SYNTHETIC BIOLOGY

# A big step toward mirror-image ribosomes

## Synthetic protein factories could one day make durable drugs the body can't break down

*By* **Robert F. Service**

All of life exists on just one side of a mirror. To put it more technically, the biomolecules that comprise living things—DNA, RNA, and proteins—are all "chiral." Their building blocks have two possible mirror-image shapes, but in every case, life chooses just one. At least so far.

This week in *Science*, researchers report they've made strides toward exploring the other side of the mirror. They re-engineered a workhorse enzyme that synthesizes RNA so it makes the mirror-image form. They then used that enzyme to construct all the RNAs needed to make a ribosome, the cellular machine responsible for constructing proteins. Other components still need to be added, but once completed, a mirror-image ribosome might be able to churn out proteins that could serve as novel drugs and diagnostics and can't readily be broken down in the body. It also sets the stage for a grander goal: making mirror-image life, a prospect that has fired the imagination of scientists ever since Louis Pasteur discovered mirror-image compounds in 1848.

"This is a major step towards re-creating the central dogma of molecular biology in the mirror-image world," says Stephen Kent, a professor emeritus of chemistry at the University of Chicago who was not involved with the work.

That dogma refers to the standard operating procedure of life: The genetic code—usually DNA—is transcribed into a corresponding sequence of RNA, which is then translated into proteins that perform much of the essential chemistry in cells. Exquisitely complex molecular machines made of proteins or, in the case of the ribosome, a combination of proteins and RNA carry out each step. And every molecule involved churns out chiral products. Chemists have long been able to synthesize opposite-handed DNA, RNA, and proteins. But they've never been able to put all the pieces together to make mirror-image life, or even enough of them to see whether such a conceit is possible.

Ting Zhu, a synthetic biologist at Westlake University in Hangzhou, China, has been building toward this vision for years. Among the first steps, as Zhu sees it, is to make a mirror-image ribosome—the factory that can make so many other mirror-image parts. That's no small feat. The ribosome is a molecular behemoth, made up of three large RNA fragments, consisting of approximately 2900 nucleotide building blocks in total, along with 54 proteins.

"The most challenging part is making the long ribosomal RNAs," Zhu says. Chemists can synthesize fragments up to about 70 nucleotides long and stitch them together. But to make the three much longer ribosomal RNA fragments in mirror-image form they needed a molecular machine that could crank them out—a polymerase enzyme. In 2016, Zhu and his colleagues took a first stab at the task, synthesizing a mirror-image version of a polymerase from a virus. The polymerase made mirror-image RNA, but it was slow and prone to errors.

For the current study, Zhu and his graduate student Yuan Xu set out to synthesize a mirror-image version of a workhorse enzyme used in molecular biology labs worldwide to synthesize long RNA strands, the T7 RNA polymerase. A massive, 883 amino acid protein, it lay well beyond the limits of traditional chemical synthesis. But an analysis of T7's x-ray crystal structure showed the enzyme could likely be split into three sections, each stitched from short segments. So, they synthesized the three sections—one with 363 amino acids, a second with 238, and a third with 282. In solution, the fragments naturally folded into their proper 3D shapes and assembled themselves into a working T7. "It was a herculean effort to put together a protein of this size," says Jonathan Sczepanski, a chemist at Texas A&M University, College Station.

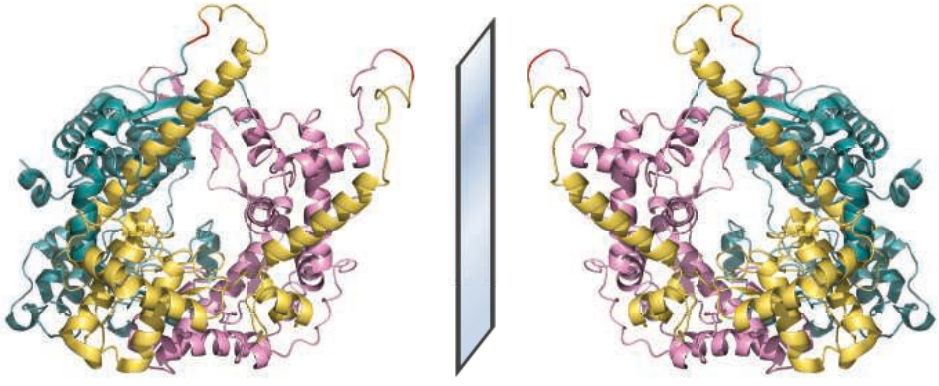
The researchers then put the polymerase to work. They assembled mirror-image genes encoding the three long RNA fragments the team hoped to make; then the mirror-image T7 RNA polymerase read the code and transcribed it into the ribosomal RNAs.

The result provided a tantalizing glimpse of the power of mirror-image molecules. The mirror-image RNAs fashioned by the polymerase were far more stable than the normal versions produced by a regular T7, the researchers showed, because they were untouched by the naturally occurring RNA chewing enzymes that almost unavoidably contaminate such experiments and quickly destroy normal RNAs.

This same resistance to degradation "could open the door to whole new types of diagnostics and other applications," including novel drugs, says Michael Jewett, a chemist and ribosome expert at Northwestern University. For example, Xu and

### Re-engineering a workhorse enzyme

Many biomolecules come in mirror-image forms, much like gloves that fit a left or right hand. But life uses just one form. Now, synthetic biologists have made a mirror-image form of an enzyme that generates RNA components of the ribosome, a key step toward building a mirror-image protein factory.



