Encapsulation as a strategy for the design of biological compartmentalization

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Abstract

Compartmentalization is one of the defining features of life. Through intracellular spatial control, cells are able to organize and regulate their metabolism. One of the most broadly used organizational principles in nature is encapsulation. Cellular processes can either be encapsulated within membrane-bound organelles or proteinaceous compartments that create distinct microenvironments optimized for a given task. Further challenges addressed through intracellular compartmentalization are toxic or volatile pathway intermediates, slow turnover rates, and competing side reactions. This review highlights a selection of naturally occurring membrane- and protein-based encapsulation systems in microbes and their recent applications and emerging opportunities in synthetic biology. We focus on examples that use engineered cellular organization to control metabolic pathway flux for the production of useful compounds and materials.

Keywords

Encapsulation, bacterial microcompartments, viral capsids, ferritins, membrane compartments

Introduction

Dynamic spatial organization across all scales, from ecological niches to molecules, is a ubiquitous property of living systems [1-3]. It has been argued that the emergence of compartmentalized chemical reactions must have played a key role in the initial emergence of life by sequestering and protecting early metabolism from the environment. At the same time, compartmentalization would allow the regulated exchange of matter and energy between the inside and outside of a protocell [4].

Modern cells, both prokaryotic and eukaryotic, face many challenges that could not be overcome without intracellular spatial control and organization. In eukaryotes, intracytoplasmic membrane systems including chloroplasts, mitochondria, vacuoles and the nucleus represent the main strategies to localize and control various metabolic processes [5], while only a select number of bacterial species with similar membrane-bound compartments have been identified thus far. Most prokaryotes use proteinaceous compartments to assert spatial control over metabolism.

In cyanobacteria and a number of carbon-fixing heteroautotrophs, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the key enzyme of the Calvin Cycle, is encapsulated within proteinaceous compartments called carboxysomes [6, 7]. Carboxysomes are bacterial microcompartments (BMCs) and are part of the carbon-concentrating mechanism employed by autotrophic prokaryotes to overcome the intrinsically low catalytic efficiency of RuBisCO. By increasing the local molarity of CO₂, carboxysomes help overcome this inherent catalytic inefficiency [8, 9]. At the same time the proteinaceous shell excludes molecular oxygen from the carboxysome lumen thereby reducing RuBisCO's non-productive oxygenase side reaction [6].

Encapsulation is also used to protect the prokaryotic cell from toxic pathway intermediates as exemplified by the 1,2-propanediol utilization (Pdu) and ethanolamine utilization (Eut)

microcompartments of various Enterobacteriaceae species [10, 11]. Some planktonic Cyanobacteria and Firmicutes store volatile compounds in gas vesicles allowing them to regulate their buoyancy [12]. Proteinaceous ferritins and membrane-bound acidocalcisomes act as storage compartments for iron and phosphorus, respectively [13, 14]. In some Planctomycetes membrane-based pirellulosome compartments are used to encapsulate the nucleoid in a similar manner as viruses use protein shells to protect their genetic information [15]. Finally, encapsulation can result in the creation of unique microenvironments in terms of pH, redox state and ionic strength required for specific processes exemplified by thylakoids and magnetosomes [16]. Protein or nucleic acid scaffolds represent another strategy for the co-localization of proteins *in vivo*. Examples include nonribosomal peptide synthetases (NRPSs) [17] and polyketide synthases (PKSs) [18], the cellulosome [19] and the ribosome [20].

The enormous diversity of intracellular encapsulation systems employed by nature precludes an exhaustive discussion of each particular compartmentalization strategy. Thus, we focus on describing a select number of naturally occurring proteinaceous (BMCs, ferritins and viral capsids) and membrane-based (acidocalcisomes, pirellulosomes, thylakoids and magnetosomes) strategies found in microbes (Figure 1). We highlight some of the recent synthetic biology applications and opportunities in creating engineered cellular organization, controlling metabolic pathway flux and producing functional materials.

