside the host that were shown to have CO_2 fixation activity and resemble the wild type organelle [45]. More recently, the same α-carboxysome operon has been successfully transferred to the gram-positive organism *C. glutamicum* [46]. It has been proposed that the transfer of the CO_2 concentrating mechanism (CCM) from cyanobacteria to plants has the potential to not only increase global carbon fixation but also increase agricultural yields. Towards this goal researchers have been able to form β-carboxysome like structures in *Nicotiana benthamiana* chloroplasts by expression of 3 proteins (CcmO-YFP, CcmK2 and CcmL-YFP or CcmM58-YFP), however it must be noted that similar structures are not seen in the absence of YFP fusions suggesting possible solubility issues [47].

Engineering BMC shells

The size and charge of the pores observed in BMC shells dictate the molecules that can pass into and out of the organelle [27]. The repurposing of BMCs for biotechnological applications depends upon the ability to modify the substrate specificity of the shell. Recent work has shown that mutations to pore residues do indeed result in altered pore properties while still allowing for incorporation in both carboxysomes and Pdu metabolosomes [42]. Similarly, a trimeric BMC shell protein has recently been engineered to bind a [4Fe-4S] cluster in the pore allowing for the potential of passing or receiving electrons to or from a BMC [35]. The work to date

provides a foundation for the *de-novo* design of pores to facilitate the construction of internalised metabolic pathways.

The high degree of structural and sequence similarity of BMC shell proteins has enabled the production of chimeric BMC shells, that offer the potential for bespoke functionality over their wildtype counterparts. Expression of the α-carboxysomal shell protein CsoS1 in a CcmK2 deletion mutant, unable to form β-carboxysomes, formed hybrid carboxysome shells [42]. Similarly, it has been shown that EutM is able to integrate into a PduA deletion mutant in *S. enterica* [48]. It has also been shown that expression of both the *Pdu* and *Eut* operons in *S. enterica* hybrid BMC shells were produced incorporating both Eut and Pdu shell proteins [49]. Overall, this suggests that shell proteins can easily be interchanged to allow for the development of BMCs with different permeability properties.

Confirming the presence of assembled recombinant BMCs

Wild-type BMCs have a number of defining characteristics when observed both *in vivo* and following purification by TEM and fluorescent microscopy techniques. In the case of fluorescent microscopy it has been shown that fluorescent proteins such as GFP can be fused to specific shell proteins, allowing the BMCs to be visualised as fluorescent puncta within the bacterial cytoplasm. However, the observation of fluorescent puncta within the cell can also be caused by aggregation of recombinant proteins and therefore BMCs should be confirmed through a combination of experimental approaches. We outline, in Textbox 1, a number of key characteristics that should be fulfilled in order for researchers to claim successful BMC formation. The criteria for confirmation of BMC formation should include an analysis by TEM to show the size of the organelle (50-200 nm in diameter), shape (polygonal, straight edges, angular facets) and number (from 5 to 50 per cell). Fluorescent microscopy can be used to show the presence of puncta within the cell and the targeting of cargo to the BMCs, and the purified BMCs should have a characteristic protein profile when analysed by SDS-PAGE. During recombinant production of BMCs some publications have claimed to show the presence of BMCs with structures that clearly do not fill these criteria, but more closely resemble protein aggregates or lipid vesicles. Such contaminants are likely due to the relatively crude purification methods used to extract BMCs. The most problematic are lipid vesicles, which are known to be of

approximately the same diameter as BMCs and are as such often mistaken for BMCs.

Empty BMCs and their refunctioning

The application of BMCs for commercial and industrial applications depends on the ability to not only redesign the properties of the outer shell, as discussed previously, but also on the ability to repurpose BMC shells and the encapsulation of new processes. Following the transfer of the entire *Pdu* operon from *C. freundii* and expression in *E. coli* it was shown that through the expression of a minimal set of proteins empty Pdu BMC shells could be produced opening up the possibility for the re-purposing of these bacterial organelles (Figure 3B) [44]. This process appears to work well with the Pdu system. Several attempts have been made to recombinantly produce Eut BMCs, however, the structures observed lack distinctive BMC features and more closely resemble lipid vesicles [40, 50, 51]. Recently it has been shown that through expression of the shell proteins of a BMC of unknown function from *Haliangium ochraceum* in *E. coli* regularly shaped BMCs can be visualised following purification (Figure 3C) [52]. Intriguingly, structures were not observed *in vivo* possibly due to a lack luminal electron density hindering visualisation, alternatively it may be that BMCs are forming during purification, as a result of an imbalance in protein stoichiometry *in vivo*. Nonetheless this BMC has been crystallised as discussed below [53] and provided fundamental insights into the structural arrangements of shell proteins within these remarkable macromolecular assemblies.

