CHEMICAL ENGINEERING

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Accelerating Industrial Biotechnology with Cell-Free Technologies

ABSTRACT: Industrial biotechnology is one of the most attractive approaches to address the need for low-cost fuels and products from sustainable resources, and nearly all current investments in biomanufacturing (almost $4B in 2018) are based on production using cells. However, chemical production from biological systems is often insufficient for manufacturing at the optimal concentration, rate, or yield because natural sources are difficult to optimize and may not scale easily (e.g., plants grow slowly). Thus, engineers seek to design enzymatic reaction schemes in microorganisms to meet manufacturing criteria. Success in these endeavors depends upon identifying sets of enzymes that can convert readily available molecules (e.g., glucose) to high-value products (e.g., medicines), with each enzyme performing one of a series of chemical modifications. Unfortunately, this is difficult because design-build-test (DBT) cycles—iterations of re-engineering organisms to test new sets of enzymes—remains costly, risky, and slow. This is because cells themselves impose inherent limitations on the effective synthesis of bioproducts. One key limitation is that cellular survival objectives are often diametrically opposed to the objectives of chemical engineers. As a result, a typical project today might only explore dozens of variants of an enzymatic reaction pathway which is often insufficient to identify a commercially relevant solution. With nearly half of approved small molecule drugs being derived from natural products and nearly all chemicals being produced from petroleum, it is essential that we speed up the biochemical engineering pipeline.

To address this opportunity, we developed a new in vitro prototyping and rapid optimization of biosynthetic enzymes approach (termed iPROBE) to inform cellular design (Fig. 1). Cell-free systems provide many advantages for accelerating DBT cycles. For example, the open reaction environment allows for direct monitoring and manipulation of the system to study enzymes and pathway performance before

implementing those reaction schemes in cells. The foundational principle of the iPROBE approach is that we can construct discrete enzymatic pathways through modular assembly of cell lysates containing enzymes produced in vitro rather than by living organisms. This reduces the overall time to build pathways from weeks (or even months) to a few days, providing an increased capability to test numerous enzymatic pathway combinations. A key conceptual innovation is that the DBT unit can now be cellular lysates rather than genetic constructs, allowing us to perform DBT iterations without the need to re-engineer organisms or reconstruct DNA.

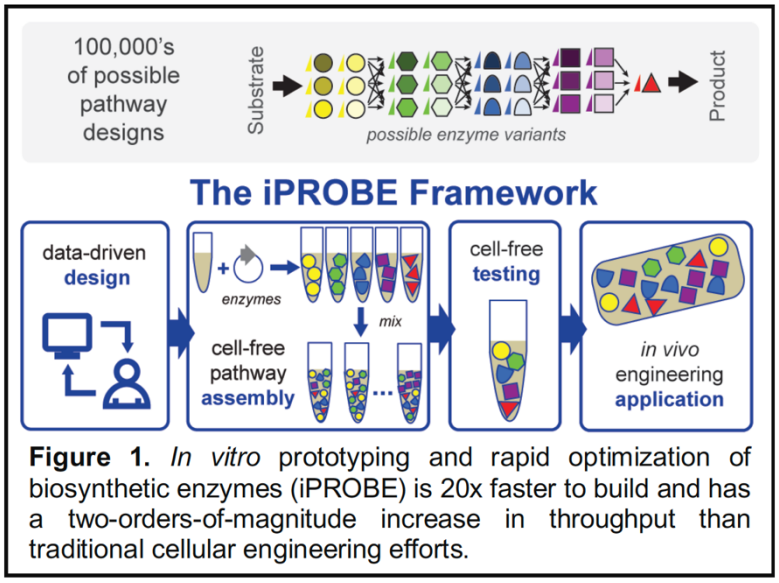

Figure 1. *In vitro* prototyping and rapid optimization of biosynthetic enzymes (iPROBE) is 20x faster to build and has a two-orders-of-magnitude increase in throughput than traditional cellular engineering efforts.

In this talk, I will show you how we've reimagined R&D for industrial biotechnology. In the first part, I will define our iPROBE approach and describe how we've used it to engineer and improve biochemical production in non-model organisms (e.g., clostridia). Specifically, by screening over 250 different pathway permutations using data-driven design we were able to show a strong correlation (r = 0.8) between cell-free and cellular performance and scale up our best performing pathway in an industrial strain of Clostridium to produce the highest reported cellular titers of 3-hydroxybutyrate, a platform chemical. We anticipate that iPROBE will continue to facilitate efforts to define, manipulate, and understand metabolic pathways and many other parameters confounded in living organisms for accelerated DBT cycles in the cell-free environment before engineering organisms. In the second part, I will highlight our developments toward expanding the scope of cell-free biomanufacturing which would enable production of molecules at a complexity and scale that are orders of magnitude beyond what is feasible today, establishing a range of innovative technologies to transform commercial applications and society.

BIOGRAPHY: Ashty Karim currently serves as a Research Fellow and Assistant Scientific Director in Michael Jewett's group at Northwestern University. His research focuses on developing cell-free tools to enhance metabolic engineering efforts with the goal of transforming industrial biotechnology. Dr. Karim finished his B.S. degrees in Chemical Engineering and Biology from The University of Texas at Austin and his Ph.D. in Chemical Engineering from Northwestern University. His work has been published across many journals including *Metabolic Engineering*, *Nature Chemical Biology*, and *ACS Synthetic Biology*, and has been recognized by the ACS BIOT Peterson Award as well as Northwestern Chemical Engineering's Distinguished Graduate Researcher Award. In addition, Dr. Karim advocates for science in the community having served as a mentor for Chicago-area school teachers and students, a EURAXESS LINKS North America Science Slam Finalist, and a U.S. Delegate to the 67th Lindau Nobel Laureate Meeting dedicated to Chemistry.

LECTURE 1:00 - 2:00 Zoom
Networking Hour on Zoom Following

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