Computational Modeling and Evolutionary Implications of Biochemical Reactions in Bacterial Microcompartments

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Abstract:

Bacterial microcompartments (BMCs) are protein-encapsulated compartments found across at least 23 bacterial phyla. BMCs contain a variety of metabolic processes that share the commonality of toxic or volatile intermediates, oxygen-sensitive enzymes and cofactors, or increased substrate concentration for magnified reaction rates. These compartmentalized reactions have been computationally modeled to explore the encapsulated dynamics, ask evolutionary-based questions, and develop a more systematic understanding required for the engineering of novel BMCs. Many crucial aspects of these systems remain unknown or unmeasured, such as substrate permeabilities across the protein shell, feasibility of pH gradients, and transport rates of associated substrates into the cell. This review explores existing BMC models, dominated in the literature by cyanobacterial carboxysomes, and highlights potentially important areas for exploration.

Highlights:

- Computational modeling of BMCs enlightens experimentally hard-to-measure features.
- Significant exploration remains of catabolic BMC capabilities.
- Oxygen sensitivity is relatively common across BMC-contained enzymes.
- BMC shell proteins are selectively permeable to negatively charged metabolites.

Introduction:

Once thought of as simple ‘sacks of enzymes’, it is now clear that the internal organization of bacteria rivals that of eukaryotes. For example, photosynthetic cyanobacteria not only contain internal photosynthetic membranes responsible for producing the oxygen that

has transformed Earth's atmosphere enabling eukaryotic evolution and survival, they also contain carboxysomes. Carboxysomes are a class of protein-based organelles, termed bacterial microcompartments (BMCs), that resemble icosahedral viral capsids [1]. BMCs, defined by a common shell architecture, contain different suites of enzymes in their core and have been found across at least 23 diverse bacterial phyla [2]. They fall within two main categories, catabolic metabolosomes and anabolic carboxysomes (**Figure 1**). Both categories of BMCs create a chemical and physical microenvironment inside the bacterial cytoplasm. BMCS are optimized to perform chemical reactions which would otherwise be toxic to other cellular components, create volatile intermediates, or involve enzymes, coenzymes, or intermediates that are inactivated by the presence of oxygen. BMCs are of interest for their potential applications in modulating the flux and selectivity of biochemical reactions, containment of toxic intermediates from industrial reactions, antigen display for vaccine development, and creation of nanoparticles in chemotherapy targeting [3,4]. Recent reviews have delved into a wide variety of topics such as BMC positioning [5], BMC repurposing for industrial uses [6], evolutionary relationship of shell proteins across different BMCs [7], and BMC protein stoichiometry [8,9].

