Step-wise Assembly of Complex Genetic Circuits in Synthetic Minimal Cells

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Synthetic minimal cells, or liposome bioreactors, are becoming an increasingly popular tool in the study of a wide range of biological systems. These areas include the study of biochemical reactions, the processing of biomolecules, as well as in the study of basic biological and evolutionary principles. These recent advancements in applications of liposomes are driven in part by new developments in microfluidics and cell-free protein expression.

Synthetic minimal cells utilize liposomes' ability to provide isolation of the inner reaction conditions and gene expression systems from the outside environment. By employing this property, coupled with programmable fusion of liposomes, we are able to construct encapsulated genetic circuits. These systems offer a high degree of control over both reaction conditions as well as a timing and stoichiometry of gene addition at each intermediate step of the pathway, enabling close mimics of biological pathways with similar complexity and controllability to natural systems.

We initially show the potential to construct liposome-based genetic circuits by reconstituting a ten-component pathway using nine populations of liposomes. These liposomes undergo eight total fusion events, with each one necessary for the formation of the final product. Next, in order to more closely represent the complexities of biological pathways, we constructed a branched genetic circuit. In this system, we were able to tightly control fusion of five different liposome populations, enabling the programmable formation of one protein end product over another. This work lays the foundation for the construction of complex genetic circuits composed of distinct liposome bioreactor populations, with each population optimized for expression of a particular gene. These populations can then be fused in a controlled order to yield a complex and complete genetic pathway.

While maintaining the simplicity and ease of working with an *in vitro* system, synthetic cells offer a new method for constructing complex genetic circuits that offers an unprecedented degree of control over reaction conditions. This programmability will allow us to construct models that approach the complexity of natural biological networks and systems.