Rehydrate -- your RNA needs it

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Water, that molecule-of-all-trades, is famous for its roles in shaping the Earth, sustaining living creatures and serving as a universal solvent. Now, researchers at the University of Michigan and the Academy of Sciences of the Czech Republic have uncovered two previously unknown roles for water in RNA enzymes, molecules which themselves play critical roles in living cells and show promising medical applications.

The researchers' findings will be published online in the *Proceedings of the National Academy of Sciences* (PNAS) this week.

RNA enzymes, also known as ribozymes, accelerate chemical reactions inside cells, just as their better-known protein counterparts do. And just as a protein enzyme is not a static structure, a ribozyme also changes shape, cycling back and forth between active and inactive forms (called conformations).

In earlier work, a team led by U-M's Nils Walter, associate professor of chemistry, found that modifications made anywhere on the ribozyme molecule---even far from the site where the chemical reaction occurs---affect the rates at which the enzyme changes conformation and catalyzes the reaction. Something similar had been seen in protein enzymes, but never before in RNA enzymes.

The earlier finding, published in PNAS two years ago, suggested that information about changes in distant parts of the ribozyme travels through some sort of network to the core of the molecule, where chemical reactions take place. The latest work shows that water molecules trapped inside the ribozyme's core are essential components of that network.

The network acts like a jostling crowd at a cocktail party, where hydrogen bonds---weak, electrostatic attractions between molecules or parts of molecules---take the place of handshakes. Water molecules trapped in ribozymes can form hydrogen bonds with other water molecules or with parts of the ribozyme molecule.

"The way we interpret the data is that in ribozymes, a chemical modification introduced at one place changes the local structure slightly," Walter said. The building blocks making up the ribozyme wiggle into different positions and in the process must let go of some hydrogen bonds and form others, just as partygoers shift position and engage with other guests.

"As a consequence, their hydrogen bonding partners---some of which are water molecules---also rearrange. Then their hydrogen bonding partners also rearrange, creating a domino effect, where a local modification spreads throughout the molecule and modifies the structure elsewhere, even at quite a distance," Walter said. Water facilitates the process by increasing the number of hydrogen bonds and making the ribozyme behave as an interconnected whole.

Walter and coworkers also found evidence that water is directly involved in catalyzing reactions in the ribozyme's core, another previously unknown role. The research team explored the new roles of water molecules using a combination of computational simulations and a technique called single-molecule fluorescence resonance energy transfer (FRET), which allowed the researchers to directly observe and measure how quickly the ribozyme switched forms and how the rates changed when various parts of the molecule were altered.

The situation in ribozymes contrasts with what happens in protein enzymes, which repel water from their cores and rely on direct contact, rather than a network of hydrogen bonds, to communicate structural changes from one part of the molecule to another.

So far, the researchers have focused on one particular ribozyme, but Walter predicts the findings will apply to other RNAs. If so, those findings should be of great interest to scientists who are learning more all the time about the diverse roles of RNA. Once thought to be only a passive carrier of encoded genetic information, RNA is now known to regulate gene expression and other important cellular processes and to act as a sort of sensor---detecting cellular signals and carrying out appropriate reactions in response. In fact, there are many more so-called non-protein coding RNAs in the cell (around 100,000 in humans), which are not translated into protein, than there are protein coding messenger RNAs (about 25,000), making these vast numbers of RNA molecules central players in our bodies.

Work is also underway in academic and industrial labs around the world to engineer RNA for medical purposes. The engineered molecules, called RNA aptamers, are selected for their ability to bind to particular proteins involved in certain diseases, blocking key steps in the disease process.

"It's likely that water helps mediate the binding between these aptamers and their disease-causing protein targets, ultimately keeping the protein away from where it can wreak havoc," Walter said. "So the fundamental understanding we are gaining of the role of water in RNA almost certainly will have relevance in the treatment or prevention of disease."

Source: University of Michigan