A Systems-Level Investigation of Low-Light Acclimation in the Marine Diatom *Phaeodactylum tricornutum*.

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**Project Goals:** Our overall goal is to reprogram metabolic networks using *in vivo* synthetic modules to increase the flux of energy and carbon into biofuel precursors in the marine diatom *Phaeodactylum tricornutum*. This is broken down into 3 sub-goals: 1) Profile the transcriptome, proteome and metabolome to investigate cell responses to physiologically relevant conditions. 2) Identify and manipulate key factors involved in the control of inorganic C assimilation, photosynthetic efficiency and carbon partitioning. 3) Create screen and genotype a forward genetic library generation. These approaches complement our development of *Phaeodactylum* genome reconstruction modeling and our development of novel synthetic genomic tools to achieve our overall goal of increasing photosynthetic productivity.

The natural light environment is highly dynamic. Photosynthetic metabolism is regulated at the physiological and genetic level to respond to these changes. Diatoms, which are eukaryotic algae, are capable of adjusting their metabolism to maximize light harvesting efficiency in low light and minimize oxidative damage in excess light. Most studies have focused on the stress response during acclimation to excess light, but low light photoacclimation has received less attention. We observed changes in the transcriptome, proteome, metabolome and in the photophysiology of *Phaeodactylum tricornutum* following a shift from excess light fluxes to low light (880 to 80 µmol photons/m²/s) over 24 hours. We observed an increase in cell pigments and in pigment precursors using established visible spectroscopy methods and by our non-targeted metabolomics methods. We developed novel statistical methods for analyzing time-series based –omics data. In general, we saw increases in light harvesting complex (LHC) transcripts following the shift to low light this was trailed by a significant increase in total LHC protein abundance. However, changes in an individual LHC’s protein abundance were not necessarily predicted by changes in transcript. This pattern was frequently observed when comparing transcript and protein abundance and we will discuss the implications of inferring metabolic changes from transcriptomics alone. Catabolic pathways quickly responded to lower light. Energy storage molecules, such as triacylglycerols and chrysolaminarin (carbohydrate storage polymer) decreased. The relative abundance of many hexoses, disaccharides and fatty acids began to increase within 20-40 minutes and this was accompanied by rapid up-regulation of the TCA cycle, but without major increases in measured respiration rates. Lipid classes associated with both the thylakoid membrane and other structural lipids increased in relative abundance during acclimation to low light. This correlated with an increase in transcripts encoding components associated with fatty acid elongation. Interestingly, organic carbon per cell only
dropped by 15% during photoacclimation and growth rates remain unchanged. These results suggest that *Phaeodactylum* can rapidly adjust its metabolism to maintain fitness in a rapidly changing environment. These findings will be integrated into a new genome reconstruction model in order to improve our understanding of energy and carbon partitioning. Ultimately, this study will provide guidance for our engineering efforts to increase the production of biofuel precursors.

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