**SYNTHETIC BIOLOGY**

**Made in translation**

Evolution of highly functionalized DNA could enable the discovery of artificial nucleic acid sequences with different properties to natural DNA. Now, an artificial translation system has been designed that can support the evolution of non-natural sequence-defined nucleic acid polymers carrying eight different functional groups on 32 codons.

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The dominance of proteins over nucleic acids in biological receptors and catalysts is generally explained by the presence of 20 amino acids as compared to the four bases found in DNA and RNA. But what if the chemical diversity of nucleic acids was increased through the addition of large numbers of protein-like side chains? Would the synthesis of highly functionalized DNA libraries enable the evolution of nucleic acid molecules with enhanced functional properties or allow for the discovery of chemical activities that cannot be achieved with natural DNA?

Although fundamental questions such as these often provide rich fodder for evening conversations about the origins of life and the emergence of biopolymer function, they also offer a possible glimpse into what the next generation of receptors, catalysts and materials might look like.

A long history of pioneering efforts has shown that it is possible to evolve chemically modified DNA with user-defined properties that include ligand binding and catalysis. Using a technique commonly known as in vitro selection (or SELEX), researchers mimic the natural process of Darwinian evolution by carrying large pools of diverse sequences through iterative rounds of selection and amplification. In these experiments, molecules that fold themselves into shapes that can perform the desired function are captured and replicated to produce progeny molecules with characteristics that are similar to the parent sequence. Although in vitro selection has produced a number of notable successes, including the evolution of artificial genetic polymers with entirely non-natural sugar-phosphate backbones, the process itself is ultimately limited to the subset of monomers that can be recognized by a polymerase, which include nucleoside triphosphates with natural and chemically modified bases.

Consequently, the ensuing libraries explore a much smaller region of chemical space than would otherwise be possible if DNA synthesis were performed outside of these constraints.

Moving beyond polymerases requires the ability to encode and decode chemical information in synthetic polymers where each sequence carries a precise set of building blocks that appear in a specific order. In principle, this step could be performed using non-enzymatic template-directed polymerization chemistry, where activated monomers are chemically synthesized on nucleic acid templates. However, in practice, this approach is too inefficient to support the synthesis of DNA-scaffolded libraries carrying functionally rich side chains. Recognizing this problem, researchers have sought to re-engineer the central dogma of biology so that information stored in DNA can be translated into sequence-defined synthetic polymers with side chains that mimic the amino acids found in proteins.

Now, writing in *Nature Chemistry*, a team led by David Liu describes an artificial translation system that enables the synthesis and evolution of highly functionalized nucleic acid polymers (HFNAPs). This system, which was inspired by nature, provides a clever solution to the synthetic biology problem of how to synthesize non-natural nucleic acid polymers when the desired chemical building blocks are not substrates for a polymerase. To achieve this goal, the team designed their own genetic code, which consists of 5′-phosphorylated DNA trinucleotides that are modified at the first nucleotide position with a chemical side chain. In total, 32 codons were constructed for eight different side chains (4 codons per side chain). The set of eight side chains include a range of chemical groups, many of which are not found in proteins. Using the codons as monomeric building blocks, bacteriophage T3 DNA ligase was used to translate DNA information into HFNAPs by stitching the codons together in the order specified by the DNA template. To complete the replication cycle, the HFNAPs were separated from their DNA templates, purified, and reverse translated back into cDNA using Q5 DNA polymerase. Reverse translation enables regeneration of the starting DNA templates by copying functionally rich sequences back into their encoding cDNA strands.

By introducing a selective amplification step into the replication cycle, HFNAPs can be evolved with desired properties, such as the ability to bind a protein target. The utility of this synthetic biology approach was nicely illustrated by the in vitro selection of HFNAPs against the therapeutic targets PCSK9 (an enzyme) and IL-6, (a protein involved in cell signalling) both of which have been implicated in human diseases. Subsequent directed evolution of a highly active PCSK9 member led to the isolation of PCSK9-Evo5, which was found to antagonize the binding of PCSK9 to its cognate low-density lipoprotein (LDL) receptor. Liu and co-workers note that the inhibitory effect of PCSK9-Evo5 is similar to a known PCSK9-neutralizing monoclonal antibody, which suggests that HFNAPs could one day be used to inhibit the harmful effects of upregulated endogenous proteins implicated in human diseases. The team also showed that the DNA scaffold alone fails to inhibit PCSK9 binding to the LDL, demonstrating that the phenotypic effects of PCSK9-Evo5 are due to the precise arrangement of the functional groups on the DNA scaffold.

Although Liu and co-workers do not answer the original question of whether HFNAPs can exceed the functional properties of natural DNA, their study along with a related paper on peptide-modified DNA show that highly functionalized nucleic acids are capable of Darwinian evolution. This important first step provides the methodology needed to answer fundamental questions about the chemical space explored by sequence-defined non-natural polymers. As these studies continue, we are likely to see improvements in HFNAP function that could include the emergence of HFNAP catalysts. Such studies have the potential to produce enzymatic activities that cannot be achieved by natural nucleic acids — or possibly even proteins — as HFNAPs are not limited to the functional groups found in nature. Other pursuits worthy of
Investigation includes solving the structure of an in-vitro-evolved HFNAP receptor bound to its protein target. Though challenging, structural information would solidify the mechanism of binding and help guide the discovery of new HFNAPs that function with even greater activity. As these studies continue, it is clear that HFNAPs will shed new light on many long-standing questions concerning the evolutionary potential of functionally rich sequences. Accomplishing this feat could lead to the next generation of receptors, catalysts and materials with future applications in synthetic biology, biomedicine and materials science.

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