# Review of: "Single-particle cryo-EM analysis of the shell architecture and internal organization of an intact α-carboxysome"

Wei Dai[1], Jennifer Jiang[1]

1 Rutgers University

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Evans *et al.* characterized the structure of native α-carboxysome isolated from a marine cyanobacterium *Cyanobium* by cryo-electron microscopy single particle analysis. The sample was composed of intact carboxysomes, broken shell fragments and “free-standing” proteinaceous components likely originating from the broken carboxysomes. The authors reported a 2.9Å resolution structure of form 1A RuBisCO enzyme and identified a density in the active site potentially corresponding to the substrate RuBP.

To characterize the ultrastructure of the α-carboxysome shell, single particle analysis was also performed on intact carboxysomes. The authors classified particles based on diameter and applied icosahedral symmetry to the shell to improve resolution. Given known heterogeneity in both size and morphology of the carboxysomes, applying icosahedral symmetry may lead to loss of structural information on the degree of icosahedral symmetry caused by potential variations in triangulation numbers among different faces of the particle. To resolve the potential continuum of carboxysome shell size and morphology variations, cryo-electron tomography may be better positioned to address both limitation by particle numbers and the heterogeneity of carboxysome complexes.

Using an integrative modeling strategy, the authors proposed an atomic model for the structural architecture of α-carboxysomes and interior organization of encapsulated proteins. We appreciate that the co-evolution analysis produced insights into molecular interactions between shell proteins and the internal packing and distribution of enzymes within the carboxysomes. However, since the analysis is correlational and the shell map lacks structural features to guide modeling, the manuscript may benefit from a discussion on modeling validation or limitation of their approach. We are also curious as to why a synthetic β-carboxysome was used for modeling of hexamer and pentamer orientation since it is well-established that α- and β-carboxysomes differ in structural organization, assembly, and protein composition.

In conclusion, we acknowledge the authors’ contributions to expanding our understanding of carboxysome structural architecture and internal arrangement of encapsulated enzymes. This study yields structural insights into the structural basis of carboxysome architecture and advances exploration of BMCs for biotechnological purposes.