Lipidomics analysis of the emerging model 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 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 us in the redesigning and engineering of the metabolism of in *C. zofingiensis*. This presentation will focus on the lipidomics analysis of the *C. zofingiensis* trophic transitions to help us understand the key metabolic pathways involved in lipids synthesis and degradation.

Abstract text: The emerging model green alga *Chromochloris zofingiensis* is a promising producer of lipids and astaxanthin, in which lipids can comprise up to 50% of its dry biomass. One of its unique characteristics is that its photosynthesis can be switched off under the supplement of additional carbon sources (e.g. glucose)[1]. The changes of the metabolism result in the decomposition of thylakoid membranes and accumulation of lipids, e.g. triacylglycerols (TAGs). The abundance of TAGs increased over 20-fold within a few days with glucose making it ideal for the extraction of lipids as the biofuel precursor. In addition, thylakoids are reassembled and photosynthesis is resumed when the carbon source is depleted. This unique metabolism switch phenomenon offers us a great opportunity to reveal the key metabolic pathways of the algal lipid synthesis.

To gain insights into this metabolic switch we are developing lipidomic approaches for analyzing the algal lipids using high performance liquid chromatography coupled to Q Orbitrap Exactive mass spectrometry. Critically, we are also developing the cheminformatic capabilities to analyze the complex lipid profiles. MetaboliteAtlas is our main software tool and it requires a manually defined "Lipid Atlas" with defined mass-to-charge ratios and retention times. To date we have characterized algal lipids, e.g. phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine, lysophosphatidylethanolamine, and glycerolipids, to create the requisite Lipid Atlas, which currently includes ~1300 lipids divided into more than 14 lipid subclasses. Once complete, we will use this approach to analyze a time series experiment of *C. zofingiensis* to observe its trophic transitions by the addition of glucose. The resulting information will be used to refine and develop predictive models that will provide new biological insights into how this important organism is able to re-route flux during trophic transitions.

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References

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