Researchers develop new ligase for biomedical use

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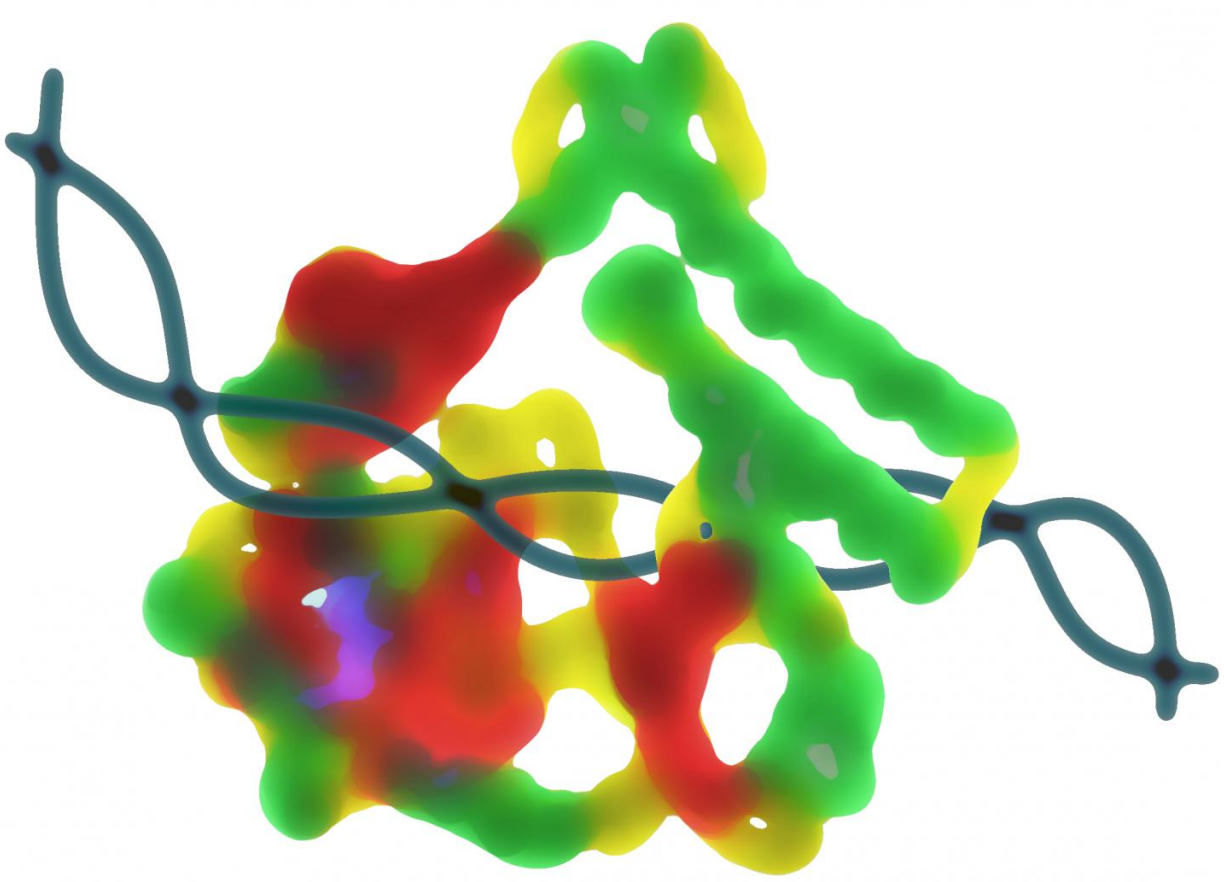

Brown researchers have developed a new ligase for laboratory use that could aid in in-vitro diagnostics and sequencing. Credit: Lei Zhang / Brown University

Ligases are enzymes that serve critical functions in cells, helping to fuse

together broken strands of DNA and RNA. These enzymes are also important bioengineering tools, useful in genetic sequencing, mutation detection and other applications.

Researchers in Brown University's School of Engineering have now developed a new RNA ligase for laboratory use. Derived from a microbe that thrives near volcanic thermal vents, the new ligase, called KOD1Rnl, can work at the high temperature desirable for some laboratory procedures. It's also the most active in the presence of certain RNA structures, called templates, which makes it useful for RNA sequencing and detection.

"This new ligase has all the properties we think are desirable for manipulating RNA," said Lei Zhang, a biomedical engineering graduate student at Brown and lead author of a paper describing the work. "We think this could be a useful tool to add to the biomedical engineering toolbox."

The paper is published in the journal *RNA Biology*.

Zhang works in the lab of Anubhav Tripathi, professor of engineering at Brown and senior author on the paper. "The focus of our group has always been in the engineering of medical diagnostics and molecular therapies, particularly dealing with RNA," Tripathi said. "In many recent studies, we engineered novel molecular assays and platforms to rapidly detect viral infections and the presence of viral mutations. While we design and build the microfluidic platforms and the assays in-house, we always relied on off-the-shelf reagents to power these assays. As our designs became more complex, we grew increasingly frustrated at the lack of specialized enzymes, such as ligases."

In particular, there are few RNA ligases that are thermostable, that is, ones that can operate at high temperatures. Working with RNA at higher

temperatures can make things easier, Tripathi says. At room or body temperature, RNA molecules are a tangled mess. But when heated, those tangles loosen up and the molecule straightens out.

"When working with RNA, you often want to hybridize a complementary nucleic acid to it," Tripathi said. "That's much easier when the molecule is linear. When it's tangly, a lot of the sites for the hybridization are blocked."

The problem is that the temperatures needed to straighten out the RNA are often too high for the ligase to be effective in fusing molecules. But KOD1Rnl is active up to nearly 140 degrees Fahrenheit, which enables its use in laboratory work above body temperatures.

The enzyme's template dependency is also important. A template-dependent RNA ligase will fuse two RNA strands only when one strand is aligned adjacently with the other strand onto a template. This trait is used by scientists to detect and sequence RNA. For their paper, Zhang and Tripathi used KOD1Rnl to ligate engineered probes in the detection of lab-made Ebola RNA transcripts.

Template dependence is also useful in detecting mutations in RNA. This is done by creating RNA probes—strands of RNA that contains a mutation of interest. The probes have a small break, or "nick," at the point along the strand where the mutation occurs. The probes are then paired or "hybridized" with a target RNA strand in the presence of template dependent ligase. If the mutation is present in the target strand, the RNA probes will hybridize properly, and the nick will be sealed by the ligase. If the mutation is not present, the structure of the hybrid molecule is disrupted and the ligase will not seal the nick. By looking at whether or not the nick has been sealed, scientists can tell if the mutation is present.

This all depends on the extent to which the ligase is template dependent and its structural specificity. Some ligases are inherently more specific than others. "We found that this is a highly specific RNA ligase," Zhang said of KOD1Rnl. "It's more specific, in fact, than existing enzymes used in the lab."

The researchers have patented the new enzyme and are hoping to work with an industry partner to bring it to market.

"The development of KOD1Rnl is the first of a family of enzymes we are developing for the biomedical community," Tripathi said. " As biomedical engineers, our dream is to translate the results of our research into the clinic, and this is an important milestone to hasten us to that goal."

More information: Lei Zhang et al, Archaeal RNA Ligase from Thermoccocus Kodakarensis for Template Dependent Ligation, *RNA Biology* (2016). DOI: 10.1080/15476286.2016.1239688

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