Engineering bacterial microcompartments in *Clostridium autoethanogenum* to overcome bottlenecks in sustainable production of synthetic rubber

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

Project Goals: To investigate bacterial microcompartments in *C. autoethanogenum* and engineer them to compartmentalize synthetic metabolic pathways.

One promising route to sustainable bioproduction of fuels and chemicals is the engineering of organisms such as acetogens to efficiently convert abundant and low-cost carbon monoxide (CO) or carbon dioxide (CO_2) and hydrogen (H_2) containing gases to desirable products at high efficiency and low cost. This approach not only provides an avenue for repurposing greenhouse gases (GHG), but also minimizes the necessity for harsh chemicals and hazardous byproducts common in petroleum-based processes. However, many biochemicals are not yet produced biologically due to roadblocks in the cellular biosynthesis process. These roadblocks can include toxicity of intermediates, redox imbalances, and/or loss of product to off-pathway reactions. Nature uses spatial organization strategies, such as sequestration in organelles, to alleviate these issues. In bacteria, organization occurs in protein organelle-like structures known as bacterial microcompartments.

We will investigate the native regulation, assembly, and function of microcompartments in the industrially relevant non-model host *Clostridium autoethanogenum*. In the *C. autoethanogenum* genome, two unique clusters (Pdu and Cut) that resemble MCP operons, including the presence of putative hexamers, trimers, pentamers, and enzyme encapsulation sequences have been identified. RNAseq data showed functional genes in the Pdu cluster to be significantly upregulated ($p<0.001$) in the presence of specific substrates compared to control conditions. These findings are corroborated by electron microscopy of *C. autoethanogenum* grown in the same conditions, which shows distinctive polyhedral shapes within the cells indicative of MCP formation.

Our goal is to sequester key biosynthesis enzymes from two distinct metabolic pathways into microcompartments to make compounds involved in rubber production to showcase the power of the strategy for reducing toxicity and product losses due to side reactions. We will also couple modeling with experiments to understand the native system and identify the most promising targets for compartmentalization. If successful, this work would 1) provide insight into the native function of these structures in this organism, 2) be the first direct demonstration of this feature of a bacterial microcompartment in a non-model organism, and 3) would provide a detailed method for repeating this success in other organisms and with other pathways. Ultimately, this will lead to the cost-efficient production of chemicals that are currently derived from petroleum.

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