Prototyping a Discovery Pipeline for Enzymes Using Cell-Free Systems

Enzymes are key to unlocking modern-day biotechnology, whether as an additive in detergent, a step in a biosynthetic pathway, or a replacement for chemical catalysis. Traditionally, producing enzymes requires cells and cellular fermentation. Although cellular fermentation is effective for producing large quantities of certain enzymes, it may not be the best solution for discovering new enzymes. Researchers have searched for a simpler method — in essence, a prototyping system — that could accelerate the discovery process without requiring fermentation.

In 2012, Richard Murray, a bioengineer at California Institute of Technology (Caltech), and graduate student Zachary Sun demonstrated a method of prototyping biological circuits without fermentation. “A key element of the design process in areas such as aeronautical, mechanical, and electrical engineering is the ability to perform rapid prototyping of early design concepts, and this was missing for biological engineering,” said Murray. Murray and Sun created a “biomolecular breadboard” based on a cell-free system of crude lysates derived from , which are capable of transcription and translation. Consequently, they are able to speed engineering cycles multifold for building complex genetic circuits such as feedforward loops and oscillators.

“Seeing the success of our cell-free prototyping concept, I thought, ‘Could we apply this to the discovery and engineering of enzymes?’” says Sun. He theorized that eliminating the need for cloning, transformation, and growth of cells could yield multifold improvements in scale, time, and costs for producing enzymes and identifying candidates to move forward. Sun cofounded Tierra Biosciences in part to perfect the science of cell-free systems; today, he serves as the company’s CEO.

Although the cell-free prototyping technology harnessed by Tierra stems from the earlier Caltech system, it needed to be adapted to ýt the enzymes market, where producing enough material for downstream assays is key. With National Science Foundation (NSF) suppo(NSF10 -pletely ex vivo process of computa-tional protein design, DN DNA assembly, cell-free expression, and analytical detection methods.

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