Genome-scale Metabolic Model of *Chromochloris zofingiensis*, an Emerging Model Green Alga for Sustainable Fuel Production

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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 large-scale multi-omics systems analysis to understand the genomic basis for energy metabolism partitioning as a consequence of 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 the redesign and engineering of metabolism in *C. zofingiensis.* The Boyle laboratory is tasked with developing and utilizing a genome scale metabolic network reconstruction to predict intracellular carbon fluxes which will then be compared to fluxes measured experimentally using ^{13}C-MFA. The Northen group is focused on the exometabolomics and lipidomics analysis of the *C. zofingiensis* to help us understand the algal metabolite uptake preference and intracellular lipid synthesis during trophic transitions.

C. zofingiensis is an emerging model system for the production of biofuels and bioproducts. It is an especially attractive system because it produces astaxanthin along with a large amount of lipids. Astaxanthin is a high value product (~$7,000 per kilogram) that has uses in the pharmaceutical, nutraceutical, and cosmetic industries[1-3]. It also demonstrates high levels of triacylglycerol accumulation and low photosynthetic productivity when additional organic carbon sources are provided[4], making it ideal for metabolic or genetic engineering focused on increasing algal lipid production.

In order to investigate the metabolic capacity of this organism for both biofuel and astaxanthin production, we generated a genome-scale metabolic network reconstruction. The current reconstruction includes 3500 metabolic reactions and 2832 metabolites. In order to formulate an accurate biomass formation equation, we measured both the macromolecule composition of *C. zofingiensis* (DNA, RNA, protein, lipid, carbohydrate) and the composition of each in photoautotrophic and photoheterotrophic growth modes. By combining these compositions with

growth curves and uptake measurements, we also predicted fermentation products and flux distributions. These predicted products are currently being experimentally validated.

To gain phenotypic data for model refinement, we conducted a *C. zofingiensis* time-course experiment, by measuring alterations in media composition resulting from algal growth and by measuring changes in algal lipid composition under different conditions. These experiments show a clear nutrient (carbon/nitrogen source) preference order during the growth of *C.zofingiensis.* Interestingly, we also observe utilization of organic compounds as energy and nitrogen sources under photoautotrophic and heterotrophic states. During these same transitions we find that the abundance of triacylglycerols increased up to 26-fold, whereas there are significant decreases in monogalactosyldiacylglycerols, digalactosyldiacylglycerols, and sulfoquinovosyl diacylglycerols, the major lipids that constitute thylakoid membranes. Additionally, we found an increase in phosphatidic acid but a decrease in phosphatidylinositol and phosphatidylserine. We are now incorporating these exometabolomic and lipidomic data to improve our understanding of the algal nutrient demand and lipid metabolisms, and the combination of the model and the data has great potential to elucidate dramatic metabolic shifts within the organism.

References

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