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

Michelle F. Meagher (mmeagher@mymail.mines.edu)[1*], Yuntao Hu[2,3], Melissa S. Roth[3], Alex J. Metcalf[1], **Krishna Niyogi[3]** ,Trent R. Northen[2,4], and Nanette R. Boyle[1]

[1]Colorado School of Mines, Golden CO, [2]Lawrence Berkeley National Laboratory, Berkeley, CA; [3]University of California, Berkeley, CA; [4]Joint Genome Institute, Berkeley, CA

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

Project Goals: Our overarching research goal is to engineer the green alga *Chromochloris zofingiensis* for the production of biofuels. 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 group is focused on the development of computational models of cellular metabolism in this organism to study carbon partitioning on a genome wide scale. The Northen group is focused on the exometabolomics and metabolomics analysis of the *C. zofingiensis* to help us understand the algal nutrient preference and metabolism 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) with uses in the pharmaceutical, nutraceutical, and cosmetic industries. It also demonstrates high levels of triacylglycerol accumulation and low photosynthetic productivity when additional organic carbon sources are provided[1], making it ideal for metabolic or genetic engineering focused on increasing algal lipid production.

To create a genome-scale metabolic model of this organism, we implemented a new computational algorithm, Rapid Annotation of Photosynthetic Systems[2] (RAPS). This model was used to perform flux balance analysis (FBA) of this organism with experimentally determined constraints for photoautotrophic and heterotrophic growth on glucose. The results of these FBA studies show highly variant metabolic flux distributions for these different growth conditions. Using experimentally determined constraints of nutrient uptake, it was found that glucose consumption

was higher than that required to match experimental growth rate data. Model simulations predicted the excretion of lactate, among other products, as an outlet for the carbon imbalance. We were able to validate this assumption with metabolomics analysis.

To gain phenotypic data for model refinement, we conducted a *C. zofingiensis* time-course experiment, by analyzing its metabolome and measuring alterations in media composition resulting from algal growth. Our results showed that *C. zofingiensis* secreted more diverse exometabolites during its heterotrophic growth stages when supplemented with glucose. Although *C. zofingiensis* has a clear nutrient (carbon/nitrogen source) preference order, it will recapture its secreted metabolites when the exogenous glucose is limited. During the heterotrophic growth of *C. zofingiensis*, we observed a decreased concentration of intermediates of the tricarboxylic acid cycle and amino acids, indicating a possible redirection of energy flux toward triglyceride synthesis. We are now incorporating the exometabolomic and metabolomic data to improve our understanding of algal metabolism and nutrient demand. The metabolic model that has been developed, used in combination with this extensive data set, has great potential to elucidate dramatic metabolic shifts within the organism and will enable informed strain engineering strategies to maximize lipid productivity in this organism.

References

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