

## Multifactorial Nutrient-Dependent Proteomics Elucidates Lipid Accumulation and Regulation of Photosynthesis

Tim L. Jeffers\*<sup>1</sup> (tim\_jeffers@berkeley.edu), Sam Purvine<sup>2</sup>, Carrie Nicora<sup>2</sup>, Sean D. Gallaher<sup>3</sup>, Ryan McCombs<sup>1</sup>, Sabeeha S. Merchant<sup>1,4</sup>, Mary Lipton<sup>2</sup>, **Krishna K. Niyogi**<sup>1,4,5</sup>, Melissa S. Roth<sup>1</sup>

<sup>1</sup>Department of Plant and Microbial Biology, University of California, Berkeley; <sup>2</sup>Pacific Northwest National Laboratory; <sup>3</sup>UCLA DOE Institute for Genomics and Proteomics, University of California, Los Angeles; <sup>4</sup>Lawrence Berkeley National Laboratory; <sup>5</sup>Howard Hughes Medical Institute, University of California, Berkeley

<https://sites.google.com/view/czofingiensis/home>

**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*. Our strategy involves using large-scale multi-omics systems analysis to understand and model the genomic basis for how the energy metabolism of the cell is redirected based on the carbon source. Enabled by cutting-edge synthetic biology and genome-editing tools, we will integrate the systems data in a predictive model that will guide us in redesigning and engineering the metabolism of *C. zofingiensis*. Here, we focus on elucidating nutrient-dependent effects on regulation of photosynthesis and metabolism to ultimately improve production of biofuels and bioproducts.**

Microalgae have the potential to become a major source of biofuels and bioproducts without exacerbating environmental problems. Photosynthetic microbes can utilize solar energy, grow quickly, consume CO<sub>2</sub>, and 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. Insight into regulation of photosynthesis and metabolism will enable bioengineering of microalgae to maximize production of biofuels and bioproducts.

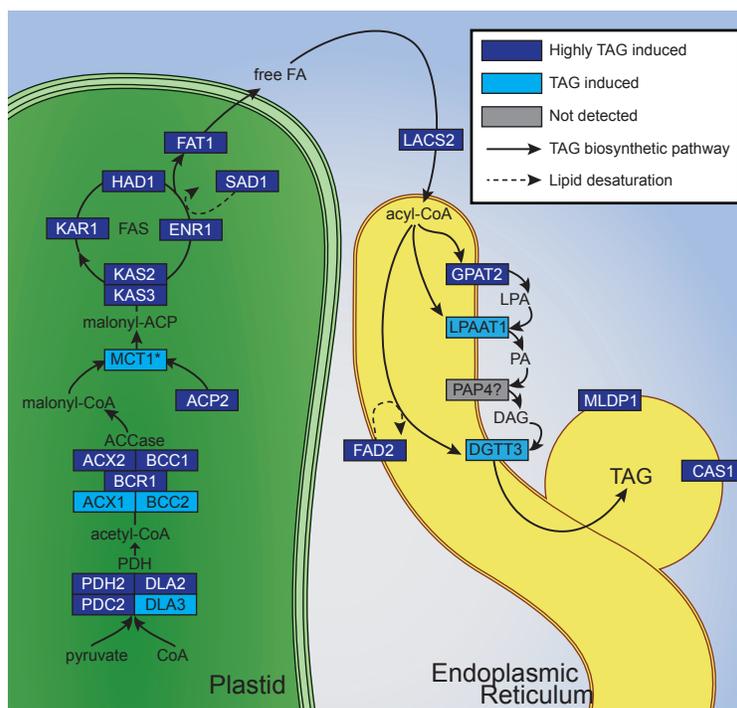
Nutrient regulation of photosynthesis and lipids can be leveraged for enhancing biofuel production. The oleaginous green alga *Chromochloris zofingiensis* has a reversible nutrient-dependent switch for photosynthesis and lipid accumulation (1) that is dependent on hexokinase (HXK1) (2). Here we show that this photosynthetic switch is dependent on iron and that replete iron supplementation can activate photosynthesis in the presence of photosynthesis-repressing glucose. To elucidate regulation of trophic states and the accumulation of triacylglycerols (TAGs), we conducted a full combinatorial proteomic analysis of the wild type vs. two independent *hvk1* mutant strains grown with and without glucose (+Glc) and in iron-replete (+Fe) and iron-limiting (-Fe) conditions (n = 3-4, 47 total samples). Isobarically labelled proteomes were analyzed via mass spectrometry, detecting peptides corresponding to ~80% of protein-coding gene models, with 52% being detected in all 12 conditions. A targeted analysis revealed 116 proteins that are highly induced by TAG-accumulating conditions (WT+Glc), capturing most *de novo* fatty acid biosynthesis enzymes, lipid droplet components, and uncharacterized proteins that are novel candidates for engineering increased TAG accumulation in autotrophic cells (**Figure 1**). We also found 782

proteins specifically responsive to the heterotrophic state (WT-Fe+Glc), including ~88% of the photosynthetic electron transport chain (ETC) subunits, which were depleted when photosynthesis was switched off. The proteomic data are consistent with a physiological explanation of iron's role in regulating photosynthesis, showing prioritization of the Fe-rich respiratory ETC over the photosynthetic ETC and revealing a putative allocation of Fe to the mitochondria by organellar transporters. This systems-level factorial experiment confirms the power of using *C. zofingiensis* for gene discovery related to nutrient physiology, photosynthesis, and bioengineering targets for metabolic redesign for sustainable algal biofuels.

## References

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2. Roth MS, Westcott DJ, Iwai M, Niyogi KK (2019) Hexokinase is necessary for glucose-mediated photosynthesis repression and lipid accumulation in a green alga. *Communications Biology* 2: 347.

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**Figure 1. Known players involved in TAG accumulation are enriched in WT+Glc proteomes.** A concerted upregulation of the *de novo* fatty acid biosynthesis proteins occurs in the plastid in response to +Glc and is dependent on HXK1. Enzymes of the Kennedy pathway and lipid droplet biogenesis that act in the endoplasmic reticulum to deposit storage lipids are also induced in the TAG-accumulating conditions. This figure also includes highly induced players in lipid desaturation.