Streamlined Construction of the Carboxysome for the Development of Modular Protein-Based Nanoreactors

C. Raul Gonzalez-Esquer1, Tyler B. Shubitowski1 and Cheryl A. Kerfeld1,2,3,4,5
1MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan, USA; 2Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, USA; 3Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA; 4Department of Plant and Microbial Biology, UC Berkeley, Berkeley, California, USA; 5Berkeley Synthetic Biology Institute, UC Berkeley, Berkeley, California, USA.

Introduction
The carboxysome is a self-assembling cyanobacterial organelle that encapsulates the enzymes ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase (CA) within a proteinaceous shell. Carboxysomes increase the local concentration of CO2 in the vicinity of RuBisCO, thereby favoring its carboxylation activity.

Carboxysomes have captured the interest of bioengineers interested in photosynthetic organisms because of their proposed potential for enhancing CO2 fixation in photosynthetic organisms besides cyanobacteria. More broadly, carboxysome-based architectures may be redesigned as novel nanoreactors for the production of renewable chemicals (Kerfeld and Erbilgin, 2015). Potential uses of engineered carboxysomes include metabolic compartmentalization to increase pathway efficiency, segregation of toxic or volatile intermediates, etc.

However, carboxysomes are complex machines comprised of thousands of copies of at least 6 gene products; this creates significant challenges for the redesign of carboxysomes and for their transfer, regulation, assembly into heterologous systems. Hence, to overcome this bottleneck, a redesign of the carboxysome is necessary.

We designed a chimeric protein (CcMC) that structurally and functionally replaces four gene products required for carboxysome formation in the cyanobacterium Synechococcus elongatus PCC 7942.

The chimeric protein CcMC
Assembly of the native carboxysome occurs from the inside out (Cameron et al, 2013) through the protein-protein interactions (B) shown in (A). The funneling the essential domains from the core protein into a single chimeric protein that mimics the native assembly (C), we are able to form functional genetically and structurally streamlined carboxysomes (B).

CcMC can structurally replace the core proteins CcmM, M35, CcmN and CcaA
We confirmed the assembly of the chimeric carboxysomes through fluorescence and transmission electron microscopy in deletion strains expressing CcMC (COREA2: deletion of CcmM and CcmN and COREA3: deletion of CcmM, CcmN and CcaA).

Conclusion
CcMC acts as a multi-protein carboxysomal core that nucleates RuBisCO, around which the shell assembles. Streamlined carboxysomes can support photosynthesis. Our domain-fusion approach makes the design of synthetic bacterial microcompartment (BMC) cores readily tractable. We propose that (re)engineering carboxysomes by focusing on domain structures and interactions can be used to build new subcellular architectures.

Implications
Carboxysomes play the key role of carbon fixation in cyanobacteria. As such, they hold great potential for industrial carbon capture. Our strategy for repurposing carboxysomal architectures opens new realities in the realm of synthetic biology: the carboxysome shell with a redesigned core could increase the efficiency of an encapsulated pathway, sequester volatile intermediates or separate toxic intermediates from the cell lumen, etc. for diverse engineered biosynthetic applications. (Kerfeld and Erbilgin, 2015). Carboxysome-based nanoreactors will be especially valuable for the efforts in renewable chemical production from photosynthetic microbes.

Methods
Strain generation. Synechococcus elongatus PCC 7942 (Syn 7942) cells were transformed by double homologous recombination with plasmid design to replace CcmM, CcmN and CcaA with antibiotic resistance markers. Afterwards, a plasmid containing the fusion gene ccmC was transformed into the deletion strains, removing the selection marker using growth in air as positive selection. Finally, cells were transformed with a plasmid containing RbcL-GFP. Syn 7942 carboxysomes were visualized from cultures at exponential phase. GFP fluorescence was used for fluorescence microscopy in a Zeiss Axio Observer.D1 inverted microscope. Electron microscopy was performed on cells embedded in 1%M-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan, USA; 2Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, USA; 3Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA; 4Department of Plant and Microbial Biology, UC Berkeley, Berkeley, California, USA; 5Berkeley Synthetic Biology Institute, UC Berkeley, Berkeley, California, USA.

References
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Detailed data and methods from this poster can be found at: Gonzalez-Esquer CR, Shubitowski T, Kerfeld CA (2015) Streamlined construction of the cyanobacterial CO2-fixing organelle via protein domain fusions for Use in Plant Synthetic Biology. The Plant Cell, DOI: 10.1105/tpc.15.00329

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