Employing Bacterial Microcompartments To Create Privileged Redox Pools for Biofuel Production

Charlotte H. Abrahamson[1*] (cabrahamson@u.northwestern.edu), Sasha Shirman[1*] (aleksandra.shirman@northwestern.edu), Md Aminul Islam Prodhan[1*] (md.prodhan@northwestern.edu), Svetlana P. Ikonomova,[1] Bastian Vögeli,[1] Andre G. Archer,[1] Taylor M. Nichols,[1] Keith E.J. Tyo,[1] Niall Mangan,[1] and **Danielle Tullman-Ercek**[1]

[1]Northwestern University, Evanston, Illinois

https://dtelab.northwestern.edu/research/#nanobioreactors

Project Goals: To compartmentalize metabolic pathways along with enzyme cofactor recycling pathways to increase the yield and efficiency of bioproduction processes

Metabolic engineering holds great promise for creating efficient, competitive routes for the production of biofuels and biochemicals without the necessity for harsh chemicals and hazardous byproducts. Successes in biochemical engineering include Dupont's Sorona fiber, which is made using bacterially-produced 1,3-propanediol from glucose. However, roadblocks to biosynthesis prevent many biochemicals from being produced biologically given current technology. Nature uses compartmentalization (eg in organelles in eukaryotes and in bacterial microcompartments in prokaryotes) to solve issues such as intermediate leakage, toxicity, and byproduct formation. Here we propose to deploy compartmentalization as a strategy to overcome a critical roadblock: the requirement for redox cofactor recycling. In traditional systems, redox cofactors are lost to cellular growth and maintenance needs. By compartmentalizing redox cofactors with the biochemical synthesis enzymes, we anticipate increasing the thermodynamic efficiency and preventing the loss of valuable intermediates and cofactors. If successful, it would be the first direct demonstration of this feature of a bacterial microcompartment, and would provide a tool for improving metabolic pathway performance for all enzymes with redox or other cofactors.

With this poster, we will describe results from coupling modeling with experiments to guide experimental design and facilitate the engineering of novel pathways in microcompartments (MCPs). Understanding of the native 1,2-propanediol utilization MCPs will help improve the performance of our target metabolic pathways: 1,3-propanediol production and medium-chain fatty acid synthesis via reverse β-oxidation (rBox) pathway. We used computational simulation to determine the optimal experimental sampling times, creating an assay for pathway function. The mathematical method uses an eigenvector decomposition of the state space model manifold to estimate the sensitivity of experimental timepoint and metabolite sampling to MCP properties. We applied this assay first to the native 1,2-propanediol MCPs. To gain more in-depth characterization of the composition of MCPs and strains expressing MCPs, we used tandem mass tag (TMT) based proteomics on purified MCPs and cell lysates containing MCPs. We identified proteins that showed significant fold-change in relative quantity between purified MCPs and lysates. Additionally, we made headway in the encapsulation of the non-native metabolic pathways. We incorporated cell-free protein synthesis and cell-free metabolic engineering to test function of a partially encapsulated pathway. Butanoic acid and hexanoic acid were produced

when we supplemented MCPs containing a partial pathway with unencapsulated enzymes in an *in vitro* assay.

This material is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DE-SC0019337.