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Engineering nanoreactors using bacterial microcompartment architectures

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Bacterial microcompartments (BMCs) are organelles that encapsulate enzymes involved in CO_2 fixation or carbon catabolism in a selectively permeable protein shell. Here, we highlight recent advances in the bioengineering of these protein-based nanoreactors in heterologous systems, including transfer and expression of BMC gene clusters, the production of template empty shells, and the encapsulation of non-native enzymes.

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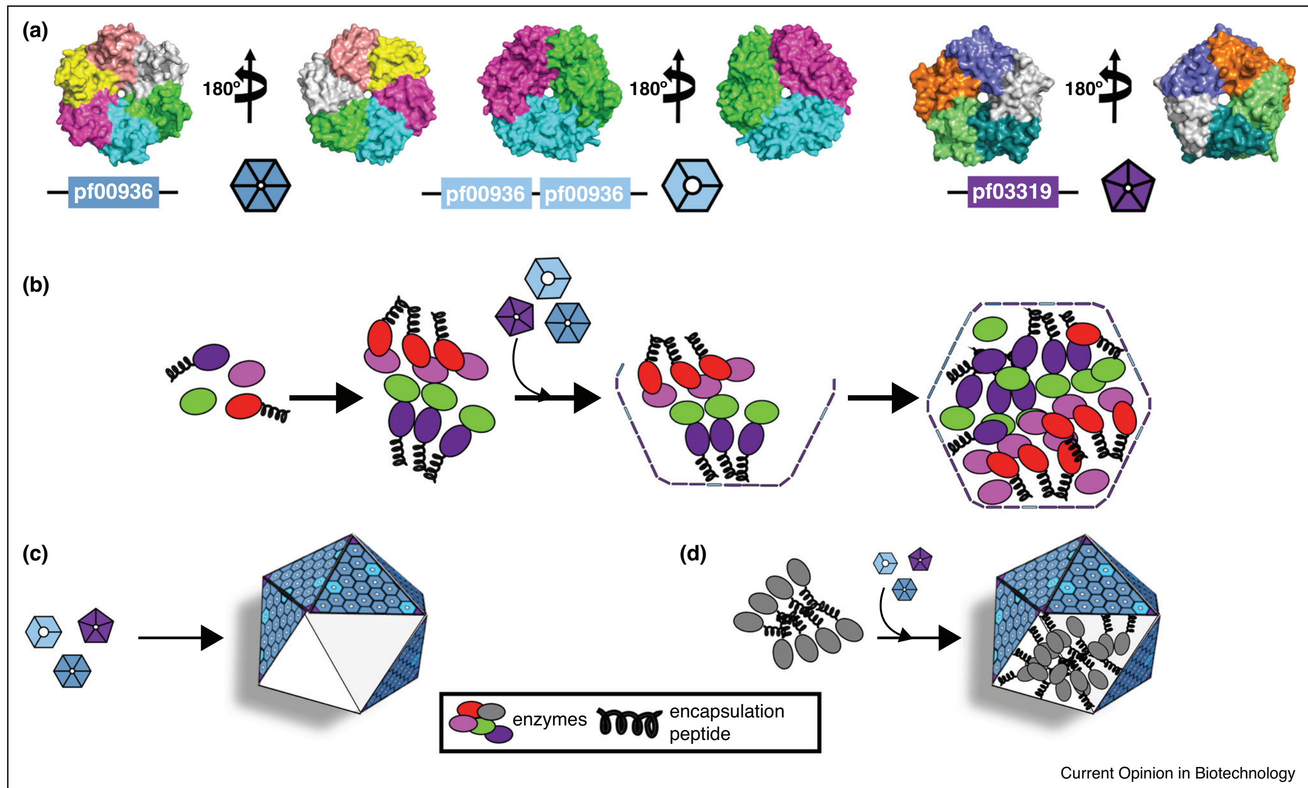
Introduction

A key goal of synthetic biology is to engineer metabolic pathways to produce bulk chemicals for medical, agricultural, and industrial purposes using microbial cell factories. Factors that reduce the efficiency of engineered pathways include crosstalk of metabolites, toxic intermediates, and inhibitory products. Eukaryotes have evolved compartmentalizing organelles to overcome these obstacles. Bacteria also have organelles, known as bacterial microcompartments (BMCs) [1–3]. BMCs contain enzymes that catalyze sequential reactions and a private pool of cofactors (e.g. NAD^+/NADH, coenzyme A, and ATP) within a protein shell. The BMC shell serves as a selectively permeable interface between the encapsulated pathway and the cellular environment. Because they self-assemble entirely from proteins, BMCs are becoming a viable platform for engineering novel nanoreactors.