Protein-based encapsulation in prokaryotes and viruses

Bacterial microcompartments (BMCs)

BMCs are proteinaceous, icosahedral compartments, between 40-500 nm in diameter that create a separate and distinctive microenvironment inside a bacterial cell (Figure 2A) [21]. BMCs encapsulate a number of metabolic pathways, thereby optimizing the function of specialized enzymes and chemical transformations. Their selectively-permeable protein shells consist of (pseudo)-hexameric and pentameric proteins that form the facets and vertices of an icosahedron, respectively [22]. Pores

present at the center of each hexamer are likely involved in the movement of substrates, products and cofactors across the BMC shell [22]. The fact that homologs of BMC shell proteins have been found in 17% of sequenced bacterial genomes spanning 13 phyla suggests that encapsulation using proteinaceous compartments is a common feature among a wide range of prokaryotes [23, 24]. The three best studied BMCs are carboxysomes [6, 7], the Pdu BMC [10] and the Eut BMC [11].

BMCs may provide a flexible and robust platform to engineer encapsulation with the aim of either improving their natural function (e.g. carbon-fixation efficiency of carboxysomes) or to create artificial metabolons for the production of high value chemicals, fuels and drugs [25]. Initial studies aimed at exploiting the genetic modularity of many of the known BMC systems by heterologously expressing them in model organisms. One study focusing on the recombinant engineering of BMCs successfully expressed several Pdu shell components from *Citrobacter freundii* in *E. coli* [26]. Despite the absence of all lumen proteins and a number of shell components, intact empty Pdu BMC shells could be observed. Eut BMC shell proteins from *S. enterica*, when heterologously expressed in *E. coli*, are also able to assemble into empty Eut compartments and EutS alone is able to produce a compartment that is morphologically similar to wild type Eut BMCs [27]. This finding raises the possibility of using a minimal BMC based on only one shell protein as a platform for synthetic biology applications.

The production of functional *Halothiobacillus neapolitanus* α -carboxysomes in *E. coli* has been achieved by transferring the complete α -carboxysome operon to the heterologous host [28]. This opens up the possibility to engineer and optimize carbon-fixation in *E. coli*. Finally, in a recent study empty geometrically uniform BMC shells from a BMC system of so far unknown function in *Haliangium ochraceum* could be produced in *E. coli* [29]. With a diameter of only 40 nm this compartment is the smallest BMC identified so far. In addition to heterologously produced native and minimal BMC shells, chimeric shells containing the α -carboxysome protein CsoS1 (*Prochlorococcus marinus*) within a β -

carboxysome shell have been produced in *Synechococcus elongatus* [30]. This approach likely results in modulated BMC shell permeability and represents a useful strategy for future engineering attempts.

One of the features that makes BMCs attractive for the construction of bionanoreactors is the presence of selective 4-7 Å pores formed by the (pseudo)-hexameric proteins that make up the facets of the icosahedral shell [31]. For BMCs to be enzymatically active, pores must allow the entry and exit of substrates, cofactors and products while at the same time excluding metabolites and proteins that would be detrimental to the encapsulated pathway. A number of studies have reported the selective influx and retention of BMC substrates and intermediates, respectively [32, 33]. In addition, iron-sulfur clusters occupying BMC shell pores were recently identified in the Pdu and glycyl-radical propanediol (Grp) BMCs that are likely involved in redox equivalent transfer across BMC shells [34, 35]. Ethanolamine may serve as a negative allosteric regulator of the shell protein EutL, which would confirm the presence of a gated pore in the Eut BMC [36, 37].

BMC size plays a key role in function; modulating BMC size influences both the total number of lumen components that can be encapsulated as well as the surface to volume ratio of the compartment. Deleting the short isoform of the β -carboxysomal matrix protein CcmM in *S. elongatus* leads to the formation of a small carboxysome-like structure (< 100 nm) [38] while fusing a reporter to CcmM generally results in abnormally large carboxysomes (> 175 nm) [39]. This shows that carboxysome size is influenced by its cargo. This fact might be exploited in future efforts to engineer carboxysomes as nanobioreactors using compartment size to control protein packing and small molecule diffusion rates and thus the activity of encapsulated enzymatic pathways.

For synthetic biology and biotechnology applications, one important factor is the overall stability of the assembled compartment in response to time, temperature and pH changes. A recent study of the Pdu BMC from *S. enterica* showed that this compartment is remarkably stable, retaining its native

structure for several days *in vivo* and *in vitro* when stored at room temperature [40]. While being more sensitive towards pH changes below pH 6 or above pH 10, the *S. enterica* Pdu BMC can tolerate temperatures up to 60°C without structural degradation.

