1. Requested Information
Technical Summary for DOE Award No. DE-SC0006394
Recipient: The Regents of the University of California
Title: Engineering self-assembled bioreactors from protein microcompartments
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2. Assessment of Project Milestones vs. Achievements
The original goals of our work were focused on deciphering how bacterial microcompartments (BMCs) assemble in cyanobacteria in order to facilitate their engineering for novel synthetic pathways. These goals were summarized in the following aims:

> **'Aim 1: To define BMC assembly and function within cyanobacteria**. How BMCs function *in vivo* is poorly defined. Here, we will use a model BMC to define the nature and mechanism of particle formation and degradation. In related work, we will probe the interplay between BMC function and cellular growth and metabolism *in vivo*.
> **Aim 2: To develop a model BMC for synthetic biological applications**. We will apply our understanding of BMC assembly and function to the long-term goal of creating self-assembling cellular bioreactors, or synthetic organelles. We will develop a minimal and modular BMC chassis for further protein engineering functionalization. We will then define how proteins are targeted to BMCs and use this as a means of creating a novel BMC capable of catalyzing the model pathway of ethanol fermentation.'

Our achievements mirrored these aims in many ways, although did deviate in specifics, as outlined below. **An important early achievement was the**

demonstration that we could heterologously express the alpha carboxysome BMC from *Halothiobacillus* (not cyanobacterial) in *E. coli*, thus opening the door to novel compartmentalization routes and synthetic CO2 fixation pathways (ref. 7). This large achievement ultimately led us to focus on this particular BMC, rather than the cyanobacterial beta carboxysome as originally proposed. In parallel publish a number of papers investigating the function of beta carboxysomes *in vivo* in cyanobacteria. **Major advances here included the observation that carboxysome assemble in a cargo-dependent fashion (refs. 5,6), the discovery of a largely uncharacterized signaling pathway in cyanobacteria controlling physiology of light-dark transitions (ref. 2), and a quantitative model of carboxysome function *in* vivo (ref. 1).** The latter two achievements were not originally proposed but were derived from and related to studies on carboxysome function. Finally, it should be noted that in the early stages of our work the Kerfeld Laboratory published a paper directly related to experiments in our original Aim 1, (Cameron et al. 2013 Cell), which made pursuing some of the original experiments in cyanobacteria unnecessary.

Our work on synthetic compartmentalization has focused on both the alpha carboxysome (related to original proposal) and a smaller, simpler class of compartments known as encapsulins (not in original proposal). The major advance in the first area has been the delineation of a critical protein, **CsoS2, as a critical building block of the alpha carboxysome (ref 4).** Since the alpha carboxysome has remained recalcitrant to engineering thus far, we have focused on an alternative class of protein compartments, the encapsulins, as a simple protein compartment for synthetic compartmentalization. **Here, we have isolated the cargo-loading signal sequence and demonstrate a robust biochemical strategy for loading and obtaining large amounts of pure, homogenous preparations of synthetically loaded compartments.** In future work, we intend to continue our studies of the alpha carboxysome focusing on CsoS2 and leverage our ability to engineer encapsulins.

3. Major Accomplishments

Our major accomplishments during the project timeframe were as follows:

- We demonstrated that 10 genes are sufficient to produce a carboxysome in a heterologous host, thus opening the door to improved CO_2 assimilation in new organisms such as plants (ref. 7).
- We identified a poorly characterized carboxysomal protein, CsoS2, as critical to assembly and answered a 30+ year old question in the field as to how multiple isoforms of this protein are expressed from a single gene (ref. 4)).
- We discovered new mechanisms used by the host to regulate the CCM. We found that the bacterial stringent response is a master regulator of cyanobacterial metabolism during light-dark transitions (ref. 2).

Relatedly, we developed a whole-cell mathematical model of the CCM and discovered that dynamic changes in cytosolic pH play a critical – and entirely unappreciated – role in photosynthesis (ref. 1).

- We demonstrated that encapsulins, a poorly characterized class of capsid proteins, can be used to understand the function and significance of protein compartmentalization (ref. 3).

5. Cost Status
Not applicable. Budget attached.

5. Schedule Status
Not applicable.

6. Reporting Period Changes
Not applicable.

7. Problems and Delays
Throughout the project there was minor re-prioritization of goals based on positive/negative results of experiments and the publication of concurrent overlapping results from other groups (see above).

8. Key Personnel Changes
Not applicable.

9. Products and Deliverables
Advances and highlights are covered on: www.savagelab.org

Publications supported by this project were:

1. N. M. Mangan, A. Flamholz, R. D. Hood, R. Milo, D. F. Savage, pH determines the energetic efficiency of the cyanobacterial CO2 concentrating mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E5354–62 (2016).
2. R. D. Hood, S. A. Higgins, A. Flamholz, R. J. Nichols, D. F. Savage, The stringent response regulates adaptation to darkness in the cyanobacterium Synechococcus elongatus. *Proc. Natl. Acad. Sci. U.S.A.* (2016), doi:10.1073/pnas.1524915113.
3. C. Cassidy-Amstutz *et al.*, Identification of a Minimal Peptide Tag for in Vivo and in Vitro Loading of Encapsulin. *Biochemistry*. **55**, 3461–3468 (2016).
4. T. Chaijarasphong *et al.*, Programmed Ribosomal Frameshifting Mediates Expression of the α-Carboxysome. *J. Mol. Biol.* **428**, 153–164 (2016).
5. R. Yokoo, R. D. Hood, D. F. Savage, Live-cell imaging of cyanobacteria. *Photosynthesis Research*. **126**, 33–46 (2015).
6. A. H. Chen, A. Robinson-Mosher, D. F. Savage, P. A. Silver, J. K. Polka, The bacterial carbon-fixing organelle is formed by shell envelopment of

preassembled cargo. *PLoS ONE*. **8**, e76127 (2013).
7. W. Bonacci *et al.*, Modularity of a carbon-fixing protein organelle. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 478–483 (2012).
8. A. H. Chen, B. Afonso, P. A. Silver, D. F. Savage, Spatial and temporal organization of chromosome duplication and segregation in the cyanobacterium Synechococcus elongatus PCC 7942. *PLoS ONE*. **7**, e47837 (2012).

Plasmid reagents from our work are available at:
https://www.addgene.org/David_Savage/

Computer source code is available at: https://github.com/savagelab