A chemical cloak of invisibility could reveal RNA's secrets

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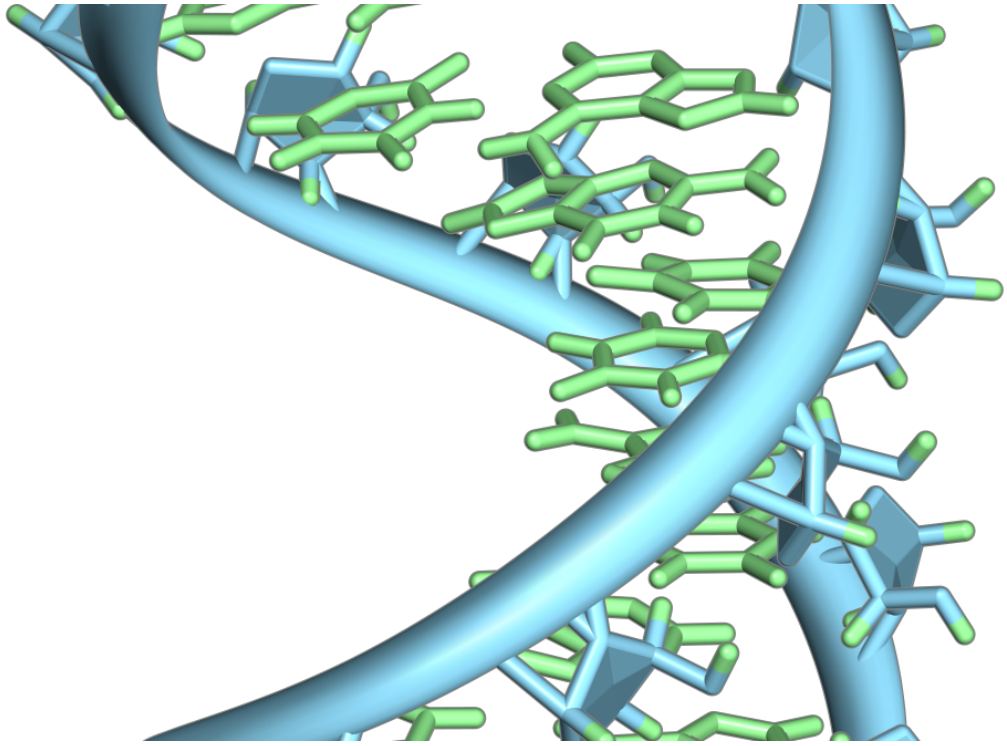

A hairpin loop from a pre-mRNA. Highlighted are the nucleobases (green) and the ribose-phosphate backbone (blue). Note that this is a single strand of RNA that folds back upon itself. Credit: Vossman/ Wikipedia

Biologists used to think they knew DNA's less famous cousin, RNA, but in the last two decades it's become clear the molecule is keeping far more secrets than it has ever revealed. Recent discoveries have it taking

on never-before-anticipated roles in regulating how a cell functions.

Stanford scientists report in the journal *Angewandte Chemie* they've now developed a tool that could help uncover some of those secrets, essentially by hiding RNA molecules from the world. What this new tool reveals about RNA could help biologists better understand the inner workings of our cells in both sickness and health.

"RNA to me is still one of the big mysteries in the cell," said Eric Kool, the George and Hilda Daubert Professor of Chemistry and a member of Stanford Bio-X and Stanford ChEM-H. "We used to think of RNA pretty simply, but we know now that there are many kinds of RNA, dozens of classes of RNA, and we don't know what maybe 90 percent of them do in the cell."

To address that problem, Kool; Anastasia Kadina, the paper's first author and a postdoctoral fellow in Kool's lab at the time the research was done; and postdoctoral fellow Anna Kietrys developed what they call RNA cloaking, a simple, reversible method that could help biologists better understand the range of unknown operations RNA is up to in the cells of living things.

DNA's unstable cousin

Just 15 or 20 years ago, scientists believed there were just a few types of RNA, and that they all served one goal: Read the genetic code written in DNA and use it to build the proteins that all living things need to survive. Over time, however, it became clear that there were other types of RNA that weren't just reading genes and building proteins – but what they were up to was anyone's guess.

The challenge, researchers found, was that the same thing that makes RNA so multifunctional and interesting also makes it deeply frustrating

to work with. It will react with pretty much anything – a small molecule, an enzyme or even itself – meaning that it will break apart at the slightest touch, or just curl itself into a little ball without warning. As a result, it's hard to keep RNA samples stable, let alone get them under enough control to study them.

Hiding RNA under a chemical blanket

The solution, the team found, was to hide RNA from other molecules using a special chemical cloak, one that would cover up RNA without folding, breaking or otherwise messing up the underlying molecule's structure.

"It's like throwing a blanket on it," Kool said, "like Harry Potter's cloak of invisibility." The chemical hides RNA from proteins, enzymes and other molecules. The blanket itself is made of a chemical relative of vitamin B3 that the lab has been developing over the last several years. Based on that work, Kadina worked to find the right conditions – the right temperature, the right blend of liquids to mix with the cloaking agent and so on – to get the chemical blanket to cover most or all of an RNA molecule.

To really get a handle on what specific RNA molecules do, however, researchers would like to be able to turn RNA reactions off and on again – that is, they need to be able to take the blanket off, too. So Kadina also developed an uncloaking method that returns RNA to its former, unruly self. Crucially, both cloaking and uncloaking work regardless of the size of an RNA molecule, something that was not previously possible, Kool said.

Studying RNA in the real world

Because of its reversibility and flexibility, RNA cloaking could help researchers study not only the functions of a wide range of RNA molecules – in theory, any RNA molecule at all – but also how the timing of RNA reactions affects those functions. Still, one of the most pressing potential applications is among the simplest: simply keeping RNA stable in a lab for extended periods of time, something that RNA cloaking could do very well.

Then, "we want to move into living systems," Kool said, and use cloaking and uncloaking to study the function of particular RNA molecules in cells. Roughly, the idea is to cloak RNA in a protective blanket in the lab, inject it into a living cell, then uncloak it, turning on whatever cellular functions that piece of RNA controls. The team members will need to show that their uncloaking agent does no harm to the cells they're trying to study, but the method could help biologists better understand how RNA reactions work. The researchers are also looking at ways to localize the effects of an RNA cloak to a specific tissue or location in a biological sample.

Longer term, Kool said, RNA cloaking could become a standard tool for biologists. The method is simple compared to other tools developed over the years to rein in RNA, so it would be easy for nonspecialists to learn and make use of in their labs.

More information: Anastasia Kadina et al. RNA Cloaking by Reversible Acylation, *Angewandte Chemie* (2018). DOI: 10.1002/ange.201708696

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