

## Genome-scale Metabolic Model of *Chromochloris zofingiensis*, an Emerging Model Organism 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 <sup>13</sup>C-MFA.

*C. zofingiensis* is an emerging model system for the production of biofuels and bioproducts. It is an especially attractive system attractive 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<sup>1-3</sup>. In order to investigate the metabolic capacity of this organism for both fuel and astaxanthin production, we generated a genome-scale metabolic network reconstruction. The current reconstruction includes 3522 metabolic reactions and 2880 metabolites. In order to formulate an accurate biomass formation equation, we are also measuring both the macromolecule composition of *C. zofingiensis* (DNA, RNA, protein, lipid, carbohydrate) as well as the composition of each in photoautotrophic and photoheterotrophic growth modes. Predicted carbon flux distributions for each growth mode will be presented.

To enable faster reconstruction efforts of new organisms in the future, we also developed an automated reconstruction algorithm specifically designed for photosynthetic microorganisms. This approach leverages the manual curation efforts of published genome scale network reconstructions to minimize duplicate efforts for well-characterized pathways. We will present our algorithm, Rapid Annotation of Photosynthetic Systems (RAPS), and discuss the performance of automated reconstruction compared to other automated algorithms.

### References

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