ESTABLISHING CELL-FREE SYNTHETIC BIOLOGY FOR THE PRODUCTION OF THERAPEUTIC GLYCOPROTEINS AND CHEMICALS

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Imagine a world in which we could adapt biology to manufacture any therapeutic, material, or chemical from renewable resources, both quickly and on demand. Industrial biotechnology is one of the most attractive approaches for addressing this need, particularly when large-scale chemical synthesis is untenable. Unfortunately, current approaches to engineering organisms remain costly and slow. This is because cells themselves impose limitations on biobased product synthesis. It is difficult to balance intracellular fluxes to optimally satisfy a very active synthetic pathway while the machinery of the cell is functioning to maintain reproductive viability. Further, chemical reactions take place behind a selective barrier, the cell wall, which limits sample acquisition, monitoring, and direct control. In addition, cells are adapted to a relatively simple chemical operating system (i.e., a few common sugars, 20 amino acids), which presents researchers a limited set of accessible molecules with which to work. To overcome these limitations, my group is developing new strategies that widen the aperture of the traditional model of biotechnology. Specifically, we seek to create a new paradigm for engineering biocatalytic systems using cell-free synthetic biology. The foundational principle is that we can conduct precise, complex biomolecular transformations in crude lysates without using intact cells. This concept represents a significant departure from cell-based processes that rely on microscopic cellular 'reactors,' and provides an unprecedented freedom of design to modify and control biological systems. In this presentation, I will discuss my group's efforts to establish cell-free systems for biomanufacturing. In one example, I will describe the development of a novel cell-free expression platform for the synthesis of homogeneous glycoproteins. Specifically, we have combined Cell-Free Protein Synthesis (CFPS) and SelfAssembled Monolayers for Desorption Ionization Mass Spectrometry (SAMDI-MS) to characterize a cytoplasmic N-linked glycosyltransferase (NGT) in vitro and determine its peptide and sugar acceptor specificities at unprecedented depth and throughput with >3000 unique peptides. We then used insights provided by these peptide screens to direct the efficient installation of N-linked glycans onto small, robust acceptor sequon motifs (GlycTags) in three heterologous proteins. This work broadens the glycoengineering toolkit, facilitates discovery of the structural and functional consequences of glycan attachment, and makes possible new applications in glycoprotein therapeutics and conjugate vaccines. In another example, I will report a new cell-free framework that enables systematic optimization and debugging of biosynthetic pathways for metabolic engineering. In our framework, cell-free cocktails for synthesizing target small molecules are assembled by mixing-and-matching crude cell lysates containing one or more overexpressed pathway enzymes, enabling the parallelized construction of combinatorial designs. We use this framework to screen enzyme variants, optimize enzyme ratios, and explore cofactor landscapes for improving production of limonene. Our approach facilitates efforts to define, manipulate, and understand metabolic pathways for accelerated design-build-test cycles without the need to re-engineer organisms. In summary, our approach to gain direct access to and control of the inner workings of the cell dramatically increases the resolution at which we can manipulate catalytic ensembles to create new design rules for constructive biology that look beyond what does exist to what can exist. Collectively, our work in cellfree systems provides exciting opportunities to profoundly transform biochemical engineering and become a driver of global innovation.