Improving the production of biofuels by understanding metabolic pathways in microalgae

The objective of the proposed research is to quantitatively assess the metabolic capabilities of microalgae to identify inefficiencies that limit cell growth and lipid synthesis. The long term goal is to create a strain of microalgae that is maximized for biofuel production. Currently, plant biodiesel is the main source of biofuels. However, the demand for oil has outpaced the amount of biodiesel that can be produced in this manner. Microalgae are a viable alternative to plants due to their ability to produce up to 370 barrels of oil per hectare, which is more than 100 times greater than the oil produced through soybeans- the main crop for biofuel.[1] Even so, efforts to enhance their biofuel- producing capability is still needed in order to realize their industrial viability. One of the biggest challenges with algae biofuels is the understanding of mechanisms that influence the production of triacylglycerol (TAG), a precursor to biodiesel.

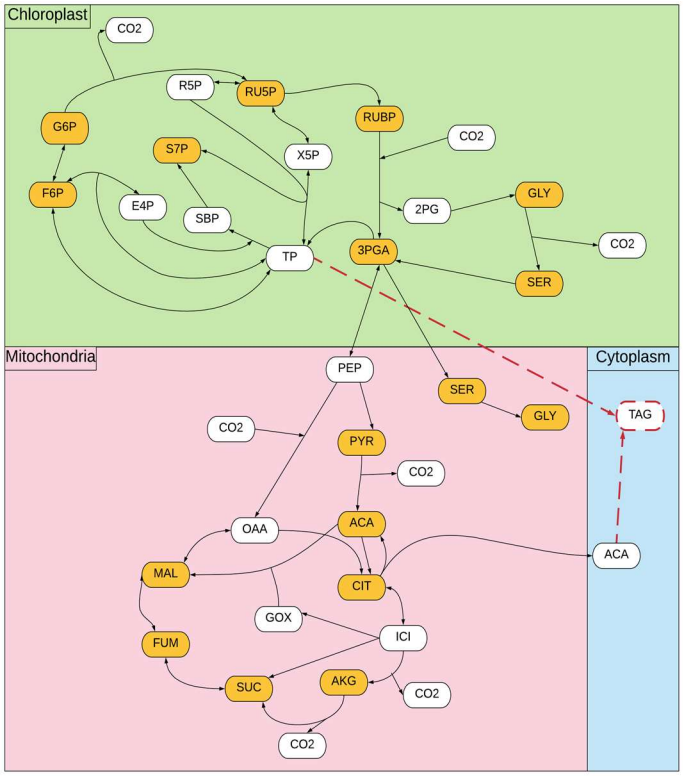

Figure 1: Metabolic network for Pt
The colored boxes indicate different cellular compartments. Dark yellow circles indicate the metabolites that can be measured using GC-MS and LC-MS. While circles indicate metabolites that cannot be measured. Red dotted lines indicate that reactions exist between TP, ACA and TAG.

The objective of the proposed research is to develop a platform to identify reactions that limit TAG production in the central carbon metabolism of algae (Fig 1). The microalgae, Phaeodactylum tricornutum (Pt), is ideal for biofuel production: 1) they efficiently fix atmospheric CO_2- responsible for absorbing at least 25% of the total amount of carbon dioxide processed by the seas, 45-50 billion tons of organic carbon [2] 2) they accumulate up to 45% of their dry cell weight in TAG [3].

In recent years, advances in genomic tools for Pt such as genome editing[4] and stable delivery vectors[5]. allow us to further enhance the ability of Pt to produce TAG. However, the genes that correlate to these metabolic inefficiencies are currently unknown so gene alterations are conducted through educated guessing.

Stable isotope tracers, such as ^{13}C, are added to biological systems to track patterns of isotope incorporation into numerous products synthesized by the cell, including TAG. Coupling tracers with Isotopically Nonstationary Metabolic Flux Analysis (INST-MFA), the metabolic fluxes in central carbon metabolism as well as the transport rates across cellular compartments (Fig 1) can be determined. With this knowledge, reactions that divert carbon away from TAG can be identified and subsequently targeted for gene editing. Through this method, we will be able to create a strain of Pt that is optimized for TAG production.

The proposed work will be broken down into two major tasks: 1) Describing the central carbon metabolism of Pt using INSTA-MFA 2) Highlight metabolic targets for deletion and assess its effect on TAG production.

Task 1: Developing flux map of central carbon metabolism in Pt The objective is to find which reactions contribute to TAG production using INSTA-MFA. INSTA-MFA combines of computational and experimental methods in order to describe the metabolism of an organism. This is due to the infeasibility of measuring all metabolites within an organism. Therefore, the metabolites that cannot be explicitly measured (white circles in Fig 1) must be interpolated using a model. In the computational portion, a model is compiled from literature and biochemical databases focusing on the central carbon metabolism. Central carbon metabolism the main focus of our model because these pathways contain the major carbon reactions. Carbon enters the organism as CO_2 and is turned into biomass or TAG.[6] TAG is synthesized from ACA and TP (red dotted line Fig 1). We have created this model for Pt which includes all of the measurable metabolites and the major carbon cycles such as the Calvin, Tricarboxylic Acid Cycle and Pentose Phosphate Pathway (Fig 1).

On the experimental side, metabolites are tracked by feeding the ^{13}C tracer as sodium carbonate (yellow circles Fig 1), allowing us to track the pathway of the carbon through the organism. The metabolites containing the tracer in the organism will increase over time as it becomes incorporated into the major cycles. This pattern of incorporation over time is called the Metabolite Labeling Data (MLD).

In INSTA-MFA, MLD is combined with the computational model to understand the labeling pattern of unmeasurable metabolites. The results gives us the fluxes through all of the reactions present in the model, also known as a flux map. Using the flux map, we can identify bottlenecks, which are genes corresponding to reactions that direct CO_2 away from TAG.[6]

Task 2: Highlight metabolic targets for deletion and assess its effect on TAG production The objective of the second area is to produce the diatom with the maximum amount of TAG production by targeting the genes correlated to the bottlenecks.

From our simulation created in Area 1, we can identify reactions that improve TAG production through gene alteration. However, there is a gap in knowledge on how these gene mutations affect the metabolism of the diatom. Using INSTA-MFA, we can predict which genes limit TAG production by looking for bottlenecks. Then, we can delete those genes with help from our collaborators at Colorado State University, who have developed methods for genome engineering in diatoms, to produce desired mutants. After the mutants have been synthesized, we can assess the changes in metabolism and identify additional bottlenecks by using INSTA-MFA again. Through this iterative process, we can create a strain of diatoms that produce the maximum amount of TAG.

Broader Impacts: Within the scientific community, this project will help us understand how CO_2 is incorporated into photosynthetic organisms. On an industrial scale, increasing the efficiency of biofuel production in Pt will eliminate our reliance on fossil fuels. INST-MFA allows us to understand the metabolism of Pt by combining ^{13}C isotope experiments and simulations to create a unique flux map. From this map, we can identify genes that can be mutated to increase TAG production. By understanding how metabolism within Pt functions, we can create a diatom that produces the maximum amount of TAG physically possible.

Sources: [1] Chisti *Biotechn Adv* **25**, 294-306 (2007). [2] Young et al.*Metab Eng* **13**, 656-665 (2011). [3] Falkowski, et al. *Aquatic Photosynthesis*. 2nd edn, (Princeton University Press, 2007). [4] Hu et al. *Plant J* **54**, 621-639 (2008). [5] Weyman et.*al Plant Biotechnol J*. **13**, 460-470 (2015) [6] Cheah et al. *Systems Biology* 25-70 Wiley (2017).