System-level analysis of metabolic trade-offs and changes during diurnal cycle of *Chlamydomonas reinhardtii*

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Project Goals: The LLNL Biofuels SFA seeks to support robust and sustainable microalgae fuel production through a systems biology understanding of algal-bacterial interactions. We hypothesize that by understanding the factors that control cellular physiology and biogeochemical fluxes in and out of algal cells, particularly through the phycosphere, we can advance the efficiency and reliability of algal biofuel production. Our research includes studies of probiotic traits of phycosphere-associated bacteria, systems biology studies of model algae, and genome-enabled metabolic modeling to predict the interspecies exchanges that promote algal growth, lipid production and healthy co-cultures. Our overall goal is to develop a comprehensive understanding of complex microbial communities needed to advance the use of biological properties for practical energy production.

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Genome-scale models (GSM) used with constraint-based optimization approaches such as Flux Balance Analysis (FBA)[1] allow system-level characterization of metabolic traits such as needs for specific nutrients or the ability to produce compounds of interest such as biofuels. FBA models are constrained by experimental measurements and fundamental physio-chemical laws and solve for a feasible metabolic flux pattern that would result in the optimum value for one biological objective (e.g., growth or ATP production). To assess metabolic changes in the microalga *Chlamydomonas reinhardtii* as it transitions through its daily lifecycle, we have used a comprehensive omics dataset and a curated GSM[2], along with a suite of LLNL-developed constraint-based modeling approaches. We used transcriptomic and proteomic data within our GX-FBA[3] modeling approach to identify critical active pathways as the cell changes its metabolism from energy-rich daytime phototrophic metabolism to nighttime conditions where chemotrophic metabolism dominate. The GX-FBA approach expands the utility of FBA models by using omics data such as gene-expression measurements to constrain GSM of metabolism and optimize (within constraints of mass balance) metabolic flux variations associated with changes in enzyme concentration. Thus, GX-FBA calculates metabolic changes as a system adapts to new environments. Our results indicate that significant changes associated with production of energy, biomass, redox potential and antioxidants are needed to neutralize harmful reactive oxidative species. Furthermore, by mapping the measured nutrient flux rates within the *n*-dimensional Pareto solution space generated by our high-dimensional Multi-Objective Flux Analysis (MOFA) modeling approach, we quantified the metabolic trade-offs among critical biological objectives at various stages of the *C. reinhardtii* lifecycle. Our results show that in addition to previously identified dominant biological objectives (growth, ATP production, optimum resource allocation)[4], during energy abundant periods, the cell diverts some energy toward other biological activities such as production of byproducts. However, our simulations show that significant production of any energy rich compound such as lipids or hydrogen gas has deleterious effects on cellular growth. Future plans include examination of environmental factors that determine the choice of which metabolic byproducts are produced and their biological roles.

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