A major goal in the utilisation of BMC technology is to be able to re-purpose these intracellular organelles to provide novel functionality. The first proof of concept study was conducted which showed that an ethanol bioreactor could be produced by tagging the enzymes pyruvate decarboxylase and alcohol dehydrogenase with EPs and their coproduction with the proteins required to form an empty BMC [39]. The resulting strains produced more ethanol *in vivo* in comparison to strains producing untagged enzymes with BMC shells.

A similar attempt was made to engineer a bioreactor for 1,2-propanediol production utilising 4 enzymes and the same EPs [41]. However, in this case a 245% increase in 1,2-propanediol production was observed irrespective of the presence of shell

proteins. TEM imaging revealed the presence of large intracellular inclusion bodies which are thought to increase local enzyme concentrations and provide an environment for substrate channelling.

Bioremediation of industrial waste to remove metals and other toxic compounds has been of industrial interest for many years [54]. To this end BMCs have been thought of as potential facilitator of this due to their ability to sequester molecules from the cytoplasm enabling microorganisms to sequester greater levels of toxic molecules without impacting on their viability. Recently it has been shown that the expression of encapsulated polyphosphate kinase results in the build-up of polyphosphate that is not degraded by the expression of an exopolyphosphatase suggesting the protection of polyphosphate within the lumen of the BMC [55]. This initial investigation highlights the potential of BMCs to serve as an intracellular sink for toxic molecules.

Structure of a recombinant BMC.

The first crystal structures of individual BMC-H, BMC-T and BMC-P proteins allowed the generation of models for the outer shell of a BMC [27, 34]. More recently, a structure of a recombinant BMC, of unknown function, from *Haliangium ochraceum* has been resolved by cryo-electron microscopy and x-ray crystallography [53]. The 40 nm diameter, single layer icosahedron is composed of 60 hexameric and 20 trimeric shell proteins, with 12 pentameric tiles located at the vertices (Figure 3D). The structure reveals 4 distinct protein-protein interfaces; 2 hexamer-hexamer interactions, a hexamer-trimer interaction and a hexamer-pentamer interaction. The high degree of sequence and structural similarity means that these interactions are likely conserved across BMC systems [53].

Engineering BMC components

To date major successes have been made through the deconstruction of BMC systems and the utilization of the component parts to form novel cytoplasmic architectures. Researchers initially observed filamentous structures when constructing systems for the expression of empty Pdu BMCs (Figure 4A) [44]. These filaments, around 20 nm in diameter, were found to be composed of the hexameric BMC building block PduA. It has been shown that it is possible to target fluorescent proteins to these bundles of filaments *in vivo* utilizing BMC targeting peptides [56]. A

PduA homologue RmmH from *Mycobacterium smegmatis* was similarly found to form nanotubes however in this case the structures observed had a diameter of ~ 14 nm (Figure 4B) [57]. Researchers have shown that mutations to residues at the hexamer-hexamer interface of PduA results in the formation of more sheet-like assemblies *in vivo* offering the opportunity to re-design a PduA based scaffold (Figure 4C) [58].

Lately, a 3-component system has been developed comprising the hexameric BMC shell protein PduA and a pair of *de-novo* designed coiled-coils [59]. The resulting filamentous scaffold is arrayed throughout the bacterial cytoplasm rather than aligned as is the case with the unmodified variant (Figure 4D). By tagging fluorescent proteins with the cognate partner peptide such tagged proteins can be localised to the scaffold. Targeting the enzymes pyruvate decarboxylase and alcohol dehydrogenase to the scaffold resulted in a 221% increase in ethanol production as a result of the enhanced co-localisation of the enzymes. Additionally, it is possible to target this intracellular cytoscaffold to the inner membrane of the cell, a characteristic that may facilitate rapid channelling of intermediates between enzymes and due to the specific localisation of the scaffold/ enzyme complex faster export of products out of the cell.

