Developing episome-based gene expression platforms in the model diatom *Phaeodactylum tricornutum*

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Project Goals: We propose integration of genome-scale modeling with genome engineering to optimize energy and metabolte flux through subcellular compartments to promote efficient production of high value and fuel-related metabolites. We aim to construct streamlined artificial chromosomes encoding reprogrammed biological modules designed for *in vivo* optimization of electron flow efficiency, photosynthesis, and overall cellular growth while directing key metabolic precursors away from storage carbohydrates and into lipids or branched chain amino acids (BCAA). The underlying goal of the proposed research is to produce strains of diatoms encoding cellularly compartmentalized biosynthesis pathways on an artificial chromosome, with the natural genetic background altered to include knockouts of respective native genes as well as the installation of in vivo metabolite bioreporters. Specific goals and technical approaches are focused around four themes: 1) Modeling and Flux studies, 2) Photosynthetic efficiency, 3) Linking metabolic and regulatory networks, 4) Genome scale engineering methodology and application.

Developing genetic approaches for efficient and scalable disruption of gene expression can aid in dissecting mechanisms governing cellular processes, studying gene function, enable high throughput genome-scale screens and assist in redirecting metabolic flux towards high-value metabolites. In diatom algae, RNAi has been the most commonly used gene expression knockdown tool. The current state of the art for inducible gene expression is based on endogenous promoters that respond to different environmental conditions that also change host physiology. Here, we are developing highly efficient, target specific, tunable, and scalable transcriptional control system in the model diatom *P. tricornutum*. We designed and tested six episome-based chemical inducible gene expression systems that have been proven effective in other eukaryotic organisms. Using flow cytometry, we assess time- and dose-response dynamics of each expression system and its reporter protein (YFP). Addition of a chemical inducer/ligand to transgenic strains activates transcription with a dynamic range of up to ~450-fold. We demonstrate our transcriptional control system is tunable and reversible in a dose-and time-dependent manner. Using RT-PCR, we found that inducer dependent transcriptional activation starts within ten minutes of addition and saturates within two hours - without any detectible expression in controls. Upon inducer withdrawal, the expression of the reporter protein was nearly undetectable after 24hrs. The system described here will expand the molecular and synthetic biology toolkits in algae, facilitate gene discovery efforts and tailoring of organisms for maximum productivity.

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