Cell-free synthetic biology comes of age

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High school students use the BioBits educational kit. Credit: Northwestern University

If you ask Northwestern Engineering's Michael Jewett, the potential of cell-free gene expression has always made sense. Rip off the wall of the cell, collect its insides, and teach the cell catalyst to produce new kinds of molecules and biological processes without the evolutionary constraints of using intact living cells.

But less than 20 years ago, this burgeoning field within synthetic biology still had much to prove.

"People thought we were crazy," said Jewett, professor of chemical and biological engineering at the McCormick School of Engineering. "When I was a graduate student, the idea of making a protein therapeutic was so obscure. At best, it was something that wasn't going to be cost effective enough to be useful, or the system wasn't going to produce enough protein to do anything worthwhile."

In a review paper published on November 29 in the journal *Nature Reviews Genetics*, Jewett, director of Northwestern's Center for Synthetic Biology, explores how cell-free engineering evolved from a specialized research tool to the backbone of a variety of applications in synthetic biology that stand to dramatically impact society, from the environment to medicine to education.

Now, synthetic biology garners wide interest. "Commercial industries are popping up around these technologies. Granting agencies are seeing the importance," he said. "The time for cell-free systems is here. It's now."

A technical renaissance

While cell-free gene expression has been used as a research tool for more than 50 years, its transformative potential has been limited by several constraints, including low and variable protein synthesis yields, short reaction durations, and small reaction scale. Researchers also battled against doubts that controlling the reaction environment within cells would remain beyond reach.

However, in the last 20 years, synthetic biology researchers have gradually peeled back the curtain of cell-free gene expression's potential, uncovering new insights in the lab that have led to new efficiencies and applications outside of it—from biosensors to measure and monitor environmental contaminants in natural resources to targeted therapeutics to treat disease.

Jewett and collaborators, for example, recently developed a high-yielding one-pot cell-free protein synthesis platform derived from a genomically recoded strain of Escherichia coli. The system is not only optimized to produce the highest batch reaction expression yield of a protein to date, but the platform can make proteins with non-canonical amino acids, expanding the genetically encoded chemistry available to proteins and opening the door to create new types of enzymes, materials, and therapeutics.

"By having a platform that enables high-level gene expression in a one-pot use, the process becomes a lot more democratized," Jewett said. "That's exciting, because it will hopefully make it easier for other labs to use cell-free gene expression systems."

Northwestern at the forefront

As cell-free synthetic biology has grown in importance, so too has Northwestern's Center for Synthetic Biology. Launched in 2016 to bring together the brightest minds in the field and to provide a supportive ecosystem for research and education, the center has quickly established itself as a leader of cell-free systems research and technological development.

"The center has organically grown into one of leading centers in synthetic biology in the United States, and perhaps the world," said Jewett, Charles Deering McCormick Professor of Teaching Excellence. "As our team has come together, we've thought about research themes that not only connect us, but also position Northwestern as having a particular strength—and cell-free systems has emerged."

Recent advances by center faculty have pushed the boundaries of cell-free engineering even further. Jewett and Milan Mrksich, Henry Wade Rogers Professor of Biomedical Engineering, for example, collaborated on a method to rapidly produce enzymes and analyze their reactions. The system, which combines Jewett's cell-free protein synthesis technology with Mrksich's SAMDI mass spectrometry platform, will help synthetic biologists design more complex molecules faster than ever.

Neha Kamat, assistant professor of biomedical engineering, recently demonstrated the first instance of using cell-free systems to selectively drive the fusion of lipid nanoparticles—an emerging carrier for drug-delivery—opening the door to new and complex types of biochemical reactions. Danielle Tullman-Ercek, associate professor of chemical and biological engineering, is uncovering new rules governing the function of microcompartment systems like viruses, which could serve as vessels to deliver protein therapeutics derived from cell-free systems to targeted locations in the body. Joshua Leonard, associate professor of chemical and biological engineering, is studying the interface of synthetic biology and systems biology to achieve design-driven medicine. Earlier this year, he chaired the Sixth International Mammalian Synthetic Biology Workshop hosted at Northwestern.

The center's work also touches the startup space. Stemloop was born out of the lab of Julius Lucks, associate professor of chemical and biological engineering. The company applies Lucks's research mission to understand how cellular systems sense and respond to their environments through a platform of technologies, including one focused on environmental water quality monitoring. Jewett also recently started SwiftScale Biologics, which seeks to accelerate a drug's arrival to the market using cell-free systems.

Sherlock Biosciences, started by Center for Synthetic Biology advisory board member James Collins, uses engineering biology platforms to create better, faster, and affordable medical diagnostic tests.

"The technology is ready to be applied outside the lab to address societal issues, and companies are emerging to give them a fair shake in the marketplace," Jewett said. "What people thought was once completely impossible is proving to be more than possible."

A glimpse of what's to come

The next decade will welcome even greater milestones, thanks in part to growing research collaborations, Jewett said.

Northwestern is working with clean-energy startup LanzaTech and Oak Ridge National Laboratory on a multi-year project supported by the Department of Energy to leverage clostridia, a bacterium that metabolizes carbon, to produce sustainable fuels. Jewett and his lab, joined by Keith Tyo, associate professor of chemical and biological engineering, and Linda Broadbelt, Sarah Rebecca Roland Professor of Chemical and Biological Engineering, are using computational design algorithms and cell-free engineering to rapidly prototype thousands of potential biosynthetic pathway designs that could optimize clostridia's production of biofuels.

"What would take LanzaTech months to engineer and test, our lab can do in days, thanks to cell-free systems," Jewett said.

Jewett also envisions an expansion of synthetic biology education through experiential learning opportunities in middle school and high school classrooms. Hundreds of schools around the world want to incorporate his suite of BioBits educational kits—developed in collaboration with MIT and the Wyss Institute at Harvard—into science curriculums. The interactive kits, now used in dozens of Evanston and Chicago classrooms, equip students to conduct synthetic and molecular biology experiments by adding water and simple reagents to freeze-dried cell-free reactions.

"It's critically important that we provide training opportunities to students, so they're excited about supporting and contributing to the emerging bio-economy," he said.

And what about the first FDA-approved therapeutic supported by a cell-free system? As companies improve the ability to scale the production of engineered proteins, Jewett is hopeful that engineered therapeutics will move into clinics.

"An FDA-approved product will certainly be a watershed moment," he said, "and I believe it is coming in the next decade."

More information: Adam D. Silverman et al, Cell-free gene expression: an expanded repertoire of applications, *Nature Reviews Genetics* (2019). DOI: 10.1038/s41576-019-0186-3

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