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Hacked molecular machine could pump out custom proteins

Engineered ribosomes pave way for designer enzymes and synthetic cells.

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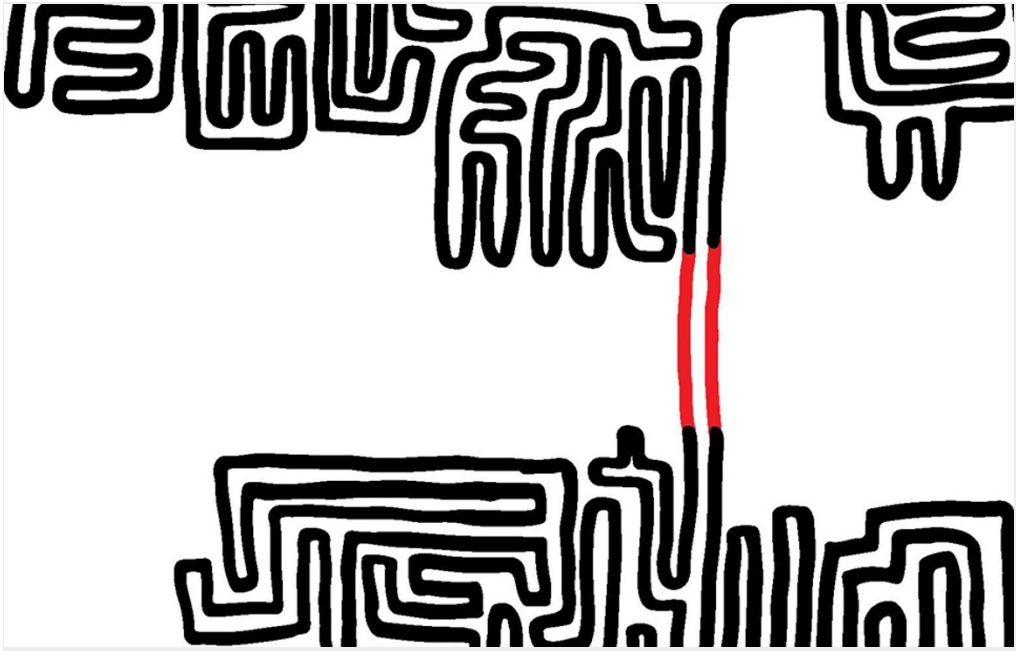

Erik Carlson

An engineered ribosome with a permanent connection between its subunits (red) can operate side-by-side with a cell's own protein production machinery.

By hijacking the cellular machinery that makes proteins, bioengineers have developed a tool that could allow them to better understand protein synthesis, explore how antibiotics work and convert cells into custom chemical factories.

All life owes its existence to the ribosome, a huge, hardworking molecular machine that reads RNA templates transcribed from DNA, and uses the information to string together amino acids into proteins. A cell requires functioning ribosomes to survive — but they are difficult to engineer. If the engineered molecules deviate too far from the standard design, the cell will die.

“An engineered ribosome learns to do better what you want, but it starts to forget how to do its normal job,” says biochemist Alexander Mankin of the University of Illinois in Chicago.

Mankin teamed up with biochemical engineer Michael Jewett of Northwestern University in Evanston, Illinois, and others to create a ribosome that engineers could tinker with. The results of their handiwork are published in *Nature*[1].

Mega-machines

Ribosomes are conglomerates of RNA and protein, hundreds of times larger than typical enzymes. RNA is thought to be responsible for the bulk of a ribosome’s work, which is is considerable — it produces protein at a rate of up to 20 amino acids a second with a remarkably low error rate. “The ribosome deserves all possible respect,” says Mankin.

It is these properties that draw the attention of bioengineers such as Jewett. These researchers would like to create ribosomes that could do other chemical reactions and spit out novel polymers, or incorporate unnatural amino acids into proteins that could be used as drugs.

Each ribosome contains two clumps of snarled RNA molecules, a small subunit and a large one. The subunits come together to translate a messenger RNA sequence into protein, and then separate. They assemble again when it is time to make another protein, although not necessarily with the same partners. “In a way they are very promiscuous,” says Mankin.

Arranged marriage

That promiscuity hindered efforts to engineer ribosomes to incorporate unnatural amino acids or other compounds. Engineered and natural subunits mixed and matched, reducing the cell's ability to produce normal proteins.

The solution, Mankin and Jewett's team decided, was to marry together two engineered subunits. It was unclear whether the approach would work: it was thought that ribosomes exist in two distinct units because it is necessary for their function.

The researchers used a strand of RNA to tether the large and the small subunit together, toiling for months to get the length and location of the link just right so that the machine could still function. “We certainly came close, several times, to saying ‘OK, biology wins',” says Jewett.

The team screened its tethered ribosomes in *Escherichia coli* cells that lacked functioning RNA, and eventually found engineered ribosomes that worked well enough to support some growth, albeit slow. They then tested their platform to confirm that a tethered ribosome could operate side-by-side with natural ribosomes.

The result unlocks a molecular playground for bioengineers: by tethering the artificial subunits together, they can tweak the engineered machines to their liking without halting cell growth, says Joseph Puglisi, a structural biologist at Stanford University in California. Puglisi hopes to harness the system to study how the ribosome functions. James Collins, a bioengineer at the Massachusetts Institute of Technology in Cambridge, says that his lab may use the system to study antibiotics — many of which work by binding to bacterial ribosomes.

Jewett wants to see what the system can do for synthetic biology, perhaps producing new antibiotics or unnatural polymers. “We’re just at the leading edge,” he says. “We're going to try to expand the genetic code in unique and transformational ways.”

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References

1. Orelle, C. *et al*. *Nature* http://dx.doi.org/10.1038/nature14862 (2015).