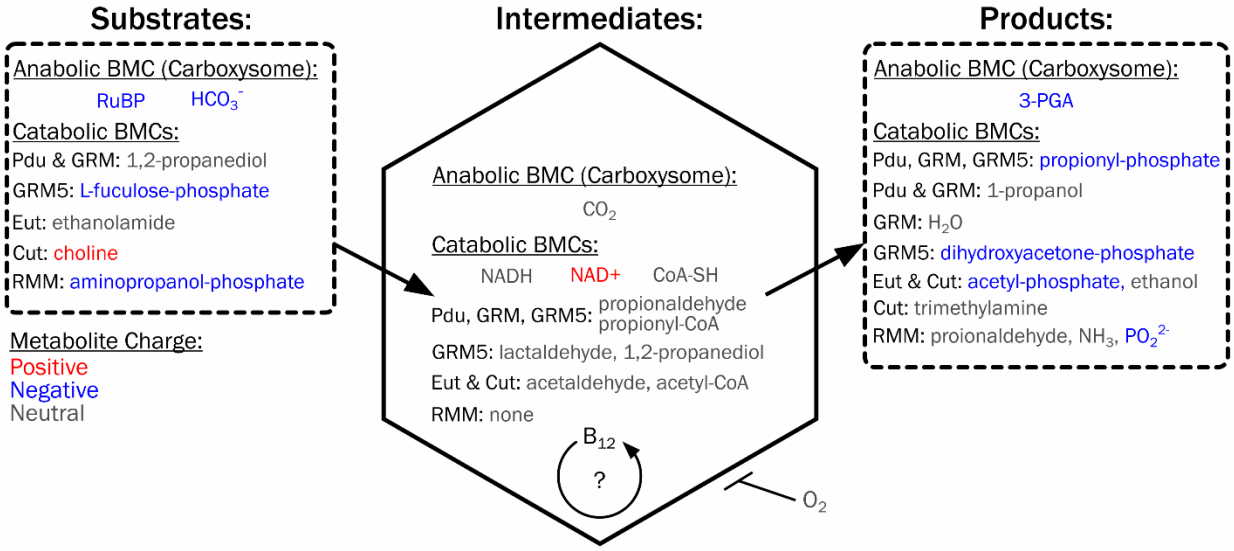

Figure 1. Diagram of the substrates, intermediates, and products of carboxysomes and the most studied catabolic BMCs [10–13]. The charge of each metabolite is indicated with color; positive in red, negative in blue, and neutral in grey. Nearly all the intermediates are nonpolar whereas substrates and products, which transverse the protein shell, are predominantly negatively charged or nonpolar. There is theorized inclusion of B_{12} cycling inside some catabolic BMCs as well as a common exclusion of oxygen [11,13]. Abbreviations: BMC, bacterial microcompartments; RuBP, ribulose-1,5-bisphosphate; Pdu, 1,2-propanediol utilization; GRM, glycyl radical enzyme-associated microcompartments; GRM5, fuculose-associated microcompartment; Eut, ethanolamine utilization; Cut, choline-utilization; RMM, 1-amino-2-propanol utilization; 3-PGA, 3-phosphoglyceric acid.

Despite this intense focus on synthesizing what is currently known about BMCs, many important details remain unknown about the function(s), assembly mechanisms, stability, and

ultimate use in enhancing the biochemical reactions that they encapsulate. Due to their small size (40-600nm)[14,15] and sub-cellular heterogeneity (e.g. intact/functional, assembly intermediates, and degrading stages (**Figure 2b**)), the chemical environment inside BMCs remains challenging to experimentally measure with standard technologies. Recent work tracking the position and activity of individual BMCs for the first time through single-cell lineages addresses this heterogeneity and points towards a solution for understanding how the chemical environment of BMCs dictate activity and degradation [16].

While empirical knowledge of how BMCs function *in vivo* and *in vitro* is hard won, computational modelling can be used to develop hypotheses about the encapsulated pathways using existing knowledge of enzymatic reaction rates, metabolite concentrations, and transport phenomena. Models can be designed to focus on specific aspects of BMC function with a relatively simple framework containing the relevant components or expanded to include whole cell physiology. However, the uncertainty of these predictions increases along with the number of variables in the associated model, leading to potentially misleading conclusions [17–20]. Thus, one of the most challenging aspects of modeling is determining what should and should not be included and what assumptions are reasonable to make in simplifying the model. In this review, we synthesize existing BMC models, focusing on what assumptions were made in their construction, and emphasizing areas for further investigation. We focus on models of carboxysomes due to their long history and a recent spate of new advances. With these models we can explore the parameter space that BMCs may functionally occupy, test evolutionary pressures leading to formation, and help focus the direction of future experimental work. Ultimately, these quantitative understandings could enable the *de novo* design and engineering of novel BMCs for diverse functions [21,22].

Carboxysomes:

Found in cyanobacteria [23] and chemoautotrophic bacteria [24], carboxysomes are the only known anabolic BMC and contain the enzymatic machinery to fix carbon dioxide (CO_2) from the atmosphere into organic compounds needed to generate biomass (**Figure 2A**). Carboxysomes are notable for their role in the cyanobacterial CO_2-concentration mechanism (CCM) which allows cyanobacteria to contribute nearly 25% of fixed carbon globally [25]. The intact carboxysome shell is thought to be largely impermeable to CO_2 [26–29] and oxygen (O_2) [28,29] but permeable to bicarbonate (HCO_3^-) (**Figure 2**). Therefore, an increased local concentration of carboxysomal CO_2 sourced from HCO_3^- can be created around Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the rate-limiting enzymatic step of CO_2-fixation, alongside a simultaneous exclusion of O_2, a competitive inhibitor. The RuBisCO oxygenation reaction produces 2-phosphoglycolate, a competitive inhibitor of triose-phosphate isomerase [30], and requires metabolization through an energetically expensive pathway called photorespiration to recover invested CO_2 and nitrogen while regenerating ribulose-1,5-bisphosphate (RuBP). While not aiding directly in sugar production, photorespiration may play an important role in preventing reactive oxygen species production, production of serine and cysteine, and assimilation of sulfur and nitrogen [31–33]. Recent experimental work identified

