

## **Modeling carbon metabolism of the diatom *Phaeodactylum tricornutum* during nitrogen starvation and during high light and low light conditions**

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**The diatom *Phaeodactylum tricornutum* (Pt), a model photosynthetic eukaryotic microbe, has the ability to store up to 45% of dry cell weight as triacylglycerol (TAG), a neutral lipid and precursor to biodiesel<sup>1</sup>. To take advantage of this innate ability, we need to understand how metabolic pathways adjust to changing environmental conditions. The long-term goal of this project is to promote efficient production of high-value and fuel-related compounds through optimization of metabolic fluxes in Pt. Building upon our expertise in <sup>13</sup>C metabolic flux analysis (MFA),<sup>2</sup> our current goal is to develop novel experimental protocols and data analysis workflows to enable <sup>13</sup>C flux analysis of Pt. We are currently investigating the metabolic adjustments of Pt to three variables, *i.e.*, light, nitrogen availability, and genetic knockout of TAG degradation enzymes, which strongly impact cell growth and lipid accumulation.**

In our first study, we varied the light intensity supplied to the Pt culture. We compared metabolic fluxes inside wild-type (WT) Pt cells grown under low-light (60  $\mu\text{E m}^2 \text{s}^{-1}$ ) or high-light (250  $\mu\text{E m}^2 \text{s}^{-1}$ ) conditions. We observed higher metabolic flux in the TCA cycle under high light relative to the low light. Further, we observed that the TAG profile at high light contained significantly more omega-3 fatty acids compared to low light.

In a second study, we investigated metabolic fluxes inside wild-type Pt and a nitrate reductase (NR) knock-out strain in response to changing nitrogen availability in the culture medium. We studied three cultures, *i.e.*, Pt-WT with nitrate (Pt-WT), Pt-WT without nitrate (Pt-WT\_N-), and Pt-NR with nitrate (Pt-NR). We found enhanced accumulation of TAG in Pt-WT\_N- and Pt-NR relative to Pt-WT. Concomitantly, the pool sizes of pyruvate (end product of glycolysis) and amino acids decreased significantly in Pt-WT\_N- compared to those in Pt-WT, whereas the pool sizes of TCA cycle metabolites and urea increased dramatically. Our results are consistent with previous findings that genes associated with urea cycle are upregulated while expression of urea-degrading urease is downregulated in WT Pt cells under N- conditions<sup>3</sup>. Interestingly, Pt-NR showed a similar trend of abundance of intracellular metabolites except a few in the TCA cycle. Our preliminary <sup>13</sup>C-MFA results have revealed remarkable differences in the metabolic fluxes between Pt-WT, Pt-WT\_N- and Pt-NR.

In a third study, we aimed to characterize metabolic changes in an acyl-CoA dehydrogenase knockout (ACAD-KO) Pt strain. When Pt cultures are switched from nitrogen-depleted to nitrogen-replete media, WT cells rapidly degrade the accumulated TAG while ACAD-KO cells retain their TAG storages. Comparing the ACAD-KO strain to WT after nitrogen repletion, we observed increases in TCA cycle labeling in the ACAD mutant. We hypothesize that the TCA cycle in the WT strain is being fed by the breakdown of the TAG, resulting in lower labeling.

Our findings based on  $^{13}\text{C}$  MFA will help us to understand how Pt metabolism adapts to various environmental conditions and genetic modifications, which will guide strain engineering efforts to maximize TAG biosynthesis in Pt.

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