PHYSICAL CHEMISTRY

The More The Merrier

New evidence proves that RNAs can take on multiple folded forms, just as proteins do

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The conventional wisdom on large biomolecules is that they each adopt a single three-dimensional form when they fold. A number of studies over the years have reported otherwise, demonstrating cases in which folded proteins take on multiple so-called native states. But such studies have not generally attracted a lot of interest or attention.

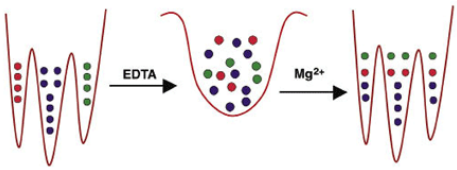
That may begin to change as more systems with multiple native states are discovered. Such a finding was made last month, when a study extended the multiple native state idea definitively from proteins to RNA for the first time. The study used single-molecule experiments to show not only that an RNA enzyme has multiple stable conformations, but that an individual ribozyme molecule in one conformation can interconvert slowly into a covalently identical molecule with a different conformation.

Multiple native states have been observed earlier in RNA as well as proteins. But although a number of the protein studies were definitive, conformational interconversion was only partial and unidirectional in the RNA studies, leaving open the possibility that the RNA states were covalently rather than just conformationally different. Molecules that differ covalently are expected to have different native states because they are, in fact, different molecules, instead of being varied shapes of the same molecule.

In the new study, postdoc Sergey V. Solomatin, graduate student Max Greenfeld, Department of Energy Secretary Steven Chu, and professor of biochemistry **Daniel Herschlag** at Stanford University School of Medicine demonstrated interconversion of multiple native states of covalently identical RNA molecules conclusively (*Nature* **2010,** *463,* 681).

The findings could lead to a better understanding of the structure, function, and "energy landscape" of RNAs and proteins and could have implications for the way such biomolecules are modeled computationally.

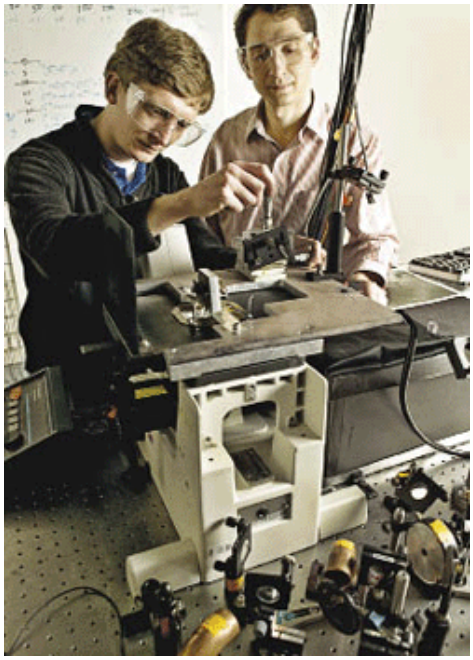
MUSICAL CHAIRS



Credit: Nature © 2010

In the ribozyme study by Herschlag and coworkers, initially (left) each folded state (red, blue, and green) had a specific energy (three separate wells). The states were unfolded (center) by adding ethylenediaminetetraacetic acid (EDTA) and then refolded by adding Mg2+. Some molecules in each state interconverted and adopted different energy wells (right). Interconversion shows that the states are conformational as opposed to covalent.

IN SEARCH OF MULTIPLICITY



Credit: Steve Gladfelter/Stanford U

Solomatin (left) and Greenfeld at the single-molecule microscope used in the Herschlag group's study of RNA native states.

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The idea of biomolecules having multiple native states goes back quite far. “The concept that biomolecules have to be able to accept many conformations to work was clearly expressed by theoretical physicist Erwin Schrödinger in lectures in 1944,” says physicist Hans Frauenfelder of Los Alamos National Laboratory. A number of “farsighted biochemists,” such as Danish scientist Kaj Ulrik Linderstrøm-Lang, voiced similar opinions, Frauenfelder says.

Frauenfelder and coworkers provided experimental evidence for multiple conformational states of myoglobin and depicted their energy landscapes in a 1975 study. Later, they further characterized such landscapes by reporting on factors that affect the size of energy barriers between conformations.

A number of other research teams have focused on multiple native states. For example, biochemist Carl Frieden of Washington University in St. Louis, School of Medicine, and coworkers introduced the concept of protein hysteresis, a time dependence in the behavior of proteins due to factors like conformational transformations. Biochemist **Franz X. Schmid** and Heidi Blaschek of the University of Regensburg, in Germany, reported that a folded intermediate in the refolding reaction of ribonuclease A has a nativelike active site and enzymatic activity similar to that of the native enzyme.