The same coiled-coil peptide system has also been used to target protein cargo to either the inside or outside of a recombinant BMC *in vivo* [60]. In this case, one of the coiled-coil peptides was attached to the N-terminus of PduA, which is located on the outer-facing (cytoplasmic) concave side of the protein. Attachment of the cognate coiled-coil peptide to a fluorescent cargo protein then directed the fluorophore to the outside of the BMC. The redesign of PduA from a BMC-H_C to a permuted conformation BMC-H_P then allows for the N-terminus to be located on the inside of the compartment, thereby allowing targeting of cargo to the interior [60]. A similar idea of using affinity handles and covalent linkages to target proteins to the lumen of recombinant BMCs has also been reported with the *H. ochraceum* system [61].

Concluding remarks

Since their first visualisation in the 1950s and subsequent rapid expansion in investigations over recent years, BMCs and related technologies have come to the

fore as a mechanism to enhance the spatial organisation of metabolic pathways. Significant work to date has focussed on not only understanding the characteristics of wild type organelles but also on the re-design of such systems to provide novel functionality. On-going work has shown that it is possible to produce BMCs in a recombinant and functional fashion and to direct new protein cargo to their lumen. Engineering different sized pores and the properties of the shell proteins all indicate that the redesign of BMCs for bespoke applications is a tangible, realistic and achievable challenge. The introduction of carboxysomes into plants is of great interest as it holds the potential to enhance crop yields and some initial steps have been taken towards this goal. Future efforts will undoubtedly expand on this early work. Similarly proof of concept studies have shown how recombinant BMCs can be utilised for the production of commodity chemicals and the sequestering of toxic compounds from the cellular cytoplasm. The development of shell proteins into cytoplasmic scaffolds and the use of EPs to promote shell-less aggregation highlight ways in which BMC technology is being harnessed for engineering purposes (Figure 5). Such studies have shown the potential of BMC technology and provide a platform for its industrial application. Despite the wealth of knowledge that has been generated in the field a significant number of fundamental questions remain (see Outstanding Questions). Genomic analysis has revealed 23 BMC subtypes in a diverse variety of species, however, few have been experimentally characterised. Such uncharacterised systems may possess bespoke functionality, further our understanding of native systems and offer new opportunities for the reprograming of such organelles for synthetic biology applications.

There also remain a number of outstanding general basic scientific questions concerning the appearance of BMCs, including how and why they evolved. BMCs represent one of the most complex proteinaceous structures within the bacterial cell and there is still much to learn not only about their assembly but also their dis-assembly. Finally, metabolosomes, especially, represent unique multi-enzyme congregations and there is a pressing need to understand the kinetics of these parochial communions.

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Figure Legends

Figure 1
Functional characteristics of wild-type BMCs. **LHS** cartoon representation of a bacterial cell containing BMCs. **RHS** pathways encapsulated within anabolic carboxysomes (top) and metabolic metabolosomes (bottom). Highlighting the toxic and volatile intermediates sequestered by each BMC in red.

Figure 2
Classification of BMC shell proteins and their suggested functions.

Figure 3
Recombinant production of BMCs: (**A**) Thin section of *E. coli* Recombinantly producing Pdu metabolosomes. Reproduced with permission from [32] (**B**) Micrograph of negative stained, empty, Pdu BMCs. (**C**) Synthetic construction of negatively stained empty, *H. ochraceum* BMCs of unknown function. Reproduced with permission from [52] (**D**) Surface representation of the crystal structure of *H. ochraceum* BMC shown in C, BMC-H in blue, BMC-T_D in green and BMC-P in yellow. Reproduced with permission from [53] Scale bars in **A** and **B**, 200 nm, **C**, 50 nm

Figure 4
Recombinant production of hexameric BMC components in *E. coli*: (**A**) Thin section of *E. coli* expressing *C. freundii*, PduA*. Reproduced with permission from [59] (**B**) Purified, negative stained *M. smegmatis*, RmmH. Reproduced with permission from [57] (**C**) Thin section of *C. freundii,* R79A PduA* mutant recombinantly expressed in *E. coli*. Reproduced with permission from [58] (**D**) CC-Di-B tagged PduA* forming a filamentous cytoscaffold in *E. coli*. Reproduced with permission from [59]. Scale bars in **A** and **C,** 200 nm, in **B**, 100 nm and in **D**, 500 nm

Figure 5
BMC based architectures for enhancing the spatial organisation of metabolic pathways. **LHS**: Encapsulation of a metabolic pathway within a BMC by fusing enzymes to BMC encapsulation peptides. **Middle**: Arrangement of enzymes on a BMC shell protein based scaffold. **RHS**: Aggregation of a metabolic pathway into an active inclusion body upon fusion of encapsulation peptides to enzymes.

