Title: Developing, Understanding, and Harnessing Modular Carbon/Nitrogen-Fixing Tripartite Microbial Consortia for Versatile Production of Biofuel and Platform Chemicals

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Project Goals: The overall goal of this project is to design, construct, analyze and optimize a synthetic microbial consortium system consisting of three closely interacting members: a CO_2-fixing photosynthetic specialist, a N_2-fixing specialist, and a third specialist that can convert organic carbon and nitrogen generated by the first two specialists to synthesize a desired product. By integrating complimentary expertise from multiple research labs at three institutions, we are pursuing three specific objectives: i) Develop tripartite microbial consortia for carbon/nitrogen fixation and production of bio-molecules with various nitrogen/carbon ratios; ii) Investigate molecular and cellular mechanisms governing the tripartite consortia via omics study and predictive modeling; and iii) Explore alternative spatial configurations and develop scalable design principles.

Abstract:

Microbial communities are ubiquitous in nature, exhibiting incredibly versatile metabolic capabilities and remarkable robustness. Inspired by these synergistic microbial ecosystems, rationally designed synthetic microbial consortia is emerging as a new paradigm for bioprocessing and offers tremendous potential for solving some of the biggest challenges our society faces. In this project, we focus on a tripartite consortium consisting of a CO_2-fixing photosynthetic specialist, a N_2-fixing specialist, and a third specialist that can convert organic carbon and nitrogen generated by the first two specialists to synthesize a desired product. In addition to CO_2 fixation, a noteworthy feature of this design is the elimination of the requirement for nitrogen fertilizer, which has been produced through ammonia synthesis using the Haber-Bosch process and accounts for an estimated 2% of global energy expenditure. We aim to develop a modular and flexible model system capable of producing diverse bio-molecules (varying C:N ratio) as advanced biofuel or platform chemicals, to dissect this complex ecosystem using a spectrum of cutting-edge systems approaches, and to ultimately derive scalable and broadly applicable design principles for maximizing the system performance.

Our first prototype tripartite consortium employs genetically modified strains of photosynthetic cyanobacterium *Synechococcus elongatus*and and nitrogen-fixing bacterium *Azotobacter vinelandii*, that secrete sucrose and ammonia respectively, to form a symbiotic foundation for supporting a third producer member. We demonstrated supported growth for a range of producer strain candidates, including a sucrose-metabolizing *Escherichia coli* K-12 derivative strain, *Corynebacterium glutamicum*, and *Bacillus subtilis*, using a multi-chamber bioreactor system

under continuous culture conditions. We have also developed an ODE-based mathematical model to capture essential molecular, cellular, ecological and process characteristics of the experimental system.

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