Mechanism of α-carboxysomes Formation

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Carboxysomes are a family of bacterial microcompartments in cyanobacteria and chemoautotrophs. They encapsulate Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and carbonic anhydrase catalyzing carbon fixation inside a proteinaceous shell. An intrinsically disordered linker protein CsoS2 plays a central role in driving carboxysomes assembly, however, the underlying molecular mechanism is obscure. Here we used cryo-electron microscopy and tomography to study α-carboxysomes from a chemoautotrophic bacterium *Halothiobacillus neapolitanus*. By cryo-ET, we identify that Rubiscos form high-order intertwining spiral assembly inside the carboxysomes and further resolve the structures of native Rubisco at near-atomic resolutions by subtomogram averaging. Surprisingly, CsoS2 interacts only with the Rubiscos close to the shell using its N-terminal region. In addition, high-resolution cryo-EM structures of recombinant shell assemblies revealed that CsoS2 C-terminus interacts with the inner surface of shell, acting as a “molecular thread” stitching through multiple shell protein interfaces with a remarkable highly-conserved repeating motif. Taken together, our findings provide critical knowledge of the assembly principles of α-carboxysomes, which may aid in the rational design and repurposing of carboxysome structures for new functions.