Figure 1

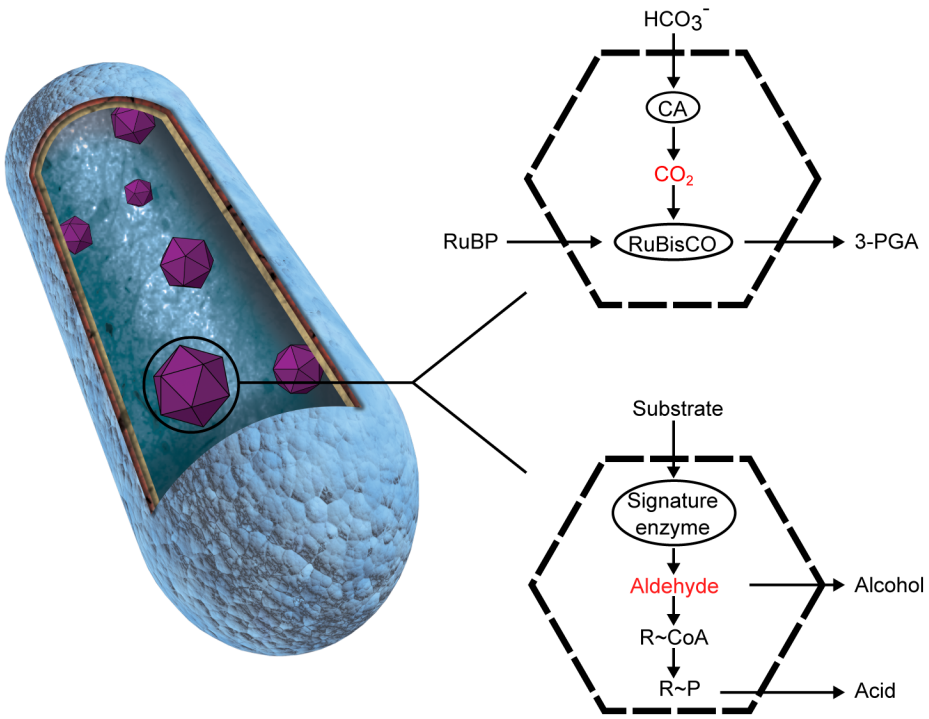

HCO3-
CA
CO2
RuBP
RuBisCO
3-PGA
Substrate
Signature enzyme
Aldehyde
Alcohol
R~CoA
R~P
Acid

