Title: Systems biology to enable modular metabolic engineering of fatty acid production in cyanobacteria

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Project Goals: This project will apply systems biology to identify metabolic control points and bottlenecks that regulate flux to lipid products in cyanobacteria. Our long-term goal is to develop strains of cyanobacteria that are optimized for producing renewable chemicals at commercially feasible rates and titers.

Abstract Text: Cyanobacteria are attractive hosts for biomanufacturing because of their ability to rapidly fix CO2, grow in nutrient-poor environments, and produce renewable chemicals directly from photosynthesis. Unlike triacylglycerol production in green algae, production of free fatty acids (FFAs) using genetically engineered cyanobacteria results in secretion of the product into the culture medium where it can be efficiently recovered. However, there is a major gap in our understanding of how lipid metabolism is regulated in cyanobacteria that limits our ability to rationally engineer high-titer FFA production in cyanobacterial hosts. The overall objective of this project is to use systems biology to identify metabolic control points and bottlenecks that regulate flux to FFAs in cyanobacteria. Our central hypothesis is that cyanobacterial lipid metabolism can be modularized into pathways that are "upstream" and "downstream" of the nodal metabolite acetyl-CoA, which can be separately studied and optimized to enhance overall FFA production. We will apply a suite of systems biology approaches including ^{13}C flux analysis, metabolomics, lipidomics, proteomics, and CRISPRi screens to rigorously define the regulation of flux within each module. As proof of principle, this modular approach will be applied to optimize cyanobacterial production of FFAs in the fast-growing, halotolerant strain *Synechococcus* sp. strain PCC 7002. The rationale for the proposed research is that a deeper understanding of how fatty acid flux is regulated upstream and downstream of acetyl-CoA will enable integrated "push-pull" metabolic engineering strategies to produce lipid products directly from photosynthetic CO_2 fixation in cyanobacteria. This research will directly contribute to DOE's mission by advancing toward biological production of renewable fuels that do not compete with agriculture.

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