To utilize BMCs as synthetic metabolons, specific enzymes must be targeted to and encapsulated by a given engineered BMC. Recent studies have identified short N- and C-terminal α -helical peptides that are used by various BMCs to specifically localize their native cargo. These targeting peptides have been successfully used to target non-native cargo to the inside of different BMCs, including green fluorescent protein (GFP), glutathione S-transferase (GST), maltose-binding protein (MBP) [41, 42] and β -galactosidase [27]. Besides the use of small helical tags a second targeting strategy employs fusions of non-native cargo proteins with natural lumen components exemplified by the successful targeting of GFP fusions to the Pdu and carboxysome BMCs [16, 43, 44]. Recently, the first functional bioreactor based on a BMC was reported [41]. The authors used the *Citrobacter freundii* Pdu BMC shell in combination with two targeting peptides to encapsulate a pyruvate decarboxylase and an alcohol dehydrogenase resulting in a functional ethanol producing bioreactor [41].

To take full advantage of BMCs as production platforms for non-native metabolic pathways it will be important to understand and control the mechanisms that drive BMC assembly. This would allow for the fine-tuning of the timing of BMC formation as well as control over BMC copy number and intracellular positioning. Initial insights into the spatial dynamics of BMCs have been gained through studies of β -carboxysomes in *S. elongatus* showing that cyanobacteria use their cytoskeleton to mediate the linear intracellular arrangement of carboxysomes and to ensure even BMC segregation during cell division [45]. Furthermore, it has been shown that the transcriptional activator PocR of the Pdu BMC system, when constitutively expressed in *S. enterica*, leads to Pdu BMC formation even in the absence of 1,2-propanediol [46]. The fact that overexpression of a positive transcriptional regulator leads to the

formation of morphologically normal Pdu BMCs may indicate that other BMCs could also be decoupled from their endogenous regulatory mechanisms.

Ferritins

Ferritins are a family of intracellular iron storage compartments consisting of 24 protein subunits (Figure 2B). They are ubiquitously distributed throughout the three domains of life. Ferritins self-assemble to form hollow protein cages with an outer diameter of 12 nm and an inner diameter of 7-8 nm [47]. Ferritins are able to store approximately 4500 iron atoms as hydrated ferric oxide particles in a soluble and bioavailable form [13]. Iron entry and exit through the protein shell most likely occurs through eight 0.4 nm pores located at the 3-fold axis of the compartment [48, 49]. Storage of iron inside ferritins not only prevents the spontaneous oxidation of intracellular ferrous iron to ferric iron which is known to produce toxic free radical species through the Fenton reaction, but is also important for cellular iron homeostasis and various regulatory processes [50-53].

The ability of ferritins to mineralize uniform inorganic nanoparticles of varied composition, the stability of their protein shell to pH (pH 2-11) and temperature (up to 80°C) changes [54, 55] and the possibility to easily modify their interior and exterior via genetic or chemical means, allows their potential use in a wide range of biomedical and materials science applications [56, 57]. The electronic, magnetic and optical properties of nanoparticles depend on uniform size and shape which can be achieved using ferritin [58, 59]. The reduced polydispersity achievable using ferritin for size-constrained nanoparticle synthesis represents an advantage over many classical synthesis methods [58, 59]. In addition, encapsulated inorganic particles are rendered water soluble and biocompatible due to the surrounding protein shell. We will focus on the use of ferritins to encapsulate and precipitate inorganic nanoparticles *in vivo*.

A study using the silver binding AG4 peptide in combination with human ferritin showed that it is possible to prepare uniform silver nanoparticles in *E. coli* [60]. The ferritin producing strain showed less reduction of cell viability in the presence of high concentrations of silver salts presumably due to reduction of Ag^+ to Ag^0 and sequestration inside ferritins. By expressing ferritin and modifying iron homeostasis at the same time, biogenic magnetization in yeast has been engineered [61]. Subsequent magnetization-based screening of gene knockouts identified TORC1 (target of rapamycin complex 1) and several carbon metabolism genes as important for magnetization. Ferritins have also been used to engineer mammalian cells that show superparamagnetic behavior [62]. Through the combination of ectopic production of human ferritin and the concomitant overexpression of the iron importer DMT1, efficient mineralization of magnetic iron oxide particles within ferritin cages was achieved. This study presents a useful technology for applications ranging from diagnostics to cell-based therapies including ferritin-based magnetic resonance imaging (MRI) for cell tracking and targeted drug delivery [56, 57]. Future efforts will focus on expanding single particle engineering to the controlled assembly of defined higher order bioinorganic hybrid materials *in vivo*.