Figure 2

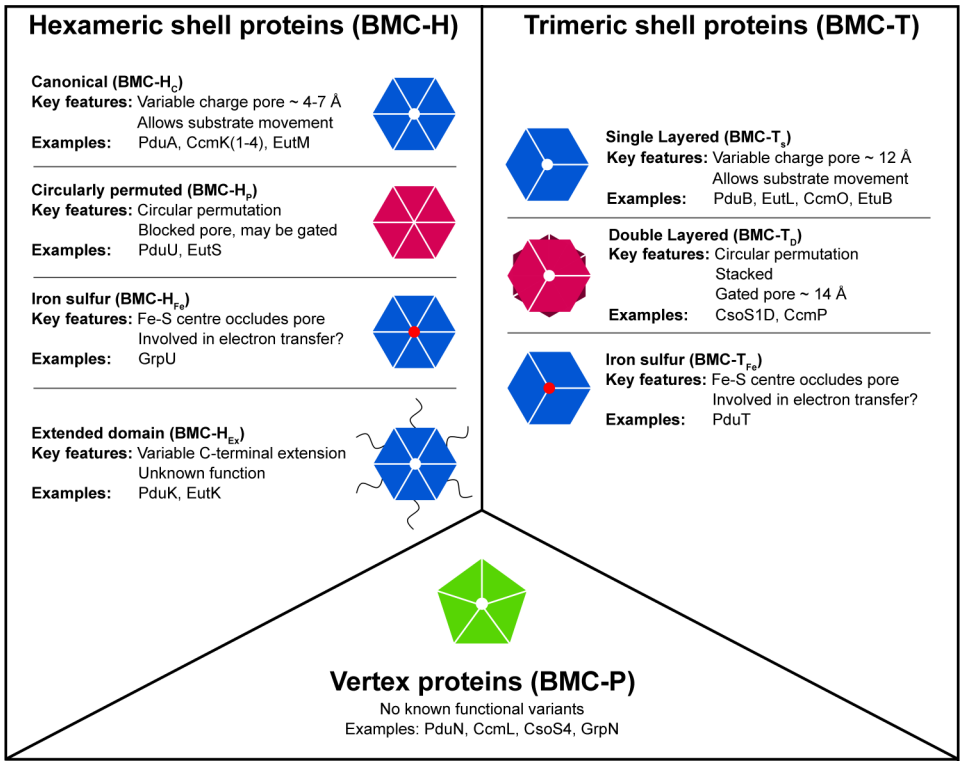

Hexameric shell proteins (BMC-H)
Canonical (BMC-H$_C$)
Key features: Variable charge pore ~ 4-7 Å
Allows substrate movement
Examples: PduA, CcmK(1-4), EutM
Circularly permuted (BMC-H$_P$)
Key features: Circular permutation
Blocked pore, may be gated
Examples: PduU, EutS
Iron sulfur (BMC-H$_{Fe}$)
Key features: Fe-S centre occludes pore
Involved in electron transfer?
Examples: GrpU
Extended domain (BMC-H$_{Ex}$)
Key features: Variable C-terminal extension
Unknown function
Examples: PduK, EutK
Trimeric shell proteins (BMC-T)
Single Layered (BMC-T$_S$)
Key features: Variable charge pore ~ 12 Å
Allows substrate movement
Examples: PduB, EutL, CcmO, EtuB
Double Layered (BMC-T$_D$)
Key features: Circular permutation
Stacked
Gated pore ~ 14 Å
Examples: CsoS1D, CcmP
Iron sulfur (BMC-T$_{Fe}$)
Key features: Fe-S centre occludes pore
Involved in electron transfer?
Examples: PduT
Vertex proteins (BMC-P)
No known functional variants
Examples: PduN, CcmL, CsoS4, GrpN

