Construction of an artificial CO_2-fixing compartment using DNA nanostructure as a scaffold

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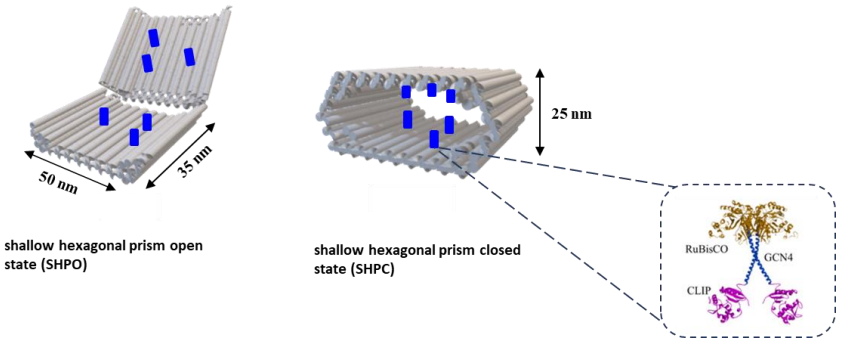
Cellular systems evolve small compartments to carry out biochemical reactions efficiently. In addition, substrate channeling is considered one of the most important factors in achieving highly efficient metabolic reactions. Despite its low enzymatic activity, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) plays an important biological role in natural CO_2 fixation. In cyanobacteria, RuBisCO and carbonic anhydrase (CA) are encapsulated in carboxysomes with diameters of 100-200 nm.[1] A large amount of RuBisCO is packed into the carboxysomes, which are proposed to be in the liquid-liquid phase separation state (LLPS). While the LLPS is proposed to modulate substrate channeling, the actual mechanism or advantage of the carboxysome structure for the RuBisCO reaction remains to be elucidated.

The DNA origami method can precisely assemble enzymes with defined spacing between enzymes.[2, 3] Recent advances in enzyme assembly systems on DNA origami scaffolds show the potential to enhance enzymatic activity.[4, 5] Here, we use dynamic shape-transforming DNA origami of the shallow hexagonal prism (SHP) to encapsulate RuBisCO in a highly packed state as an artificial carboxysome. The modular adaptor method was used to fuse CLIP-tag and GCN4 to RuBisCO (CG-RuBisCO) to assemble RuBisCO in dimeric form on SHP.[3, 6] The assembly of three dimers of CG-RuBisCO on each bottom of SHPO and subsequent closure to SHPC would create the packed state for RuBisCO. The CO_2 fixation efficiency of CG-RuBisCO assembled on SHP will be investigated to understand the characteristics of the natural carboxysome structure.



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