Natural-chirality T7 RNA polymerase | Mirror-image T7 RNA polymerase

GRAPHIC: YUAN XU AND TING ZHU

Zhu also used their mirror-image enzyme to make stable RNA sensors called riboswitches that could be used to detect molecules associated with diseases, as well as stable long RNAs that could be used to store digital data. Other researchers have shown that mirror-image versions of short strands of DNA and RNA called aptamers can serve as potent drug candidates that evade degrading enzymes and the immune system, which destroy most conventional aptamer drug candidates.

Exploiting this stability more broadly wouldn't be as simple as creating mirror-image copies of existing drugs, however, as such compounds, like wrong-handed gloves, would no longer match the chirality of their intended targets in the body. Instead, researchers would likely have to screen large numbers of mirror-image drug candidates to find ones that work.

But Jewett and others say the new work could aid that effort, because it sets the stage for making functional mirror-image ribosomes. Those could allow drug companies to more readily create mirror-image amino acid strings, or peptides, Jewett says. Because peptides draw from 20 amino acid building blocks, rather than just the four nucleic acids that make up aptamers, they offer greater chemical diversity and potentially more good drug candidates.

Now, Zhu and his team need to make the remaining components of a mirror-image ribosome. The three RNA fragments they synthesized make up about two-thirds of the total mass of a ribosome. What remains are the 54 ribosomal proteins and several proteins that work in concert with the ribosome, all of which are smaller and thus likely easier to synthesize. Then the question is whether the full parts kit will assemble into a ribosome.

Even if they do, the resulting molecular machines might still not be functional, cautions George Church, a synthetic biologist at Harvard University, who leads one of the few other groups around the world working on approaches to mirror-image life. In order to churn out proteins, ribosomes must work in conjunction with a suite of additional helper proteins. To make this work inside a living cell, Church thinks it will be necessary to rewrite an organism's genetic code so the engineered ribosome can recognize all those proteins, particularly the 20 that ferry amino acids for building new proteins. Church's group is working on this. "It's very challenging," he says.

But if everything comes together, researchers—and life—may finally be able to enter a looking glass world. ■

PUBLISHING

# Journal declares an end to accepting or rejecting papers

## Instead, *eLife* will offer to peer review selected submissions for $2000 fee, then make paper and critiques free to read

By **Jeffrey Brainard**

A publisher aiming to transform how scientists share research results has launched a new experiment. Last week, *eLife*—a nonprofit, selective, online-only journal that focuses on the life and medical sciences—announced it will cease accepting or rejecting manuscripts for publication, instead offering only peer reviews of manuscripts.

Until now, *eLife* has charged authors $3000 if it accepts their paper, which is free to read after publication. Under the new approach, *eLife* will charge authors $2000 if they accept the publisher's offer to have a submitted manuscript undergo peer review. Regardless of whether the critiques are positive or negative, the manuscript and its associated, unsigned peer-review statements will be posted online and be free to read. If the author revises the paper to address the comments, *eLife* will post the new version.

> **Critiques are "more useful to the community than our thumbs-up or thumbs-down publishing decision."**
>
> **Michael Eisen**, University of California, Berkeley

Since *eLife* was founded in 2012, it has tried other innovations. In 2020, for example, it started to require all submitted manuscripts be published as preprints. Abandoning the "accept" stamp is a logical next step, says *eLife*'s editor-in-chief, biologist Michael Eisen of the University of California, Berkeley.

Eisen, who co-founded the open-access Public Library of Science journals in 2003, says the detailed critiques written by reviewers that *eLife* recruits are its main contribution to the scientific process. The reviews, he says, are "more nuanced, more informative, and more useful to the community than our thumbs-up or thumbs-down publishing decision." He also argues that the new model will speed up a peer-review process that at other journals is often opaque and slow because it can involve multiple rounds.

Not everyone shares Eisen's vision. "I have zero interest in reading other people's peer reviews," tweeted Jason Pardo, a postdoctoral fellow at the Field Museum of Natural History. "Turning reviews into supplemental publications is silly." And the new model could struggle because in most fields, the majority of researchers do not post their manuscripts as preprints.

*eLife*'s new approach, which takes full effect in January 2023, is not entirely original. The online platform F1000Research, for example, enables researchers to post manuscripts, which others can then review. Eisen hopes *eLife* will distinguish itself in this new marketplace by the quality of its critiques.

*eLife* is still finalizing details of its new model, including how editors will decide which papers to invite for review. They will likely ask prospective reviewers for their sense of which papers will be most "useful" to critique, Eisen says, perhaps because they present a valuable new method or, conversely, represent flawed science that requires correction. Like a handful of other journals, however, *eLife* will not consider the manuscript's perceived scientific importance, leaving that to readers to assess.

The publisher plans to enable authors to declare a reviewed manuscript the final "version of record." That will allow a key group of researchers—those funded by the National Institutes of Health—to meet NIH's requirement that their work be indexed by its PubMed search engine. Authors will also be able to submit an *eLife*-reviewed manuscript to a different journal for publication, but only if they and *eLife* have not declared it final.

Eisen says that, at $2000 per review, *eLife* should cover its costs without using the subsidies from donors that have backed the publisher since its founding. And he hopes the new policy will ultimately cause the publisher to fade into the background. "I would hope that we move pretty quickly to a world where … you won't be citing *eLife* at all, because you'll be citing the author's work" as it appears on a preprint server. ■

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