Figure 3

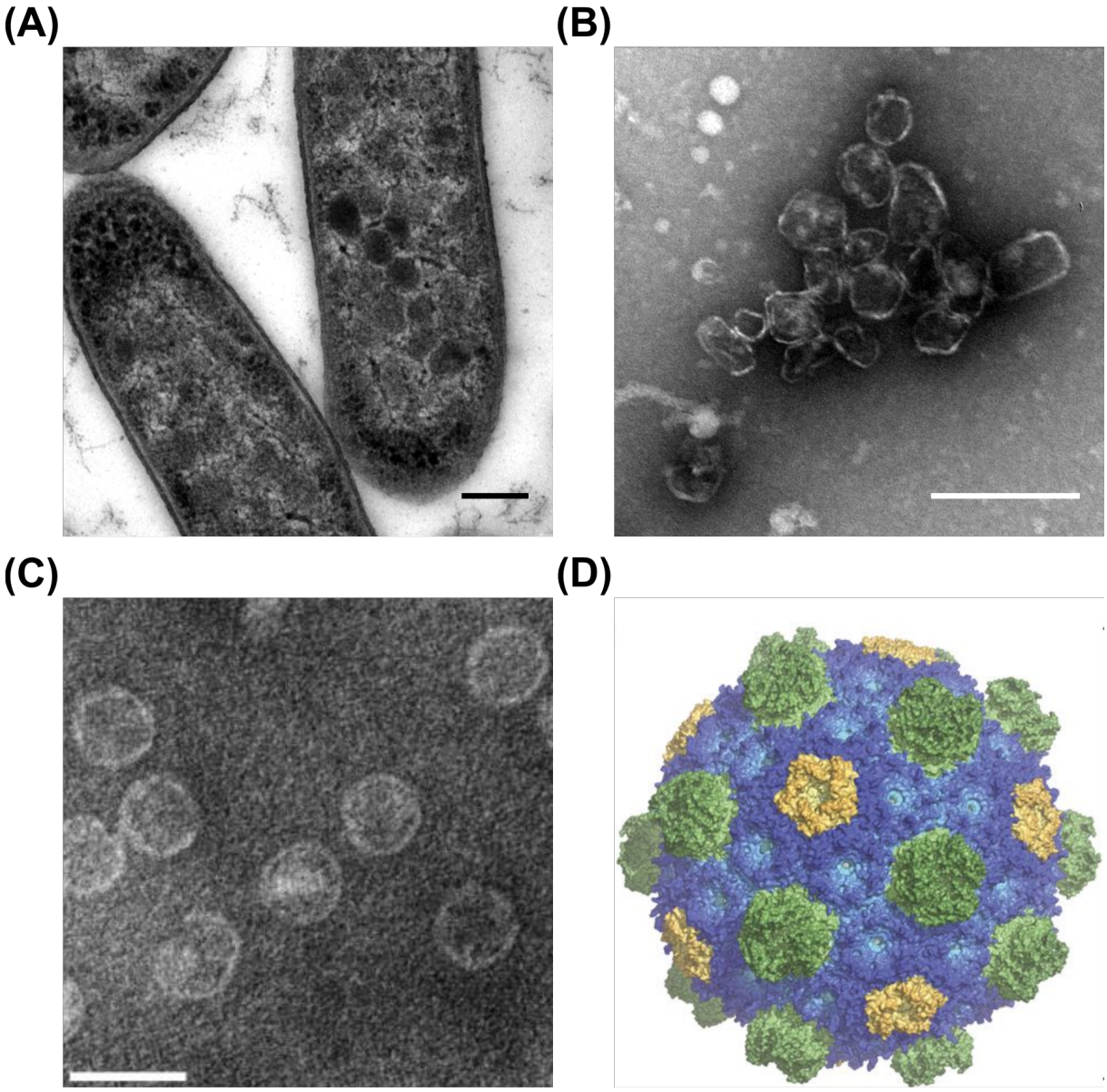


Figure 4

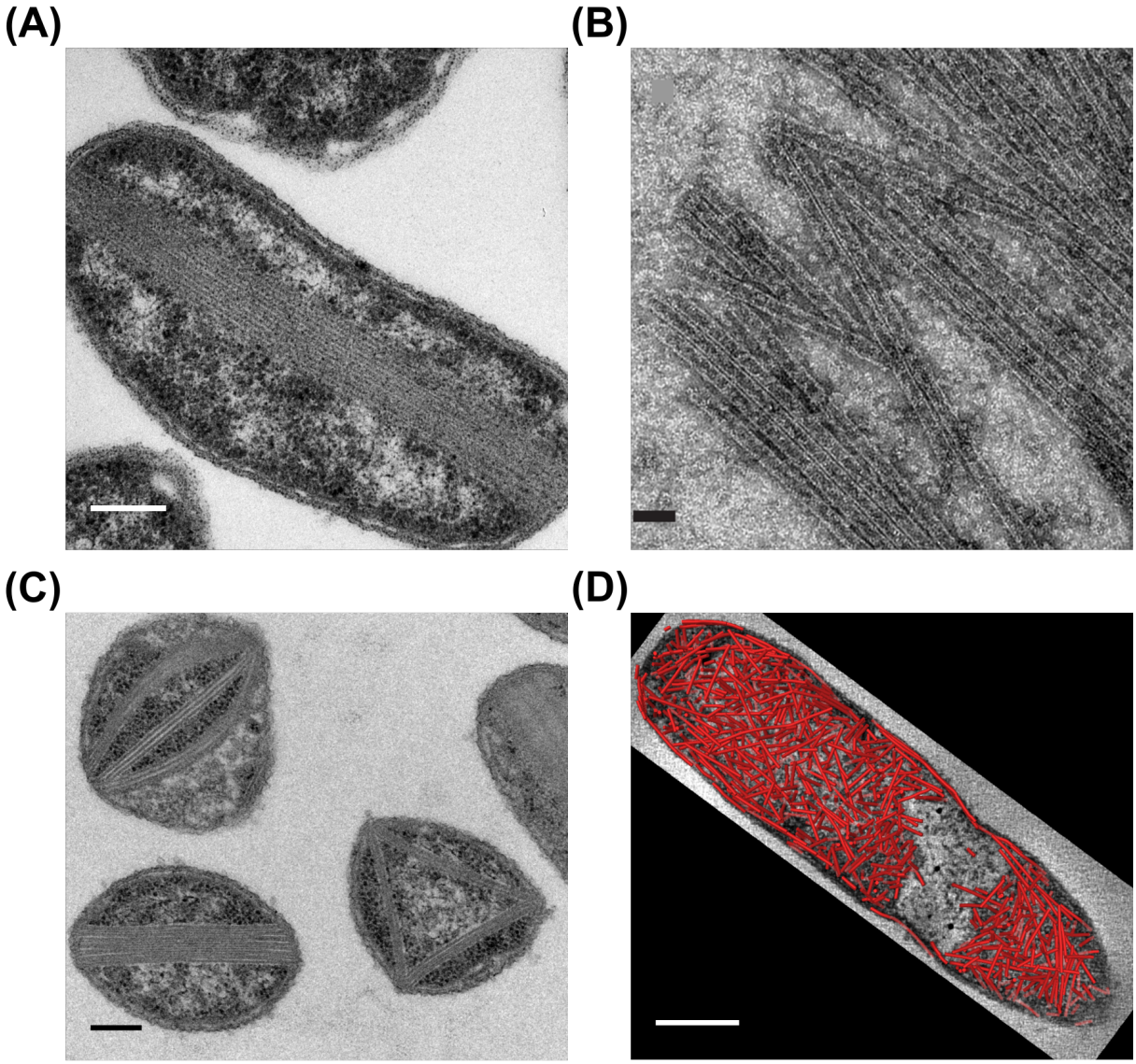
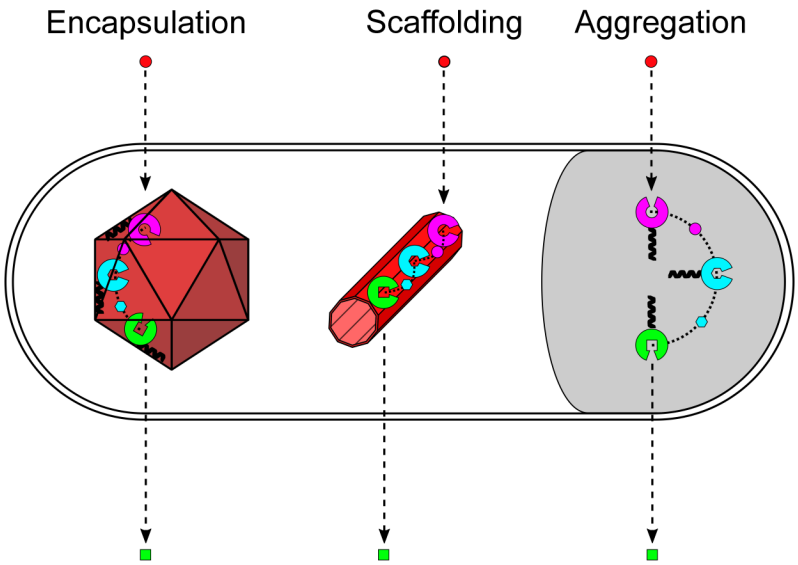


Figure 5



Highlights

- Bacterial microcompartments (BMCs) are widespread proteinaceous organelles consisting of a semipermeable protein shell and an encapsulated enzymatic pathway.
- Can be divided into the anabolic carboxysomes and the catabolic metabolosomes.
- Targeting to organelles is facilitated by short, amphipathic, alpha helical encapsulation peptides.
- Heterologous enzymatic pathways can be encapsulated within empty organelles, resulting in enhanced productivity.
- A BMC-H based cytoscaffold is able to significantly enhance biofuel production through enzyme co-location.

Outstanding Questions box

- What determines the size of BMCs? Does the shell or the cargo dictate the size?
- Do metabolosomes assemble by the same mechanism as the alpha or beta carboxysomes or by a third mechanism?
- What do encapsulation peptides interact with and how are proteins encapsulated within BMCs?
- Are some enzymes localised to the cytoplasmic surface of BMCs?
- What is the function of the BMC shell proteins that are yet to be characterised?
- How are large co-factors transported into BMCs?
- What is the role of BMCs in pathogenesis?