three separate cellular pools of RuBisCO in the same cell as a result of the dynamic assembly and degradation pathways that are hypothesized to exhibit distinct metabolic activities (**Figure 2B**) [16**].

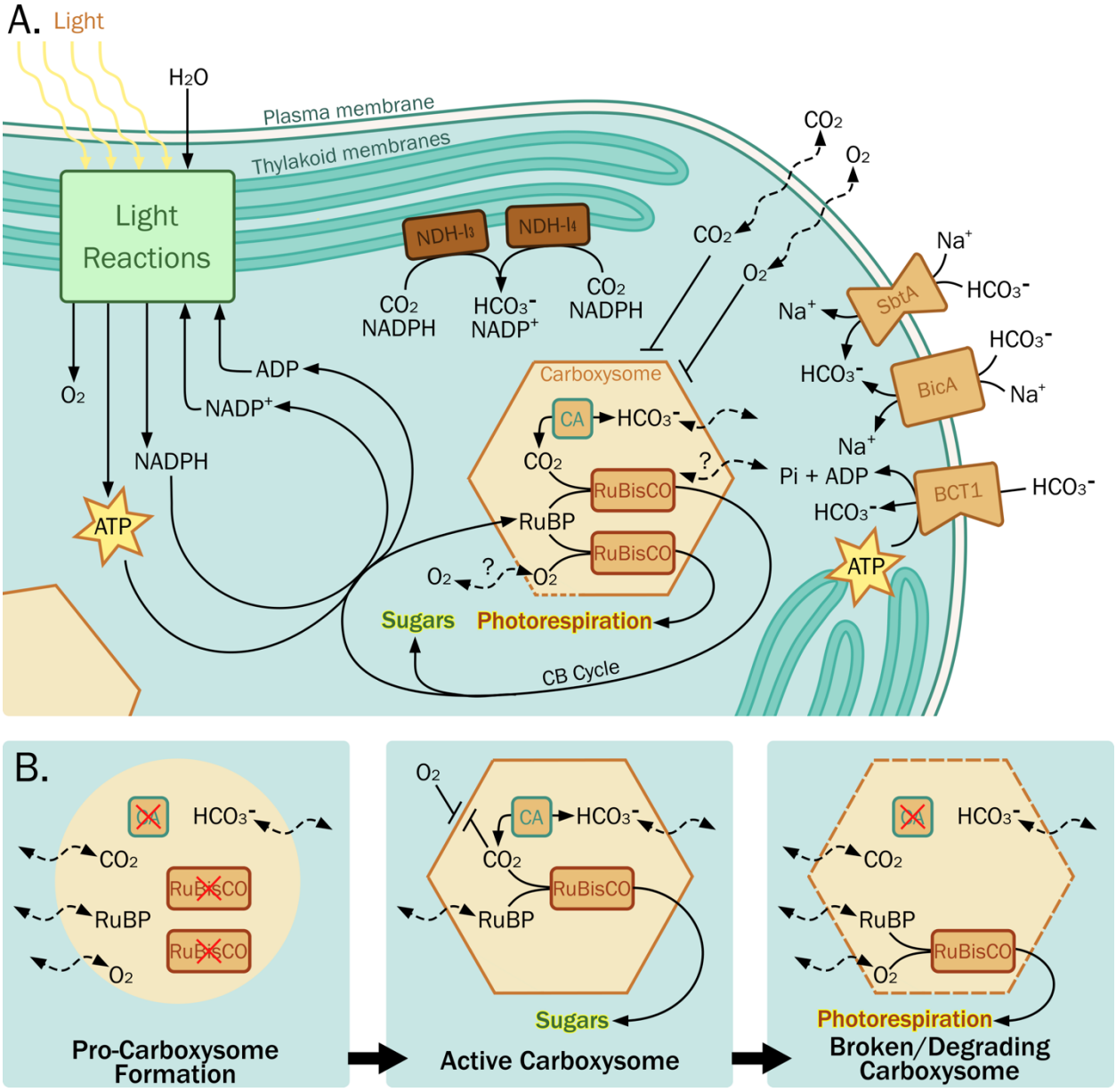

Figure 2. Schematic representations of the CO_2 concentrating mechanism (CCM) and light reactions of oxygenic photosynthesis in beta-cyanobacteria. Included are the active carbon uptake complexes, NDH-I_3 and NDH-I_4, bicarbonate transporters, BCT1, BicA, and SbtA, and carboxysome enzymes, carbonic anhydrase (CA) and Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). (A) Inorganic carbon, in the form of bicarbonate (HCO_3^-), is actively transported via transporters (BCT1, BicA, SbtA) into the cell. Carbon dioxide (CO_2) enters the cell via passive diffusion and is then converted to HCO_3^- by thylakoid membrane-localized NDH-I_3 and NDH-I_4 to increase cytoplasmic HCO_3^- concentration. Unlike CO_2 and oxygen (O_2), HCO_3- diffuses across the proteinaceous shell into the carboxysome, where it is brought into equilibrium with CO_2 by CA in a fully reversible reaction. CO_2 is

then fixed by RuBisCO using ribulose-1,5-bisphosphate (RuBP) as an acceptor molecule, resulting in the production of substrates that are converted into carbohydrates through the Calvin-Benson Cycle (CB Cycle). The CB Cycle is powered with ATP and NADPH produced from the photosynthetic light reactions. RuBisCO may require inorganic phosphate (Pi) for activation. RuBisCO can also undergo a side reaction with O_2 leading to an energetically expensive carbon recovery pathway called photorespiration, but this reaction may mainly occur in broken carboxysomes (dotted shell line) due the impermeability of O_2 to the carboxysome. (B) Three co-existing pools of RuBisCO in the same cell during the carboxysome life cycle [16] can exhibit distinct metabolic activities because of encapsulation and CA activation/inactivation based on redox environment [34] that modulates substrate accessibility and concentration. Abbreviations: CA, carbonic anhydrase; RuBisCO, Ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; CB Cycle, Calvin-Benson Cycle.

