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Editorial

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Cell-Free Synthetic Biology Special Issue

The foundational principle of cell-free synthetic biology is that precise, complex biomolecular systems can be constructed without using intact cells. Instead, purified biomolecules or crude cell lysates are used, which can be accurately monitored and modeled. This approach, which complements *in vivo* methods, provides an unprecedented freedom of design to understand, harness, and expand the capabilities of natural biological systems.

Cell-free synthetic systems are becoming important as platforms for understanding why nature's designs work the way they do and for enabling biosynthetic routes to proteins, novel biopolymers, and small molecule chemicals. In recent years, a technical renaissance has inspired new engineering foundations and applications for cell-free biology. These efforts have led to programmed circuits, spatially organized pathways, coactivated catalytic ensembles, rational optimization and prototyping of synthetic multienzyme pathways, debugging of synthetic RNA genetic circuitry, advances in minimal cells, and even the first commercial uses. For example, SutroBio Pharma has shown linear scalability for cell-free protein synthesis (CFPS) from the microliter to the 100-L scale. This special issue of *ACS Synthetic Biology* is dedicated to new advances in using cell-free systems.

One major theme in cell-free synthetic biology is the construction of minimal cells from the bottom up. In this approach, pooling together essential purified biological macromolecules, their genes, and their small molecule substrates can enable self-replication. This special issue contains three papers focusing on developments that could impact our understanding of simple cellular models for artificial cell projects. Okano *et al.* explore the effect of encapsulating CFPS systems with different compartment volumes when using constant copy numbers of DNA. Soga *et al.* study the ability to produce and insert membrane proteins into giant unilamellar vesicles. Chizzolini *et al.* use a CFPS system to evaluate and characterize *in vitro* polycistronic expression.

An emerging area in cell-free synthetic biology uses CFPS systems (or *in vitro* transcription and translation (Tx−Tl) systems) for prototyping genetic construct performance. One idea is to identify robust, reliable genetic elements (*e.g.*, promoter function, terminator strength, *etc.*) prior to putting them in hosts. In one illustrative example, Iyer and Doktycz develop and present a strategy for thrombin-mediated expression that is modulated by a DNA aptamer. In two different manuscripts, Richard Murray's laboratory advances an *in vitro* "biomolecular breadboard" prototyping concept. Sun *et al.* develop and present a strategy for stabilizing linear DNA templates in CFPS reactions. They then use this strategy to rapidly build and test circuits entirely *in vitro* from separate parts. Siegal-Gaskins *et al.* characterize the cell-free genetic circuits "breadboard" and identify system loads and resource limitations. Each of these articles highlights the potential of using *in vitro* approaches to design, build, and test dynamic biochemical circuitry.

Beyond prototyping, several papers harness cell-free systems for the production of metabolites or proteins. You and Zhang engineer a synthetic enzymatic pathway with a mini-scaffoldin complex. Lu and Ellington show a strategy to design and select a synthetic operon in CFPS to demonstrate the possibilities for optimization of *in vitro* systems. In yet a different application area, publications from Albayrak and Swartz as well as Hong *et al.* showcase new directions in site-specific incorporation of nonstandard amino acids (NSAAs) into proteins. Albayrak and Swartz report a new approach to the synthesis of active linear and branched protein polymers, opening the way to new classes of biomaterials. Hong *et al.* developed a novel CFPS platform for efficient NSAA incorporation into proteins from a genomically recoded organism lacking release factor 1 (RF1).

In summary, the collection of articles in this special issue demonstrate the potential of cell-free synthetic biology for a broad array of applications, revealing significant potential for short- and long-term impact. Looking forward, cell-free synthetic biology will continue to open new opportunities for (i) debugging and optimizing modular construction of pathways and circuits through the use of simple, well-defined experimental conditions and (ii) carrying out molecular transformations when bioconversion yields, productivities, or cellular toxicity limit feasibility of whole-cell fermentation.

Michael C. Jewett

Department of Chemical and Biological Engineering Chemistry of Life Processes Institute Member, Robert H. Lurie Comprehensive Cancer Center Affiliate Member, Institute for Bionanotechnology in Medicine Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, United States

AUTHOR INFORMATION

Notes

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