Virus capsids

Due to their unique and highly organized structures, virus capsids have long been recognized as promising biologically derived building blocks and encapsulation systems for a variety of applications including vaccine development [63, 64], gene therapy [65], directed delivery of pharmaceuticals [63, 66] and screening for biomolecular recognition events [67]. In addition, virus capsids have been exploited for the synthesis of inorganic nanomaterials [68] and have been used as bionanoreactors to carry out a number of biochemical reactions [57]. The properties that make virus capsids interesting for synthetic biology applications are their structural homogeneity, convenient production and purification, addressable functionalization and ease of modification via genetic or chemical means [57, 68]. In principle, non-native cargo can be positioned either in the inner cavity or on the outer surface of virus

capsids. When both of these approaches are combined multiple distinct or synergistic functionalities can be integrated into a single nanoarchitecture [69-71].

Virus capsids generally self-assemble from hundreds or thousands of copies of one or multiple capsid proteins. Examples of commonly used virus capsids include small and medium-sized icosahedral bacteriophages like MS2 (outer diameter (d_a): 27 nm) [72], P22 (d_a : 64 nm) [73] and Q β (d_a : 29 nm) [74], small icosahedral plant viruses like cowpea chlorotic mottle virus (CCMV, d_a : 28 nm) [75], large icosahedral insect viruses like chilo iridescent virus (CIV, d_a : 185 nm) [76] and large rod-shaped viruses like M13 (dimensions: 6.6 x 880 nm) [77] and tobacco mosaic virus (TMV, dimensions: 18 x 300 nm) [78]. Below, we focus on applications that employ virus capsids for the *in vivo* preparation of functional encapsulation systems highlighting the positioning of enzymes in the interior of virus capsids and the properties of the resulting artificially encapsulated enzymatic systems.

In general, two different approaches exist by which foreign material can be targeted to the inside of a virus capsid *in vivo*. Protein-protein interactions in combination with fusions of cargo proteins and capsid components can be used to position specific protein components inside virus capsids. Second, protein-ribonucleic acid (RNA) interactions can be exploited for *in vivo* cargo targeting using certain virus capsids that interact with specific RNA hairpin/aptamer structures.

By using *E. coli* for the co-expression of P22 capsid components together with the alcohol dehydrogenase AdhD (*Pyrococcus furiosus*) *in vivo* encapsulation was achieved [79]. Targeting of AdhD to the inside of P22 was accomplished by fusion to the scaffold protein that lines the inner cavity in the native P22 phage. About 250 molecules of AdhD were incorporated into the P22 capsid *in vivo*. Further experiments showed that encapsulated AdhD still followed Michaelis-Menten kinetics and did not show substrate inhibition, which is generally observed for free AdhD. Through heterologous co-expression about 80 monomers of the tetrameric β -glucosidase CelB (*P. furiosus*) could also be functionally

encapsulated within the P22 capsid and shown to follow the same overall kinetics as non-encapsulated CelB [80].

The first example of targeting a three enzyme pathway to the inside of a virus capsid *in vivo* was recently reported. The three enzymes that constituted the enzyme cascade were CelB, GALK and GLUK, which convert lactose to galactose-1-phosphate and glucose-6-phosphate mimicking naturally occurring reactions found in sugar metabolism [81]. GALK and GLUK were covalently fused with CelB through flexible polyglycine linkers. On average about 15 copies of the fusion protein could be localized to the inside of the P22 protein cage. Kinetic studies indicate that the overall catalytic rate depends on the efficiency of intermediate channeling between sequential enzymes.

In an intriguing approach the P22 capsid was used to produce soluble proteins that under normal circumstance would instead form insoluble inclusion bodies. The α -galactosidase GalA (*P. furiosus*) and the hemagglutinin head HA_h from influenza are known to form inclusion bodies when heterologously expressed in *E. coli* [82]. By targeting them to the inside of the P22 cage, they remained soluble and active.

