Employing Bacterial Microcompartments To Create Privileged Redox Pools for Biofuel Production

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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 how we are coupling modeling with experiments to first understand the native function of the 1,2-propanediol utilization microcompartments (MCPs), and particularly the recycling of native cofactors. Using statistical and structural analysis of our model we have determined several guidelines for experimental design, including 1) using variation in the compartment number to disambiguate between the membrane and microcompartment permeability values for future cell-based experiments and 2) optimization of sampling during time-series to capture maximally informative features. We then established an *in vitro* method for analyzing pathway performance of purified MCPs, and detected conversion of 1,2-propanediol to the intermediate propionaldehyde and product 1-propanol. Interestingly, exogenous addition of adenosylcobalamin (AdoB12) was required to activate consumption of 1,2-propanediol. Moreover, we present evidence that cofactors including AdoB12, ATP, and coenzyme A can enter the MCP lumen during the *in vitro* reaction, but also that activity depends on recycling of NAD^+ and NADH. We are currently applying the findings from the native

system towards improving the performance of our first target metabolic pathway: 1,3-propanediol production.

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