CHEMICAL BIOLOGY

Peptides made by walkin'

A DNA walker–based system enables ordered, multistep synthesis of a peptide in a single solution.

Making a peptide by hand in a chemistry lab includes hours of shepherding a bit of material through a drawer full of glassware, but the possibilities of what can be synthesized are vast. Inside a cell, in contrast, the reaction proceeds quickly with no aid from the researcher, but a trade-off in using cellular machinery to make proteins is the ribosome's specificity—which restricts the final peptide to the 20 proteinogenic amino acids, with only a bit of leeway.

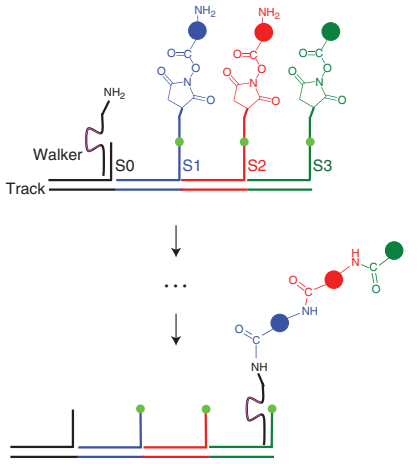
Marrying these two worlds, David Liu at Harvard University, with postdoc Yu He, developed a one-pot polypeptide synthesis method that requires no proteins. They essentially created a DNA-based ribosome mimic, called the DNAsome, that uses no cellular components but instead is derived from a man-made DNA 'walker' that has been used in nanotechnology applications.

The original walker moves along a DNA track by sequential rounds of base-pairing. For their system, Liu and He designed a single-stranded DNA track to contain codon sequences, essentially acting as an mRNA template to direct the binding of tRNA-like substrates. Analogously to tRNAs, the substrates contain unique DNA codons to bind the track, followed by a walker-docking sequence with two ribonucleotides to be cleaved by the ribozyme within the walker and, finally, one of three different activated amino acids (named S1–S3).

At the start of the reaction the walker binds the initiator substrate carrying no amino acid (S0). Then the walker binds S1, bringing its free amine group close to S1's amino acid, which results in the addition of this amino acid to the walker. After cleaving S1's RNA, the walker repeats the same maneuvers with S2, and so on.

The researchers created tracks with various combinations of codon sequences, and mass spectrometry analyses revealed the products they expected, demonstrating the specificity of the system.

Although functional, this process is very slow, on the order of several hours to synthesize the three-step product. Unlike the ribosome—which not only brings together the substrates but also positions them to enable the reactions—the DNAsome "relies entirely on effective molarity increases brought about by DNA hybridization," notes Liu. The reaction rate increase resulting from the entropy reduction is appreciable but modest compared to what the ribosome achieves by precisely placing the components and locating catalytic groups in the right position to lower transition-state energies, he adds.



DNAsome design and final product. Modified from *Nature Nanotechnology*.

But such specificity of a biological system limits an enzyme to certain substrates—a trade-off that the DNAsome circumvents. "If you tried to make the tripeptide we made with this system with a ribosome, you'd be out of luck because these aren't ribosome-compatible building blocks," comments Liu, adding, "some of the structures we used are very different from the proteinogenic amino acids."

As to future applications of this proof-of-principle work, Liu says he is "interested in applying this type of strategy to library creation, but more generally in exploring how DNA devices can manipulate themselves with synthetic organic chemistry and with functional selection screens." His team is also exploring other ways to form longer and more sophisticated products without the usual constant attendance of researchers.

Soon, this new breed of lab robots may be walking in a test tube near you.

Irene Kaganman

RESEARCH PAPERS
He, Y. & Liu, D.R. Autonomous multistep organic synthesis in a single isothermal solution mediated by a DNA walker. *Nat. Nanotechnol.* **5**, 778–782 (2010).

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