Carboxysome Models: As the first identified and prototypical BMC, most computational modeling work has been conducted on the carboxysome with the initial models published in the 1980s [35–39]. Even the most recent models have roots in these early versions, with more information being incorporated on the composition, structure, and activity of the carboxysome-encapsulated reactions as a result of scientific advancements over the last three decades.

Table 1. Comparison of parameters used in the CCM Models. Dashes indicate values not reported, NA indicates values not applicable to the set-up of the model, and double slashes indicate two different modeled values.

Parameters	Hopkinson et al. 2014 [40]	Clark et al. 2014 [41]	Mangan & Brenner 2014 [42]	Mangan et al. 2016 [43]	Boatman et al. 2018 [44]	Long et al. 2021 [45]
Environmental Species Concentrations						
HCO_3^- (μM)	0 - 2000	1955	14 [46]	100	100	10 – 25000 ** [47–49]
CO_2 (μM)	Varies	45	0.14 [46]	15	15	0.01 – 25 **
O_2 (μM)	-	400 [50–52]	260	300	300	250 – 360 ** [53,54]
Permeability and Transport Rates:						
Cell membrane permeability to CO_2 (cm/s)	0.65 ** [55]	no barrier [56]	0.3 [56,57]	0.3 [56]	0.3 [56]	NA
Cell membrane permeability to HCO_3^- (cm/s)	complete barrier	complete barrier	3.00E-04 [56,57]	2.00E-07 [56]	2.00E-07 [56]	NA
Carboxysome permeability to CO_2 (cm/s)	2.37E-05 ** ‡	complete barrier [46]	1.00E-03	1.00E-04 [39]	3.00E-05	1.00E-04 **
Carboxysome permeability to HCO_3^- (cm/s)	0.71 ** [55]	no barrier [56]	1.00E-05	1.00E-04 [39]	3.00E-05	1.00E-04 **
HCO_3^- active transport rate (molecules/s)	1.51E+04 **	1.11E+06 ** [58]	1.59E+08 ** ‡	1.14E+09 ** ‡	1.63E+04 ** ‡	NA
Compartment sizes and pH:						
Carboxysome radius (cm)	8.19E-06**	1.00E-05 [59]	5.00E-06	5.00E-06	1.50E-05	1E-04 // 5E-06 **

