

## **Iron and Nitrogen Regulate Trophic Transitions and Metabolism in the Oleaginous Green Alga *Chromochloris zofingiensis***

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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 partitioning 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 of the metabolism in *C. zofingiensis*.**

Unlike the well-studied model organism *Chlamydomonas reinhardtii*, *C. zofingiensis* can use external glucose as a sole carbon source. Here we show that interplay of glucose with iron and nitrogen concentrations drastically changes the metabolism of this organism. In iron-depleted medium with glucose in the light, *Chromochloris zofingiensis* shuts off photosynthesis to become fully heterotrophic (1), whereas replete iron rescues photosynthesis even in the presence of glucose. Furthermore, iron and nitrogen depletion in glucose medium results in production of the valuable carotenoid astaxanthin and likely the biofuel precursor triacylglycerol. To aid the study of glucose responses and the interplay of glucose with other essential nutrients, we have designed an optimized growth medium to enhance photoautotrophic growth and compensate for increased nutrient losses during growth on glucose. This defined medium will serve as a strong reference point for time course and systems analyses of the molecular responses underlying trophic transitions and accumulation of bioproducts. Through forward genetic screens on glucose, we have also generated mutants that do not trigger expected responses to iron, glucose, or nitrogen. These include mutants related to regulation of photosynthesis by iron and glucose and mutants that accumulate astaxanthin in non-inducing conditions. In total, this work positions *C. zofingiensis* as a platform for identifying the genes underlying the nutrient regulation of photosynthesis and metabolism, which will provide new targets for engineering algae for enhanced bioproduction.

### **References**

1. Roth MS, Gallaher SD, Westcott DJ, Iwai M, Louie KB, Mueller M, Walter A, Foflonker F, Bowen BP, Ataii NN, Song J, Chen J-H, Blaby-Haas CE, Larabell C, Auer M, Northen TR, Merchant SS, Niyogi KK (2019) Regulation of oxygenic photosynthesis during trophic transitions in the green alga *Chromochloris zofingiensis*. *Plant Cell*, in press.

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