An alternative targeting approach that relies on protein-RNA interactions has been recently developed using the Q β capsid and its interaction with an RNA-hairpin/aptamer present in the natural phage RNA [83-85]. By creating constructs that additionally contain a second orthogonal RNA-aptamer, it is possible to specifically target non-native cargo fused with RNA-binding protein domains to the inside of the Q β protein cage. Peptidase E and luciferase were successfully encapsulated *in vivo* and shown to be catalytically active inside Q β particles [86]. Encapsulated peptidase E and luciferase were less sensitive to inactivation by temperature shifts and could be adsorbed onto polystyrene surfaces while retaining their enzymatic activities. The same encapsulation strategy was used to position a number of fluorescent proteins in the interior of the Q β capsid [87]. The authors additionally functionalized the

exterior surface of the resulting Q β particles with a carbohydrate-based targeting ligand for the CD22 receptor and showed that the artificially encapsulated fluorescent particles could be used for specific cell labeling. Thus the variable sizes and shapes of virus capsids hold great promise as addressable building blocks for the creation of tailored nanomaterials with novel medicinal, magnetic and plasmonic properties.

Lipid-based encapsulation in prokaryotes

Membranous organelles are used for the production of specific compounds by targeting certain pathways to their interior exploiting the particular internal microenvironments. The targeting strategy is based on short sequences at the termini of a given cargo protein easily applicable in synthetic compartmentalization strategies [88-90]. Intracellular lipid vesicles have been generated through the overexpression of non-native proteins in a heterologous host. The expression of the *Acholeplasma laidlawii* glucosyltransferases aLDGS and aLMGS in *E. coli* leads to the formation of many intracellular membrane vesicles per cell with diameters of about 100 nm. Lipid composition was similar to the inner membrane hinting at the origin of the observed vesicles [91]. In a related study, the expression of eukaryotic calveolin in *E. coli* resulted in the production of 50 nm calveolae whose contents derived from the bacterial periplasm [92].

While no standard set of membraneous organelles exists in prokaryotes, as is the case for eukaryotic cells, different bacterial species can possess membrane-bound compartments providing them with unique capabilities [2]. Subsequently, we will focus on the description of a selection of intracellular membrane systems and free-standing membrane-bound compartments in bacteria highlighting their potential use as bionanoreactors and tools to study organelle biogenesis and evolution.

Acidocalcisomes

Acidocalcisomes are acidic double membrane-bounded organelles that contain high concentrations of phosphorus as polyphosphate (polyP) and pyrophosphate (PP_i) as well as calcium (Figure 3A) [14]. They have been identified in a wide range of organisms ranging from bacteria to human cells and appear as up to 200 nm spherical organelles in bacteria while their size and morphology in eukaryotes can vary more widely. Acidocalcisomes, also referred to as volutin or polyphosphate granules [93-95], have been suggested to be the only known organelle conserved over evolutionary time from bacteria to higher eukaryotes. Besides phosphate storage, acidocalcisomes have been implicated in calcium homeostasis and signaling, maintenance of intracellular pH and osmoregulation [14]. In bacteria the acidocalcisome membrane contains a signature proton pyrophosphatase responsible for the low pH encountered in the acidocalcisome lumen [96, 97]. The membrane additionally contains a species-dependent combination of other channels, pumps and antiporters while various enzymes involved in PP_i and polyP metabolism are found in the acidocalcisome interior. Due to the reported importance of acidocalcisomes in the virulence of several pathogens including *Helicobacter pylori*, *Mycobacterium tuberculosis* and *Salmonella enterica* among others as well as of parasites such as different *Trypanosoma* and *Leishmania* species they represent promising new drug targets [14].

The fact that specific enzymes have been identified inside acidocalcisomes indicates that a dedicated targeting mechanism must exist. Although proteomic analysis of acidocalcisome fractions from *T. brucei* revealed a number of proteins that localize to the acidocalcisome [98], the set of genes necessary for acidocalcisome formation has not been identified. Interestingly, high levels of other metals have been detected in acidocalcisomes of different bacteria, including aluminum, barium, cadmium, cobalt, copper, lead, manganese, mercury, nickel and strontium when cultivated in the presence of these metals [99-101]. Future applications might include the targeting of enzymes that depend on an acidic microenvironment, the synthesis of inorganic nanoparticles and polymers which

has been shown to be highly sensitive to pH, engineered heavy metal detoxification and phosphorus removal from wastewater.

