Development of a pipeline for the *in situ* assembly dynamics of a carbon-fixing bacterial nanocompartment

Samuel Hartzler[1], Lauren Ann Metskas[1,2]

[1]Department of Biological Sciences; [2]Department of Chemistry; Purdue University, West Lafayette, IN 47907
shartzle@purdue.edu

Despite being a dominant and integral part of the carbon cycle throughout the domains of life, the signature enzyme of carbon fixation (Ribulose-1,5-bisphosphate carboxylase/oxygenase or **Rubisco**) is compromised by promiscuous reactions with other substrates. This promiscuity has stimulated the evolution of various carbon-concentrating mechanisms (**CCMs**) to enhance Rubisco activity. A prominent CCM in bacteria is the alpha carboxysome (**α-CB**). α-CBs are large (~150 nm), self-assembling, polyhedral protein shell assemblies which sequester the encapsulated Rubisco enzymes away from competing substrates. While the α-CB is an attractive tool for heterologous enhancement of carbon fixation, its assembly process has not been characterized *in vitro*. Our research utilizes the powerful tools of cryo-electron tomography (**cryo-ET**) and subtomogram averaging (**STA**) to interrogate the *in vivo* molecular interactions that govern the life cycle of the α-CB. We currently are developing an environmentally driven pipeline for producing synchronized *in vitro* α-CB expression events in the autotroph *Halothiobacillus neapolitanus*. Our preliminary work suggests that environmental manipulation and resource availability results in widespread changes in both α-CBs and the surrounding cell ultrastructure. Functional reconstitution or recombinant expression of this nanocompartment is an emerging research field with a wide array of biomedical and bioengineering applications. This work provides critical insights into optimization of these processes.

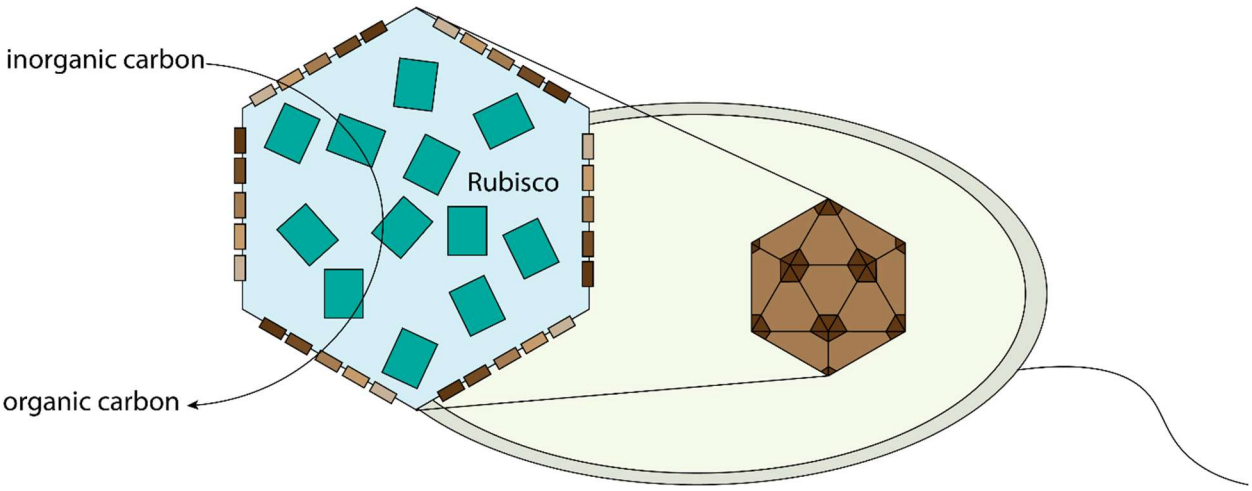

Figure 1. Schematic of Rubisco encapsulation inside polyhedral α-CB inside *Halothiobacillus neapolitanus*.