Elucidating Nutrient-Dependent Effects on Regulation of Photosynthesis and Metabolism

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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 the 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_2, 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.

Both photosynthesis and the generation of neutral lipids that can be used as biofuels are heavily regulated by nutrient availability in algae. The oleaginous green alga *Chromochloris zofingiensis* displays the ability to reversibly shut off photosynthesis entirely ($F_v/F_m = 0$, no oxygen evolution), independent of light, in the presence of glucose (+Glc) and insufficient iron (-Fe), leading to heterotrophic cultures that survive solely on glucose as a carbon source (1). This photosynthetic switch is also dependent on a functional hexokinase (HXK1) gene (2). Photosynthesis can be restored after glucose removal (1). Recently, we have found that replete iron supplementation (+Fe) can also rescue photosynthesis in the presence of glucose. With the goal of understanding the regulation of trophic states and the accumulation triacylglycerols (TAGs) in this system, we conducted a full combinatorial proteomic analysis of the wild type vs. two independent *hxk1* mutant strains grown with and without glucose and in iron-replete and iron-limiting conditions (n = 3-4, 47 total samples). Through mass spectrometry of isobaric labelled peptides, we quantified the relative abundance of 11,282 proteins across our samples, which is ~70% of the annotated proteome. In our preliminary analyses, we note large decreases, specific to the heterotrophic state, in almost all protein subunits of the photosynthetic electron transport chain, consistent with photosynthesis being turned off **(Figure 1)**. In addition, wild-type cells treated with glucose have increased abundance of most of the lipid biosynthesis pathway. Our goal is to investigate the differentially abundant proteins, including several of unknown function, in heterotrophy and TAG-accumulating conditions to find gene targets for enhanced biofuel potential of this organism.

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. doi: 10.1038/s42003-019-0577-1

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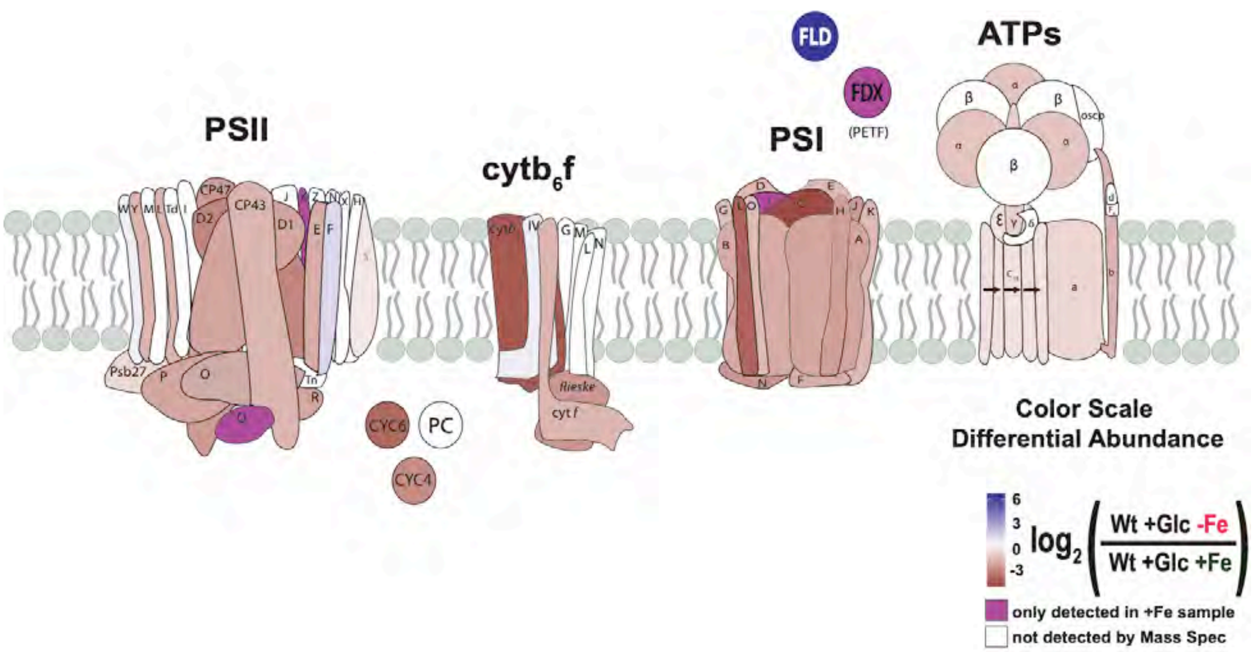

Figure 1. Universal downregulation of photosynthetic electron transport in heterotrophy (-Fe+Glc) vs. mixotrophy (+Fe+Glc). *Almost all photosynthetic complexes and electron carriers have lower protein abundance (increasing red color) in iron-deficiency-induced heterotrophy* (WT -Fe+Glc) *in comparison to the iron-replete, mixotrophic state* (WT+Fe+Glc) *that maintains photosynthesis. An exception is Flavodoxin (***FLD***) (blue circle, 90X more abundant in -Fe), an electron carrier that functions as Ferredoxin (***FDX***), but without an iron cofactor.* **PSI and PSII:** *Photosystems I and II,* **cytb$_6$f:** *cytochrome b$_6$f complex,* **ATPs:** *chloroplastic ATP synthase,* **CYC6 and CYC4:** *soluble cytochrome-c electron carriers,* **PC:** *plastocyanin.*