Text box 1

Production and characterisation of recombinant BMCs

Key characteristics that should be fulfilled in order for researchers to claim successful observation of wild type and recombinant BMC formation:

- Angular facets when observed by TEM both *in-vivo* and following purification (Figure IA and B)
- Immuno gold labelling of structures (Figure IC)
- Expected protein profile when analysed by SDS-PAGE (Figure ID)
- *In-vivo* punctate fluorescence in fluorescently modified constructs, not polar aggregates (Figure IE)
- Ability to target recombinant proteins to BMC structures by fusion to encapsulation peptides (Figure IE and F)

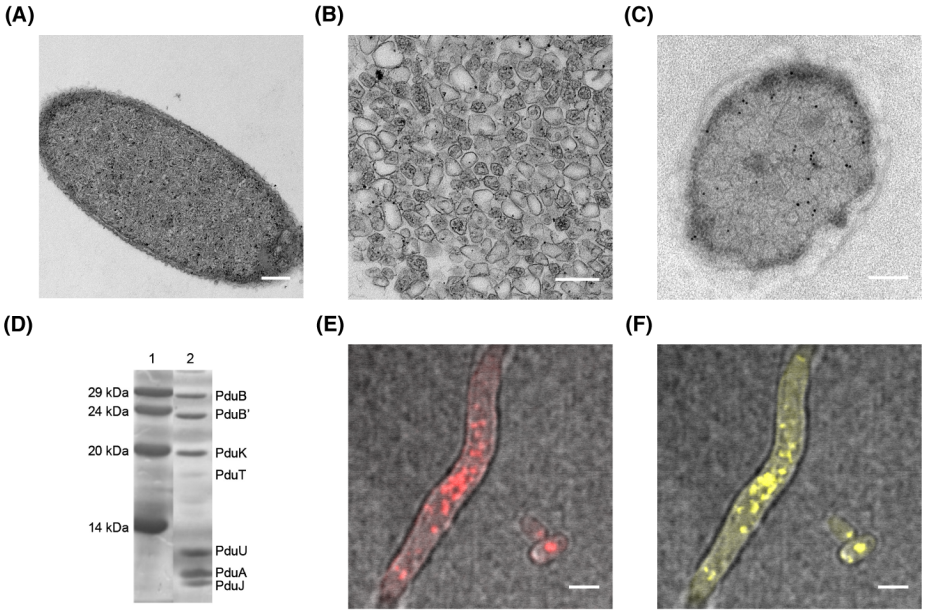


Figure I. Characteristics of recombinantly produced BMCs. (**A**) Thin section of *E. coli* cells recombinantly expressing empty Pdu BMCs (**B**) Purified, thin sectioned, empty Pdu BMCs. (**C**) Thin section of *E. coli* expressing empty Pdu BMCs cross-reacted with anti-PduA antibodies and then with a secondary antibody conjugated to 15 nm gold particles. Reproduced with permission from [44]. (**D**) SDS-PAGE analysis of purified BMCs in comparison to a molecular weight standard. Reproduced with permission from [44]. (**E**, **F**) Confocal microscopy of *E. coli* expressing mCherry labelled BMCs with D18 tagged Citrine, showing mCherry and Citrine fluorescence in E and F respectively. Scale bars in **A**-**C**, 200 nm, **E**, **F** 2 μm.

Glossary

Bacterial Microcompartment: proteinaceous organelles ~ 100 nm in diameter found in ~ 20 % of bacterial species that encases either anabolic or catabolic processes.

BMC-H: A component of the BMC shell containing a single Pfam 00936 domain, 6 BMC-H proteins come together to form a homohexameric tile that forms part of the BMC shell.

BMC-T: A component of the BMC shell containing two fused Pfam 00936 domains, 3 BMC-T proteins come together to form a homotrimeric tile that forms part of the BMC shell.

BMC-P: A component of the BMC shell containing a single Pfam 03319 domains, 5 BMC-P proteins come together to form a homopentameric tile that forms the vertices of the BMC

Carboxysome: A bacterial organelle containing the enzymes carbonic anhydrase to generate a high local concentration of CO_2 and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) to catalyse the carboxylation of ribulose 1,5-bisphosphate, the first step in carbon fixation.

Encapsulation peptide: short (~18 amino acid) peptides that form amphipathic alpha helices natively found on the N- or C-terminus of encapsulated proteins that target such proteins to the lumen of BMCs.

Metabolosome: catabolic BMCs that encase a metabolic pathway in which a signature enzyme converts a metabolite into an aldehyde which is subsequently converted into an acid and alcohol.

Signature enzyme: A metabolosome enzyme that is specific to the first substrate to product conversion.