Pirellulosomes

One of the most surprising compartmentalization strategies found in the bacterial domain are lipid-bilayer bounded compartments that contain the bacterial chromosome termed pirellulosomes (Figure 3B) [15]. So far, this organelle has been exclusively identified in the three phyla Planctomycetes, Verrucomicrobia and Chlamydiae and is surrounded by a thin layer of distinctive cytoplasm referred to as the paryphoplasm [102]. A number of *Planctomycetes* show even more complex compartmentalization of their intracellular space where the pirellulosome is further subdivided into smaller membrane-bound compartments, including an additional lipid-bilayer surrounding a highly compacted nucleoid in *Gemmata oscuriglobus* (Figure 3C) [103]. Some *Planctomycetes* contain a second membraneous compartment inside the pirellulosome referred to as the anammoxosome (Figure 3D), named for its ability to anaerobically oxidize ammonium (anammox reaction) [104, 105]. An additional unusual feature of the anammoxosome is that more than 50 percent of its membrane is composed of a highly unusual type of lipid called ladderanes, defined by the presence of two or more fused cyclobutane rings [106]. Ladderanes represent high-energy lipids that have been suggested as a biofuel, although their biosynthesis is currently unknown.

Organisms capable of performing the anammox reaction have received increased attention over the last years. The main application of biological ammonium oxidation is in nitrogen removal from wastewater [107]. The anammox process presents several advantages compared to conventional nitrogen removal from wastewater. No additional energy and carbon sources have to be added to the reactor due to the anaerobic and autotrophic nature of anammox bacteria leading to a highly energy efficient process. Very little excess sludge is produced because of the slow growth of *Planctomycetes*.

Through the formation of stable aggregated biofilms or granules reactors can be very compact with high biomass concentrations and conversion rates [108]. These factors lead to an overall cost reduction of up to 60 percent [109, 110] and lower overall carbon dioxide emission [111]. Efforts aimed at establishing genetic tools for *Planctomycetes* as well as approaches to heterologously express and engineer the anammox process in more tractable model organisms hold promise for improving this useful biotechnological process and for shedding light on a biological process that might contribute up to 50 percent of the dinitrogen produced by the world's oceans [112].

Thylakoids

Thylakoids are photosynthetic intracytoplasmic membrane systems found in cyanobacteria that are used to maximize the efficiency of oxygenic photosynthesis [113, 114]. By increasing the number and concentration of the photosynthetic protein complexes photosystem I and II (PS I and II) as well as other components needed for light harvesting and utilization, maximizing membrane surface area and creating an optimized microenvironment, thylakoids enable efficient phototrophic growth of cyanobacteria [113]. About 30 percent of the overall photosynthetic productivity on Earth is due to cyanobacterial oxygenic photosynthesis facilitated by thylakoids [115]. Although thylakoid morphology is highly variable from species to species in most cases they appear as flattened and stacked layers of elongated lipid bilayer-bounded compartments aligned with the plasma membrane (Figure 3E) [116-118].

From a synthetic biology perspective cyanobacteria represent promising hosts for the production of various chemicals, including biofuels, bioplastics, commodity chemicals and high-value pharmaceuticals using only CO₂, light and water [119]. The general approach of targeting enzymes or non-native pathways to the thylakoid aims at exploiting the high concentration of reducing equivalents in this organelle caused by the presence of high levels of photosynthetic complexes in the thylakoid

membrane [120]. In a recent study, the P450 CYP79A1, naturally localized to the endoplasmic reticulum (ER) membrane in the plant *Sorghum bicolor*, was targeted, expressed and functional in the thylakoid membrane of *Synechococcus* sp. PCC7002 [121]. Targeting was achieved by fusion of the P450 to a PS I subunit; light-driven hydroxylation of L-tyrosine by CYP79A1 could then be demonstrated. Furthermore, the three-enzyme pathway for the L-tyrosine-derivative dhurrin (*S. bicolor*) involving two P450s natively localized in the ER was transferred to the chloroplast of *S. bicolor* [122]. This resulted in the light-driven high-yielding production of dhurrin. Future efforts will likely focus on the targeting of biosynthetic pathways of high-value compounds that depend on the activity of P450s. Terpenoids are particularly interesting candidates for this strategy because their carbon skeletons are modified by a vast number of specific P450s yielding a huge set of structurally diverse natural products [123, 124]. Many terpenoids are already commercially produced including fragrances, food ingredients, therapeutics and fine chemicals, with the global market for terpenoids estimated to be in the billion dollar range [125].