Furthermore, theoretical chemist **Devarajan Thirumalai** and a coworker at the University of Maryland performed simulations to show that several folded forms of a peptide can have similar structural characteristics but different energies and can interconvert slowly. Chemistry professor **Sir Alan Fersht** of the University of Cambridge and coworkers showed that one of tyrosyl tRNA synthetase’s two subunits catalyzes adenylation rapidly, whereas the other has the same sequence but a different conformation and works 10,000 times more slowly—a phenomenon they called “half-site reactivity.” And a group led by protein folder **C. Robert Matthews**, now of the University of Massachusetts Medical School, in Worcester, showed that dihydrofolate reductase folds into four different native states that persist for long times.

In reviews of studies like those of Matthews and coworkers, enzymologists Stephen J. Benkovic and Sharon Hammes-Schiffer of Pennsylvania State University and Gordon G. Hammes of Duke University proposed that enzymes catalyze reactions as well as they do by adopting a range of conformations. “The free-energy description of enzyme catalysis cannot be described in two dimensions but requires a multidimensional free-energy landscape that is very rugged, with multiple minima and transition states,” they wrote (*Biochemistry* **2008,** *47,* 3317).

In the past dozen years or so, single-molecule experiments have helped confirm that proteins can have multiple native states with distinct properties. Spectroscopist **X. Sunney Xie** of Harvard University and coworkers have used single-molecule techniques to observe fluctuating catalytic activity in individual protein molecules and have attributed those fluctuations to multiple conformations that interconvert over time scales ranging from hundreds of microseconds to seconds.

A few years ago, Chu, chemistry professor **Nils G. Walter** of the University of Michigan, and coworkers used single-molecule fluorescence to identify four different catalytically active native states in single hairpin ribozyme molecules, thus potentially extending the multiple native state concept from proteins to RNA. However, despite diligent efforts, the group was able to observe only partial exchanges among the conformations, leaving open the possibility that they differed covalently. “Our evidence against covalent differences was strong, but not completely unassailable,” Walter says.

In their new study, Herschlag and coworkers have now used single-molecule experiments to demonstrate interconvertibility among at least six native states of a ribozyme. They took folded conformations of single ribozyme molecules, unfolded them on a surface, and showed that they could refold to form conformations that were different from their original ones. The researchers also observed single molecules for tens of minutes and found that some of them changed their behavior during that time, suggesting the molecules had undergone conformational transitions.

Such findings on multiple native states, whether of proteins or RNAs, raise a number of tricky challenges for researchers who specialize in computational modeling and structural analysis of biomolecules.

For example, molecular dynamics simulations are now typically carried out for picoseconds or nanoseconds. But if a biomolecule with multiple native states interconverts conformationally on timescales as slow as minutes, much longer simulations might be needed to model it accurately. On the other hand, if several conformations of an enzyme exist and they are all catalytically active, then it may not be necessary to always seek or predict the lowest energy structures in the computational design of new enzymes, and such efforts could therefore become easier, Thirumalai says.

And if a biomolecule has multiple native states, it remains to be seen whether a single X-ray crystal structure captures the form that is most active, least active, or somewhere in between. The presence of multiple native states suggests the need for multiple structure determinations, Thirumalai says.

The Herschlag group's study "shows nicely that three selected native states of a ribozyme catalyze the first step of splicing at the same rate," says RNA specialist **Philip C. Bevilacqua** of Penn State. "But do the native states do everything identically? Do they perceive the environment identically and respond to it identically? If the answer is no, then that inherent complexity is more interesting for biology. It allows different ways for one sequence to respond to the environment."

Walter says that "the molecular causes for persistent conformational isomers in RNA are still unknown and deserve further study"—not only to solve the mystery about why they exist but also to make it possible "to design better folding RNAs for biotechnological applications."

From a fundamental standpoint, Herschlag says, "if we're going to understand the physical behavior of complex macromolecules that fold and have set structures, we have to understand what their basic energy landscape looks like. And if they've got more energy minima and larger barriers between those energy minima than we've previously suspected, that's critical for us to know as a step toward understanding what's responsible for those differences, structurally and energetically." Further studies of multiple native states may be needed to achieve this more in-depth level of understanding of biomolecular behavior, he notes.

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