Cell/system radius (cm)	3.50E-05**	1.00E-04 [60,61]	5.00E-05	5.00E-05	3.00E-04	62 **
Number of Carboxysomes	6 [62]	1-4 [50–52]	1	1	1	1
Carboxysome pH	7.35	8	NA	7	7	<8
Cytoplasm pH	7.35	8	NA	8	8	8
Extracellular pH	8	8.2	NA	7	8.3	8
Enzyme parameters:						
RuBisCO active sites	3975 ** ‡	3351 [50–52]	2160 [63–65]	2000 [65,66]	54000	2.52E+07 // 3011 ** [48,67]
RuBisCO k_{cat} (s^{-1})	10.6 [68]	11.4 ** [50–52]	26 [63–65]	11.6 [68–70]	1.92	14.4 [48,67]
RuBisCO $K_{1/2}$ (μM)	263	185 [50–52]	270 [63–65]	340 [68–70]	145 [71]	172 [48,67]
RuBisCO specificity ($S_{c/o}$)	-	-	-	43 [68–70]	45 [71]	40 [48,67]
CA active sites	NA	NA	80 [63–65]	100 [65]	900	NA
CA hydration k_{cat} (s^{-1})	1000 ^	1.14E+05 ^ [71,72]	8.00E+04 [63–65]	8.00E+04 [65]	NA	0.05 ^
CA hydration $K_{1/2}$ (μM)	NA	NA	3.20E+03 [63–65]	3.20E+03 [65]	104.7 ^	NA
CA dehydration k_{cat} (s^{-1})	NA	NA	4.60E+04 [63–65]	4.60E+04 [65]	NA	100 ^
CA dehydration $K_{1/2}$ (μM)	NA	NA	9.30E+03 [63–65]	9.20E+03 [65]	NA	NA

** Values converted to the presented unit for comparison across models
^ Values based on establishing equilibrium between CO_2 and HCO_3^- in the carboxysome
‡ Values fitted within the model to match experimental values

Two different forms of BMCs with independent evolutionary origins are found within cyanobacterial species, alpha- and beta-carboxysomes. While structurally and functionally similar, including the presence of homologous shell proteins, the molecular scaffolds used in the assembly are distinct. Beta-carboxysomes form inside-out beginning with the aggregation and polar localization of CA and RuBisCO prior to encapsulation via shell proteins [73]. The dynamic assembly pathway and hierarchy of protein-protein interactions during alpha-carboxysome formation *in vivo* remains to be fully elucidated. Although historically, models were based on CCMs in beta-cyanobacteria, Hopkinson *et al.* explored an alpha-carboxysome-based model founded on experimental data from *Prochlorococcus sp.* They concluded that the CCM mechanism of alpha-carboxysomes functions very similarly to the more studied beta-carboxysomes [40]. The knowledge gap in carboxysome shell permeability was overcome by fitting the model to their experimental data to estimate parameter values, but this methodology ultimately limited the applicability of this model to other organisms (**Table 1**). The impact of O_2 on carbon fixation was not addressed in this paper despite the relevance of O_2/CO_2 competition with RuBisCO.