Magnetosomes

Magnetotactic bacteria possess a lipid-bilayer bounded compartment called the magnetosome (Figure 3F) [126] with which they are able to control their relative position with regards to the Earth's magnetic field. This helps them in their search for their preferred redox milieu in a process termed magnetoaerotaxis [127]. Additional possible functions of magnetosomes include respiration [128] and detoxification [129]. This unique organelle is generated from invaginations of the cell membrane and contains 50 nm magnetic crystals of either magnetite (Fe_3O_4) or greigite (Fe_3S_4) [126]. Individual magnetosome compartments are linearly aligned along the longitudinal axis of the cell by bacterial actin homologs [130]. Magnetosome biogenesis has been the focus of a number of recent studies showing that its formation relies on about one hundred genes whose products form membrane invaginations, target protein and other magnetosome components, nucleate crystal formation and finally align individual magnetosomes to form a chain [131, 132].

A first step in magnetosome engineering was the heterologous expression of the *M. gryphiswaldense* magnetosome gene cluster in the photosynthetic model organism *Rhodospirillum rubrum* resulting in morphologically normal aligned magnetosomes [133]. In an interesting study a fusion protein of the magnetosome component MamC and an antibody fragment was expressed in *M. gryphiswaldense* [134]. After isolation of magnetosomes, the respective antigen localized and bound to the antibody-presenting magnetosome opening up the possibility of using biogenic magnetic nanoparticles in various *in vitro* and *in vivo* applications. In a similar approach, a phosphohydrolase was fused to MamC and after magnetosome isolation from *M. magneticum* the resulting magnetic particles were shown to degrade a pesticide *in vitro* [135]. Additional applications of magnetosomes include drug delivery, bioinspired nanoreactors for nanoparticle synthesis, labeling and immobilization of biomolecules and in targeted imaging studies to just name a few [136, 137]. To pave the way for future biotechnological applications that require precise control over the various components responsible for magnetosome assembly and function additional studies aimed at understanding all the factors governing magnetosome biogenesis are needed. This would result in the possibility to not only engineer the size, shape and intracellular alignment of magnetic particles but also to create synthetic inorganic nanoparticles composed of various functional materials not found in nature.

Concluding remarks

Encapsulation systems discussed in this review use well-characterized compartments like viral capsids and ferritins as well as less studied compartments such as thylakoids, acidocalcisomes, pirellulosomes and anammoxosomes. While the application potential of viral capsids and ferritins is relatively mature and mainly limited by the imagination of synthetic biologists, the lack of basic understanding of compartment formation is the limiting factor for most of the other systems discussed. Thus, increased efforts are needed to better understand proteinaceous and membrane-bound compartment formation at a basic and mechanistic level. Major challenges for the use of membrane-

bound organelles include targeting of non-native components, controlling cargo stoichiometry and the secretion of products. If efforts to resolve these challenges prove successful, engineered spatial control of cellular metabolism using encapsulation could result in increased yields of valuable and useful compounds and lead to a better understanding of native biological organization.

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Figures and captions:

Figure 1: Schematic representation of all the compartments discussed in this review. The relative dimensions between the different compartments do not reflect their actual size, which is given after the respective compartment name.

Figure 2: Overview of the proteinaceous compartments discussed in this review. Above, a carboxysome model and biological assemblies of the other compartments based on the respective monomer crystal structures are shown (Ferritin, PDB ID: 4CMY, Bacteriophage MS2, PDB ID: 2IZ8, Tobacco Mosaic Virus (TMV), PDB ID: 2OM3). Monomers of the ferritin, MS2 and TMV protein cages are highlighted in orange. For TMV only a short section of the rod-shaped capsid is shown. Below, transmission electron micrographs of the compartments can be seen, (a) β -carboxysome, (b) ferritin [138], (c) MS2 capsid without RNA, (d) TMV, © 1994 Rothamsted Experimental Station.

Figure 3: Transmission electron micrographs of all lipid-based compartments discussed in this review. (a) Ac: acidocalcisome [139], (b) Pi: pirellulosome [15], An: anammoxosome [140], Nu: nucleoid, (c) Th: thylakoid, (d) Ma: magnetosome [141, 142].

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