The Center for Catalysis in Biomimetic Confinement (CCBC)
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Lead Institution: Michigan State University
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Mission Statement*: To understand the means by which Nature spatially organizes catalysis across scales using compartmentalization within selectively permeable protein-based membranes, and to use these principles to develop a modular platform for spatially organizing catalysis.*

Biology accomplishes remarkable N_2 and CO_2 fixation reaction pathways in complex environments at ambient temperature and pressure by exploiting confinement effects in charge-accumulating architectures. Confinement features range in scale from the entatic control of catalysis within the enzyme active site to the organized encapsulation of multi-step reaction sequences within subcellular organelles. While organelles have traditionally been considered the defining feature of higher (eukaryotic) organisms, recently it has become clear that bacteria also contain metabolic organelles, known as Bacterial Microcompartments (BMCs). BMCs function analogously to eukaryotic organelles like the chloroplast; by spatially sequestering sequential enzymes within a selectively permeable membrane (the shell), BMCs co-confine catalysts, establish spatial control of local reactant and substrate concentrations, sequester volatile or reactive intermediates, separate competing/incompatible pathways, and insulate catalysts from inhibitors. The most extensively characterized BMC, the carboxysome, confines ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) and carbonic anhydrase (CA) in a selectively permeable membrane (**Figure 1**) that enables concentrating CO_2 while simultaneously providing a barrier to O_2, RuBisCO's competitive inhibitor.

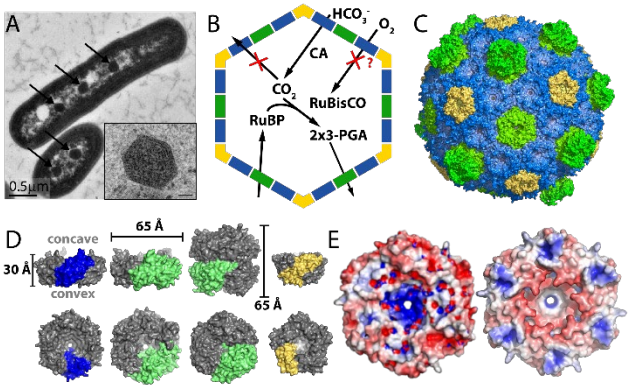
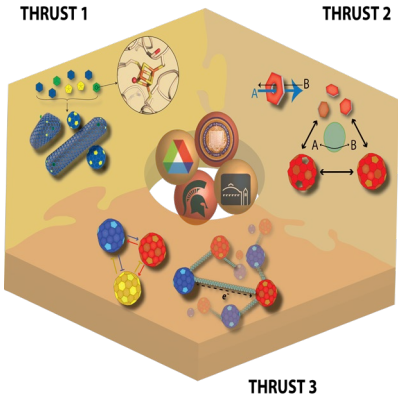


Figure 1. Structure, function, and context of BMCs. A) Cyanobacteria with carboxysomes (arrows); inset, close up view of a 200 nm carboxysome. B) Schematic of carboxysome function. C) Structure of a 6.5 MDa BMC shell determined by a combination of X-ray crystallography and Cryo-EM[3]. D) Basic building blocks of all BMC shells. E) Shell protein hexamers colored by electrostatic potential; note distinct charge around central pores of 4 and 7Å, respectively.

BMCs have evolved to naturally overcome challenges associated with the enormous complexity of metabolic pathways by confining reactions that are incompatible with the surrounding environment and by sequestering pathways to prevent unwanted metabolite crosstalk. The modularity of shell proteins and their potential for self-assembly in both heterologous expression systems and in vitro presents a remarkable opportunity to understand shell protein properties and the principles of self-assembly, as well as to develop a modular tunable framework for spatially confined chemistry. Unlike lipid-bound compartments, the protein-based membrane of the BMC can be precisely structurally defined, and the multiple shell constituents can be individually tuned for electron, substrate, product, and gas transport properties, and provide spatially controlled recognition/anchoring sites on the shell's interior and exterior surfaces for catalysts. BMCs are unique among both biological (liposomes) and synthetic (e.g., zeolites, MOFs, and mesoporous materials) compartments because their shells are essentially a chemically active material that can be tuned or evolved for site-specific/task-specific function using gene-based engineering techniques.

The CCBC is focused on elucidating the underlying principles of BMC structure and function to enable extending and repurposing shell protein building blocks as modular platforms for structuring catalysis at scales extending from the catalyst active site to hierarchical organized micron-scale assemblies of catalytically and redox-active shells. Integral to this goal is understanding the biophysical and mechanistic impact of confinement on enzymes and abiotic catalysts. The CCBC leverages National Lab facilities and bring together a multidisciplinary team comprised of structural and synthetic biologists, enzymologists, spectroscopists, computational theorists, photochemists, and inorganic and synthetic chemists to create an integrated effort addressing fundamental questions on hierarchical confinement for the control of complex, energy-transducing reaction networks. This multidisciplinary team will share a well-developed model shell protein platform combined with computational modeling and selected enzymatic and synthetic catalysts to elucidate the underlying physical principles utilized by Nature in its extraordinary variation of BMC function, thereby enabling us to apply those principles to develop new catalytic systems. This work is groundbreaking by employing BMCs as a tunable, building-block compartmentalization systems for bio-hybrid synthesis. The integration of synthetic biology, chemistry, and catalysis enables hierarchical confinement to be investigated as a general physical-chemical concept applicable for controlling non-natural biological and synthetic energy-conserving chemistries. This will be investigated at unprecedented levels of scale by using BMCs as tunable nano-reactors that can be connected into 2D and 3D hierarchical networks.



The CCBC includes a utilization of natural and synthetic catalysts, protein-based compartments, and both in vitro and in vivo assembly approaches to provide a fundamental, cross-cutting mechanistic understanding for engineering and control of catalysis in confinement. The CCBC is organized in three linked Thrusts (**Figure 2**) representing progressively increasing scales of organization. Thrust 1 focuses on characterization of assembly and microenvironments within BMC shells; Thrust 2 uses BMCs to investigate confinement effects in energy-converting chemistries; Thrust 3 investigates the mesoscale assembly and organization of compartmentalized catalysis.

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