The study published by Clark *et al.*, took a more inclusive modeling approach inspired by chemical engineering to systematically investigate how different beta-carboxysome counts (down to zero in a CCM-lacking mutant), size, and cellular position impacts CO_2-fixation rates [41]. They found that a consistent number of RuBisCO units within the cell was the most important parameter impacting CO_2 fixation (**Table 1**) [41]. An analysis using dimensionless Dahmköhler numbers was also used to identify controlling phenomena in the CCM based on transport and reaction rate considerations. Additionally, this study found that a carboxysome-lacking mutant begins to mimic a potential pre-carboxysome form of ancestral cyanobacteria, the physiology of which can be replicated with CCM knockout cell lines. Notwithstanding this insight, this paper does not explore the evolutionary implications of cells without a CCM. This model also assumes zero permeability of the carboxysome to O_2 and CO_2, although more recent evidence indicates that the transport properties of various substrates across the shell could change over the life cycle of the BMC as a result of assembly, breakage, and degradation (**Figure 2B**).

The most recent models have focused specifically on the pH dynamics of beta-cyanobacterial CCMs. Mangan *et al.*'s study explored how the CCM handled different species of inorganic carbon with a "pH-aware" model and found a lower carboxysome pH relative to the cytosol was favorable for increased carbon fixation activity [42,43]. Boatman *et al.* applied this model to an experimental study of *Trichodesmium erythraeum,* a filamentous diazotrophic cyanobacteria, with notable increases in their cell and carboxysome size parameters to match *Trichodesmium* (**Table 1**)[44]. They determined the importance of HCO_3^- and pH on CO_2 fixation rate and that elevated pH impacted CO_2 fixation through processes outside of the CCM [44]. Most recently, Long *et al.* modeled the inclusion of 'proton transport' in and out of the carboxysome via RuBP and PGA, the substrate and product of RuBisCO's carboxylase activity respectively [45]. This paper placed the carboxysome in a m^3 cytosolic solution and excluded the HCO_3^- transport aspect of the CCM (**Table 1**). This study included the generation of protons from CO_2-fixation and concluded that this creates a locally lowered pH in the carboxysome which, in turn, drives CA's equilibrium reaction towards CO_2 production [45].

These most recent models used equivalent permeabilities of the carboxysome shell to HCO_3^- and CO_2 (**Table 1**). Given the potential favorability of the pores to negatively charged ions by three orders of magnitude, equal transport of these molecules seems unlikely [29]. For example, if CO_2 is allowed to pass freely across the shell, then it would be released from the cell and collapse the functionality of the CCM similar to what was seen in classic experiments demonstrating a high-CO_2-requiring mutant by expressing CA outside the carboxysome [47]. Additionally, similar questions remain on the biological feasibility of maintaining a proton gradient across the shell within the bacterial cytoplasm.

Catabolic BMCs:

Of the catabolic BMCs, also termed 'metabolosomes' [74], the most well studied are 1,2-propanediol utilization (Pdu) and the ethanolamine utilization (Eut), which are found in *Salmonella* and *E. coli*. Less studied metabolosomes include 1-amino-2-propanol utilization

(RMM), glycyl radical enzyme-associated microcompartments (GRM), choline utilization (GRM2), and fucose and rhamnose utilization (GRM5 and PVM) BMCs [8,75]. Research remains sparse on the genes, pathways, and enzymatic reactions involved in catabolic BMCs. These pathways may also be more complex than ones in carboxysomes, being dependent on redox balancing, disproportionation, and the presence of several metals and other cofactors. One commonality in metabolosomes is the presence of oxygen sensitive metal co-factor containing enzymes, such as B_{12} cycling enzymes and glycyl-radical enzymes, with the BMC possibly acting as an O_2 exclusion mechanism [11,13].

Catabolic BMC Models: There is an overall dearth of mathematical models for the catabolic BMCs with only a pair published models [22,76] of a non-carboxysome BMC systems in recent years. Considering the special interest from industry in BMCs that could shield the cytoplasm from toxic intermediates, increase metabolic flux, or modulate reaction selectivity and product inhibition, it is valuable to explore catabolic BMC capabilities systematically [77].

