Title:

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

Type of presentation requested: poster

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

Abstract

Photosynthetic microbes are being developed as platforms for biofuel production owing to their efficient CO₂ capturing capabilities and potential to direct carbon towards synthesis of organic molecules of biotechnological interest. In cyanobacteria, carbon fixation occurs in a bacterial microcompartment (BMC) called carboxysome, that encloses ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase within a protein shell. BMCs hold great potential for industrial applications, as they inherently serve the purpose of increasing enzymatic reaction efficiency, sequestering volatile intermediates, separating toxic intermediates from the cell lumen, etc. The repurposing of carboxysomal architectures can address pressing global issues--food production, fossil fuel alternatives and climate change. However, the complexity of the carboxysomes (as well as related BMCs) present formidable challenges for their redesign. Moreover, the difficulty in transferring and regulating multiple genes in heterologous systems (eukaryotic) presents a significant bottleneck to realization of their potential. We have successfully re-engineered the carboxysome core, based on knowledge of protein domain interactions in assembly. We reduced the core of the β-carboxysome to RuBisCO and a single synthetic fusion protein (formed by key domains from 4 different proteins); the reduced carboxysome supports growth of *Synechococcus elongatus* PCC 7942 in air. The synthetic protein reduces the genomic load required to assemble a carboxysome by ~1100 bp (18% of total message required for carboxysomes). More importantly, it reduces the number of proteins and, concomitantly the need for balancing the expression levels of three different genes. Our results are a proof-of-concept of the feasibility of a domain-based approach to engineering proteinaceous organelles based on BMC architectures. This lays the foundation for generating carboxysome-based cyanobacterial nanoreactors for the production of renewable chemicals. Furthermore, the chimeric protein provides a critical building block for the construction of streamlined carboxysomes in eukaryotic organisms for increased efficiency in photosynthetic CO₂ capture.
Short description of the research and travel plan

Cyanobacteria are microorganisms that have gained significant attention for their potential as production platforms of renewable chemicals, since they efficiently fix carbon dioxide from air using sunlight as energy source. Carbon fixation in these microbes occurs in a multi-protein structure called the carboxysome. The Kerfeld group studies both basic and applied aspects of carboxysomes and other architecturally-related compartments (Kerfeld and Erbilgin 2015; Axen et al. 2014). We seek to understand their biochemical function and structure, and use this understanding for applications in biotechnology. The Kerfeld lab elucidated the steps in carboxysome assembly (Cameron et al. 2013). This enabled me to re-design the carboxysome core based on structural and assembly principles. My first achievement was the streamlining of the carboxysomal core, by creating a single fusion protein that mimics the assembly of a four-protein scaffold. This approach is unusual, as only the key regions of the essential proteins were kept intact in the fusion protein, and still the streamlined carboxysome was able to support growth of the cyanobacterium. The decrease in the number of proteins required to form a carboxysome should facilitate the transformation the full carboxysomal machinery into plants, which should increase their photosynthetic efficiency. My results serve as a proof-of-concept for the strategy of fusing proteins to retain structural characteristics resembling a native assembly, and will allow for the use of carboxysomes as microbial nanoreactors.

If selected, this grant would support my attendance to the 2015 “Algae Biomass Summit” (http://www.algaebiomasssummit.org/) to be held from September 30 to October 2, 2015 in Washington, DC. The conference is described as the largest algae conference in the world and is unique as it has organized sessions of science, policy, commercialization, etc. which allows one to obtain a very broad and comprehensive view of the advances on renewable production from photosynthetic microbes. Presenting my work in a setting like this puts me in direct contact with the industry experts and potential end users of my research, and allows me to receive feedback from their perspective. In addition, it is an excellent opportunity to establish communication and potential collaborations with industry partners, which is especially important for applied research projects and personally, as future potential employers.

References: