Systems Analysis and Engineering of Biofuel Production in *Chromochloris zofingiensis*, an Emerging Model Green Alga

Melissa S. Roth,[1,2] Sean D. Gallaher,[3] Daniel Westcott,[1,2] Tim Jeffers,[1,2] Daniela Strenkert,[3] Katherine B. Louie,[4] Benjamin P. Bowen,[4] Crysten E. Blaby-Haas,[5] Nanette R. Boyle,[6] Mary S. Lipton,[7] Trent R. Northen,[4] Sabeeha S. Merchant,[3] and **Krishna K. Niyogi**[1,2]* (niyogi@berkeley.edu)

[1]Howard Hughes Medical Institute, Berkeley, CA; [2]University of California, Berkeley, CA; [3]University of California, Los Angeles, CA; and [4]Lawrence Berkeley National Laboratory, Berkeley, CA; [5]Brookhaven National Laboratory, Upton, NY; [6]Colorado School of Mines, Golden, CO; [7]Pacific Northwest National Laboratory, Richland, WA

Project Goals:

Our overarching research goal is to design and engineer high-level production of biofuel precursors in photoautotrophic cells of the unicellular green alga *Chromochloris zofingiensis.* We will perform a comprehensive, large-scale systems analysis (transcriptome, proteome, metabolome, physiology) of *C. zofingiensis* during trophic transitions to understand and model how the energy metabolism of the cell is redirected based on the carbon source. We will integrate the systems data in a predictive model that will guide us in redesigning and engineering the metabolism of *C. zofingiensis* using cutting-edge synthetic biology and genome-editing tools to increase production of biofuel precursors (triacylglycerols) under photoautotrophic growth conditions.

Abstract:

As a core component of a sustainable bio-economy, microalgae have the potential to become a major source of biofuels and bioproducts without exacerbating environmental problems. These photosynthetic microbes utilize solar energy, grow quickly, consume CO_2, and can be cultivated on non-arable land. However, there are presently considerable practical limitations in the photosynthetic production of biofuels from microalgae, resulting in low productivity and high costs. Integrative systems biology and engineering of emerging model systems are needed to expand the possibilities of microbial production of biofuels and bioproducts. The unicellular green alga, *Chromochloris zofingiensis*, is one of the highest producers of the preferred lipid precursor for biofuel products, triacylglycerol (TAG), making it a promising biofuel feedstock. Moreover, *C. zofingiensis* exhibits an ability to dramatically switch its metabolism and divert much of its energy into making TAGs when a carbon source is added during growth. Unlike most algae, this accumulation of TAGs is in concert with accumulating biomass. However, during this metabolic switch, photosynthesis is also shut off. Recently, we have identified the molecular player hexokinase1 as a critical regulator of the photosynthetic and metabolic switch (Roth et al., in preparation). The overarching aim of this project is to design and engineer high-level production of TAGs in *C. zofingiensis.* To understand how energy metabolism is redirected

within the cell, we will perform a comprehensive, large-scale systems analysis integrated with a predictive model and using cutting-edge synthetic biology and genome-editing tools to increase production of biofuel precursors. To develop *C. zofingiensis* as an emerging oleaginous model system, we have sequenced and assembled a chromosome-level genome and high-quality transcriptome (1). The outcomes of the proposed research include a detailed, predictive, systems-level understanding of the regulation of metabolism for enhancing production of biofuels, development of tools and capabilities to enable manipulation of metabolism, and generation of engineered strains of *C. zofingiensis* to produce high levels of biofuel products. Advancing the basic science underlying regulatory 'switches' between growth and lipid production will likely be critical to developing economically viable algal biofuels.

Reference

1. Roth MS, Cokus SJ, Gallaher SD, Walter A, Lopez D, Erickson E, Endelman B, Westcott D, Larabell C, Merchant SS, Pellegrini M, Niyogi KK (2017). Chromosome-level genome assembly and transcriptome of the green alga *Chromochloris zofingiensis* illuminates astaxanthin production. Proc Natl Acad Sci USA 114: E4296-E4305.

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