New innovations in cell-free biotechnology

March 23 2018, by Stephan Benzkofer

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A Northwestern University-led team has developed a new way to manufacture proteins outside of a cell that could have important implications in therapeutics and biomaterials.

The advance could make possible decentralized manufacturing and

distribution processes for protein therapeutics that might, in the future, promote better access to costly drugs all over the world.

The team set out to improve the quality of manufactured proteins in vitro, or outside a cell, and found success across a number of fronts.

"We developed a bacterial cell-free protein synthesis system that is capable of high level expression of pure proteins containing multiple non-canonical amino acids," said Michael Jewett, associate professor of chemical and biological engineering at Northwestern's McCormick School of Engineering. "This is important because it allows us to expand the range of genetically encoded chemistry incorporated into proteins in previously unattainable ways."

The team, which brought together researchers from Northwestern, Yale University, and the Illinois Institute of Technology, reported its work in the journal *Nature Communications* on March 23.

Protein production plays a critical role in medicine, biotechnologies, and life sciences. Recombinant protein production, for example, has transformed the lives of millions of people through the synthesis of biopharmaceuticals, like insulin, and industrial enzymes, like those used in laundry detergents. Conventionally, protein production has been accomplished in living cells in large centralized manufacturing facilities.

"Alternatively, what cell-free protein synthesis does is take the cell, rip off the cell wall, and collect the guts of the cell. We then use this to make a protein without a living, intact organism," said Jewett, who is also co-director of Northwestern's Center of Synthetic Biology. "You can imagine I've taken a car and opened the hood and taken out the engine and put it over here in my driveway. Now I can use it to do something else."

Without the worry of trying to keep a cell alive, this process opens up many possibilities, including the synthesis of new classes of enzymes, therapeutics, materials, and chemicals with diverse chemistry. A living cell may balk when asked to do something it hasn't seen in its evolutionary biology, not so for a cell-free protein synthesis (CFPS) platform.

The problem up to this point, though, has been that efforts to use CFPS systems for expanding multiple non-canonical amino acids have been limited by competition with the natural machinery that terminates protein synthesis. As a result, manufacturing proteins harboring diverse chemistries with high purity and yields has presented a formidable challenge.

But Jewett and the team produced the highest yields of proteins with non-canonical amino acids ever reported for in vitro systems, suggesting that long-term commercial applications for CFPS might be realistic.

Jewett credited the breakthrough to two elements. The first was the idea of using a genomically recoded organism of Escherichia coli bacteria that lacked release factor 1.

"This is important because it provided us an open coding channel to incorporate new chemistry," he said. "Trying to develop a cell-free protein synthesis from that strain had never been done before."

The second element came courtesy of Jewett's student, Rey Martin, the lead author of the paper.

Jewett said when they first tried to use the strain, they failed to make enough protein.

"What my student Rey did in a very innovative strategy was find genes in

the chromosome of that organism that we thought were negatively affecting our ability to produce protein," Jewett said. "He functionally inactivated them to enable higher cell-free protein synthesis yields."

Jewett said that Martin didn't stop there.

"Rey optimized the cell-free environment to enable multiple identical non-canonical amino acids to be incorporated," Jewett said. Where before researchers have used just one or a few instances, he said his team was able to incorporate up to 40, site specifically with no observable truncation products.

"What that means is we can really change fundamentally the properties of protein polymers in unique and new ways," Jewett said. "And you may ask, 'well, what are you going do with that?' From a research perspective, we haven't had this exact capability before so we haven't been able to even ask the question."

Jewett imagines applications not just with medicines but also biomaterials for drug delivery systems, medical materials such as surgical sutures, and functionalized biopolymers.

"What's special about this platform is that it's already being used in so many other project areas in the lab," Jewett said. "It has the potential to open up an entirely new area of materials chemistry research for biotechnology and, more broadly, could enable new paradigms of on-demand biomanufacturing of vaccines and therapeutics."

More information: *Nature Communications* (2018). DOI: 10.1038/s41467-018-03469-5 , www.nature.com/articles/s41467-018-03469-5

Provided by Northwestern University