Functionally diverse BMCs are bioinformatically predicted to be present in at least 23 different bacterial phyla [2]. Cyanobacteria and some chemoautotrophs produce anabolic BMCs (α-carboxysomes or β-carboxysomes) that encapsulate carbonic anhydrase (CA) and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) to enhance CO_2 fixation. However, the majority of functionally diverse BMCs are catabolic (metabolosomes), utilized by heterotrophs to degrade a range of carbon compounds in niche environments. Most metabolosomes contain a signature enzyme, such as a propanediol dehydratase (PDH) [4], an ethanolamine-ammonia lyase (EAL) [5], or a glycyl-radical enzyme [6,7], that defines the function of the BMC [e.g. propanediol utilization (PDU) or ethanolamine utilization (EUT) BMCs, or glycyl-radical enzyme microcompartment (GRM)]. Metabolosome cores also typically include four conserved [1,2] enzymes: an aldehyde dehydrogenase (AldDH) [8], an alcohol dehydrogenase (AlcDH) [9], and a phosphotransacylase (PTAC) [10,11]. In addition, many BMC loci encode ancillary proteins that support organelle function, such as the transport of substrates and recycling of co-factors (e.g. ATP and vitamin B_{12}) [1,2].

The core enzymes of carboxysomes and metabolosomes are encapsulated by a shell comprised of proteins that form hexamers (BMC-H) [12,13], pseudohexamers/trimers (BMC-T) [14,15], and pentamers (BMC-P) [16,17] (Figure 1a). The hexameric shell proteins typically contain a pore at the symmetry axis, with a diameter of 4–10 Å [12,18••] and electrostatic properties [1,6,13,15,19] suited to the passage of small charged metabolites across the shell [1]. Confinement of sequential enzymatic reactions by the BMC shell facilitates substrate channeling, thereby enhancing catalytic efficiency [1,20–22,23•,24]. The shell also acts as a barrier that prevents potentially toxic/volatile intermediates from diffusing into the cytoplasm [21,22,23•]. Most BMCs are predicted to form from the inside out, the core proteins coalesce into a bolus around which a shell assembles [1,25] (Figure 1b). A short helical extension found on a subset of core proteins, the encapsulation peptide (EP), facilitates the aggregation of the core enzymes [10,26,27] and their subsequent encapsulation by the shell [1,25,28–32]. The structure and native functions of BMCs have been reviewed elsewhere [1,3,20,24,33,34]. The aim of this review is to highlight the recent efforts that adapt the BMC architectures for the development of novel nanoreactors in heterologous systems.

Figure 1



Shell proteins (a) and assembly of BMCs (b-d). **(a)** Representatives of a BMC-H protein (PDB 5DJB) (left), a BMC-T protein (PDB 5DIH) (middle), and a BMC-P protein (PDB 2QW7) (right). Pf indicates Pfam domain identification. Individual polypeptide chains are colored differently. **(b)** Cartoon representation of BMC assembly from the inside out, where the primary role of the EP is in shell recruitment [25,35••] **(c)** of empty BMC shell assembly, and **(d)** of targeting enzymes to the lumen of BMC shells using EPs [36••].

Overview of BMC engineering

Efforts to engineer BMCs have involved both the transfer and expression of BMC genes and operons in heterologous systems, the production of empty shells (Figure 1c), and the loading of cargo using encapsulation peptides (Figure 1d). Subsequent efforts have focused on building a core based on protein domain interactions and tuning shell permeability to support the encapsulated metabolism.

Heterologous expression of BMC gene clusters in *Escherichia coli*

BMCs are encoded by gene clusters, providing a ready genetic module for heterologous expression. The 21-gene PDU operon of *Citrobacter freundii* was the first demonstration of the potential for 'transplanting' a BMC into *E. coli* [37]. Electron microscopy of thin sections of strains expressing the PDU operon revealed polyhedral bodies (Figure 2a), and the recombinant metabolosome demonstrated diol dehydratase activity. Follow-up work showed that the recombinant PDU BMCs have similar morphology and mechanical properties as wildtype (WT) PDU BMCs [38]. Similarly, the α-carboxysome operon of a chemoautotroph was expressed in *E. coli* (Figure 2b), generating carboxysome-like particles and active RuBisCO [39].

Heterologous expression of BMC shells

A variety of shell proteins from both carboxysomes [42] and metabolosomes have been shown to assemble into empty shells in *E. coli* [37,40,43–45]. Compared to fully packaged native counterparts, recombinant empty shells tend to be smaller; only recombinant EUT shells, formed from all of the or a single EUT shell protein, were observed to be similar to WT EUT BMCs in size [45]. The number of these recombinant EUT shells were shown to increase when co-expressed with a putative cupin domain [46•]. When the shell protein genes of a metabolosome of unknown function (from *Haliangium ochraceum*) were expressed in *E. coli*, homogeneous, robust shells were formed and readily purified (Figure 2c) [40], enabling crystallization [41••]. The atomic resolutions structure of the 6.5 MDa empty shell is estimated to be able to accommodate three-hundred 30 kDa proteins (Figure 2d). Interestingly, the structure revealed that the

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