A notable difference between the dynamics of the carboxysome and other BMCs, such as the Pdu BMC, is that the internal reactions of the carboxysome begins with a reversible reaction while the propanediol pathway starts with an irreversible reaction potentially trapping the substrate within the shell once it has been produced. Jakobson *et al.*'s study (2017) on Pdu found that selective permeability of the BMC shell can increase the overall BMC function and highlighted the important role BMCs play in metabolite flux enhancement [76]. This model was adapted by Jakobson *et al.* (2018) to be generalizable to different cellular organizational strategies and metabolic pathways in order to guide industrial design of BMCs [22]. They did not include O_2 or the B_{12} cycling pathway, which is sensitive to O_2, in their models. Given the diversity of shell proteins and mysteries regarding their selectivity and dynamics, it is possible that additional mechanisms may allow escape or entrance of specific metabolites, co-factors, electrons, or proteins across the shell.

Conclusions:

With only a handful of BMC models proposed in the last decade [22,40–45,76], there remains a great many unanswered questions and research potential within this domain.

Many critical parameters of BMCs formation, function and regulation remain unknown. These parameters include the internal pH compared to the cytosol and the composition and stoichiometry of encapsulated proteins and co-factors. In particular, the permeabilities of substrates and products across the shell into the protein core remains largely unmeasured and speculative. The pores of the major structural shell proteins studied to date with crystallographic methods display overall positive charge, which allows for negatively charged compounds to readily pass through (**Figure 1**) [29]. However, the molecular mechanism and level of exclusion of positively charged ions, like H^+, or nonpolar substrates, like CO_2 and O_2, has yet to be determined experimentally [29]. There is debate on the internal pH, redox environment, and the creation and relevance of a pH gradient across the BMC shell [29,45,78]. Additionally, systematic errors and biases in our understanding of these mechanisms plague all

BMC models, with many of the featured parameters across all these models being based on previous measurements that may need to be revisited (**Table 1**). Therefore, it is important to combine theory with experimental data in an iterative approach to provide robust agreement between these methods.

Several mathematical models of cyanobacterial physiology not focused on BMCs take a full-cell approach, including not only carbon fixation but also photosynthetic reactions, protein production, and gene regulation [79,80]. However, these models lack the fine details of the CCM previously described in BMC models. A robust exploration, meeting in the middle of these two approaches, could yield useful insights on how the CCM is dynamically controlled in response to changes in cellular physiological conditions. In particular, several transporters relevant to the CCM are sensitive to inorganic carbon concentrations and regulated allosterically [81], potentially influencing how the CCM reacts to changing environmental conditions.

Evolutionary considerations were touched upon briefly in a few of the carboxysome modeling studies. These models could be combined with recent computational approaches in metabolic evolution [82]. This approach could allow systematic investigation on how evolutionary intermediates in CCM evolution may have responded to environmental changes during proposed times of carboxysome origination such as the Great Oxidation Event (GOE) approximately 2.3-2.5 billion years ago [83]. A dramatic increase in atmospheric O_2 concentrations across the GOE relative to slowly declining CO_2 concentrations led to an ~100 million fold increase in O_2:CO_2 ratios, which could have provided a selective pressure driving the encapsulation of oxygen-sensitive enzymes, such as RuBisCO. Recent explorations of similar questions featured a limited range of oxygen concentrations, and, as a result, may be missing important dynamics of carboxysome evolution driven by the need for oxygen exclusion [45]. Furthermore, there are undeniable structural and functional similarities between catabolic BMCs and carboxysomes, which offers an underexplored opportunity for comparative evolutionary studies. One such example is the combination of homologous shell proteins between carboxysomes and other BMCs and the frequency of highly-oxygen-sensitive coenzyme B_{12} regeneration pathways and glycyl radical enzymes being sequestered in BMCs for use in the contained reactions [11,13]. This suggests that the O_2 exclusion property of carboxysomes may be a more general property of BMCs than previously considered.

Through computational modeling of BMCs, we can relate results to experimental data, probe theoretical evolutionary pressures, and gain deeper understanding of these systems [21,77]. Given the rapidly advancing power of computation and AI in addition to new experimental techniques, we anticipate many new breakthroughs in the coming decades harnessing the capabilities of BMCs that could have major benefits for society and the environment.

Declaration of Interests: J.C.C. has equity and is co-founder and Chief Science Advisor for Prometheus Materials Inc. All other authors declare no competing interests.

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