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A Conversation with Ting Zhu

Mark Peplow

This chemical biologist is building the mirror image of nature's molecular machinery.

Despite their seemingly limitless diversity, all forms of life rely on biomolecules of a certain "handedness"—specifically L-amino acids and D-nucleic acids. Nature rarely uses the mirror-image versions of these molecules, D-amino acids and L-nucleic acids. Ting Zhu of Tsinghua University hopes to fill that gap by creating not only mirror-image (MI) DNA strands but also MI enzymes that can copy this unnatural DNA, transcribe it into MI RNA, and, eventually, translate the MI RNA into MI protein. The mirror-image system could offer a new approach to drug design and even help scientists understand the origins of life. Mark Peplow joins Zhu on a trip through the looking glass.

Credit: Ting Zhu

Why are you working on mirror-image biochemistry?

First of all, it is scientifically interesting. Mirror-image systems are independent from life as we know it, and their difference comes from one of the most fundamental chemical features of biology: chirality. They would not be just another branch on the tree of life; they would be an entirely new tree.

The work also has practical applications. For instance, because the body's molecular machinery won't recognize them, MI DNAs, MI RNAs, and MI proteins are resistant to enzymatic degradation and can largely avoid triggering an immune response, making them attractive drug candidates. Short sections of MI DNAs and MI RNAs, known as aptamers, can fold into three-dimensional structures and bind to specific biochemical targets; MI aptamers and MI peptides are already being developed as therapies for diseases such as cancer.

Which mirror-image molecules are you making?

We're trying to establish a mirror-image version of the central dogma of molecular biology—that DNA is replicated and transcribed into RNA, which is translated into protein. We have already realized the first two steps to make MI RNA, but the most difficult step will be to translate from MI RNA into MI protein. In nature, translation is carried out by the ribosome, an enormous complex of proteins and RNAs that will be extremely challenging to build in mirror-image form.

Synthesizing short strands of MI DNA is relatively straightforward—you just use mirror-image versions of DNA's building blocks in known chemical synthesis reactions. But building and replicating longer strands requires a mirror-image enzyme, a polymerase, which is trickier to make.

To build complete mirror-image polymerases, we've relied on a method called native chemical ligation, which links peptides via terminal cysteine residues, to connect synthetic peptide segments. Initially, we worked with Lei Liu's group at Tsinghua to build a mirror-image version of the smallest-known DNA polymerase, African swine fever virus polymerase X (ASFV pol X), which has 174 amino acid residues.

How well did that enzyme work?

It could copy a 44-nucleotide MI DNA strand, and it also managed to transcribe a short MI DNA sequence of six nucleotides into MI RNA. This polymerase was rather

Published: July 16, 2018

DOI: 10.1021/acscentsci.8b00432
ACS Cent. Sci. 2018, 4, 783−784

inefficient, with poor thermostability. Our second effort was to make a more efficient MI polymerase called Dpo4.

Dpo4 is thermostable so it can be used over and over again in the polymerase chain reaction (PCR), a defining tool in modern molecular biology, to amplify a given MI DNA sequence. Dpo4 is the smallest-known polymerase capable of performing PCR: From a practical standpoint, it is a lot more efficient than our first effort.

The problem is Dpo4's amino acid sequence wasn't ideal for native chemical ligation. To get around this, we mutated the enzyme to include more cysteine or alanine amino acids to make the synthesis easier, without affecting the polymerase's activity. The synthetic Dpo4 has 358 D-amino acid residues and is the largest chemically synthesized protein reported to date. This MI Dpo4 variant was able to polymerize a 120-nucleotide MI DNA strand that codes for a component of the ribosome. Recently, Sven Klussmann's group at Noxxon Pharma, in Germany, independently built a different mutant version of MI-Dpo4, also using native chemical ligation. Having different routes to synthesize this polymerase makes it more accessible to different groups.

How else do you plan to explore this mirror-image world?

For translation into proteins, we plan to build a mirror-image version of the ribosome. By enabling the translation of MI RNAs into MI protein, including building blocks for the MI ribosome itself, we can establish a complete mirror-image self-replicating system.

Tell me about the practical applications of the work you are doing in this field.

We just published a simple method for sequencing MI DNA. There are many cool applications of MI DNA sequencing, such as looking for other forms of life on Earth or on other planets, which could have a molecular handedness different from what we find here, as well as developing MI DNA aptamers for therapeutic applications.

Researchers are actively developing MI aptamer and MI peptide drug candidates for disease treatment. Currently, they develop them by synthesizing a mirror-image version of a biological target molecule and finding normal aptamers or peptides that bind to it. Then they flip the whole system to produce MI aptamers or MI peptides that will bind to the natural target. Now with MI PCR and MI DNA sequencing, we hope to set up a new method for selecting them directly. To find MI aptamers with therapeutic potential, you can synthesize large numbers of them, pull out those that bind to the target, and sequence them to see what you've got.

Can your research help answer big-picture questions about why life uses only certain chiral forms?

I think the effort to build mirror-image biology systems doesn't help us to explain directly how biology came to use just one particular set of chiral building blocks. In fact, our work could make this question even more puzzling, especially if mirror-image biology works just as well—and so far it appears so.

But mirror-image systems could help us to explore the origins of life in the lab. Catalytic RNA molecules called ribozymes could have been the basis for prebiotic self-replicating systems. Mirror-image versions of these ribozymes would be resistant to degradation by nucleases in nature, for example, so we could study their evolution for a very long time without having to worry about contamination.

Mark Peplow is a freelance contributor to Chemical & Engineering News, the weekly newsmagazine of the American Chemical Society. Center Stage interviews are edited for length and clarity.

DOI: 10.1021/acscentsci.8b00432
ACS Cent. Sci. 2018, 4, 783−784