Title: Employing Bacterial Microcompartments To Create Privileged Redox Pools for Biofuel Production

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

Abstract Text: 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 (e.g. in organelles in eukaryotes and in bacterial microcompartments in prokaryotes) to solve issues such as intermediate leakage, toxicity, and byproduct formation. We proposed to deploy compartmentalization as a strategy to overcome a critical roadblock: the requirement for redox cofactor recycling. We set out to directly demonstrate the redox recycling feature of a bacterial microcompartment (MCP) for the first time, and apply it to 1,3-propanediol production from glycerol. With this poster, we will describe how we have leveraged modeling to both inform strain design and provide fundamental insight into MCP function.

We first studied the native 1,2-propanediol (1,2-PD) utilization (Pdu) pathway to develop understanding of the principles that govern cofactor recycling in MCPs. We investigated the cofactor recycling thought to occur in the native 1,2-PD degradation pathway by running *in vitro* assays on purified Pdu MCPs. We found that while external cofactor addition is required for Pdu pathway activity, cofactor recycling enabled the pathway production to exceed stoichiometric cofactor concentrations. These findings suggest that the native system benefits from cofactor recycling but is also somewhat permeable to cofactors. Mathematical modeling of the native pathway further informed the conditions tested in these *in vitro* experiments, allowing precise fitting for parameters that govern MCP performance, such as MCP permeability. Lastly, alternative morphologies of the Pdu MCP were investigated using the native pathway *in vivo* and *in silico* for use as alternative scaffold structures.

Our insights from the native system were used to guide modeling and strain development to advance our goal of encapsulating the 1,3-propanediol (1,3-PD) production pathway. To optimize stoichiometry for 1,3-PD enzymes, we built a model to predict toxic intermediate build-up and product formation in the pathway. We quantified parametric uncertainty based on existing data, and optimized enzyme ratio in compartments based on the resulting range of intermediate and product dynamics. The expression and encapsulation of the 1,3-PD pathway enzymes *in vivo* were tuned to achieve the optimal stoichiometry predicted by the mathematical model. Combined, this work will facilitate future use of MCPs for addressing issues with pathways limited by imbalances in redox cofactors.

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