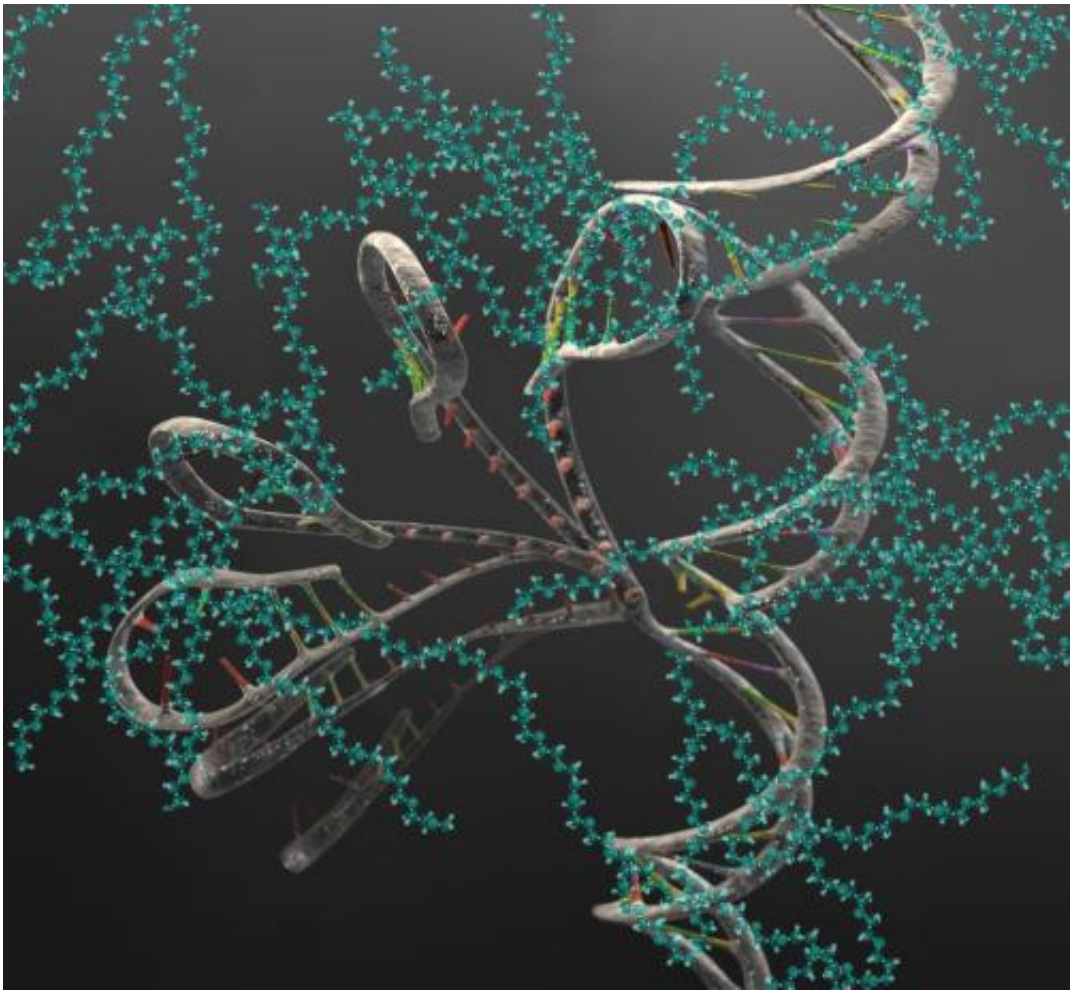
# JILA study finds crowding has big effects on biomolecules

May 21 2014, by Laura Ost



Artist's conception of an RNA molecule (large purplish ladder) in the process of folding as it is crowded by a common polymer, polyethylene glycol (turquoise strands). RNA folds more frequently--that is, the branch on the left folds upward--when it is crowded. Credit: Baxley/JILA and Talbott/NIST

Crowding has notoriously negative effects at large size scales, blamed for everything from human disease and depression to community resource shortages. But relatively little is known about the influence of crowding at the cellular level. A new JILA study shows that a crowded environment has dramatic effects on individual biomolecules.

In the first data on the underlying dynamics (or kinetics)of crowded single biomolecules , reported in *Proceedings of the National Academy of Sciences*, JILA researchers found that crowding leads to a 35-fold increase in the folding rate of RNA (ribonucleic acid), while the unfolding rate remains relatively stable.

RNA is a long chain-like molecule that contains genetic information, makes proteins and catalyzes biological reactions. It must fold into the correct 3D shape to function properly. The new results show that while RNA usually spends most of its time unfolded, in a crowded situation it folds much more often, although it remains folded for the usual period of time during each round.

"Cells are 25 to 35 percent filled with 'stuff'—proteins, nucleic acids, lipids, etc.—and the effect of crowding on simple reactions like folding of nucleic acids and proteins is not well understood," JILA/NIST Fellow David Nesbitt says. "Almost all detailed kinetic data comes from in vitro studies, that is, not in a living cell.

"But our work at the single-molecule level suggests that the rates and equilibrium constants (where folding and unfolding rates are equal) for simple nucleic acid folding processes may be shifted by up to 400,000 percent or more from what one might expect from such uncrowded solution studies."

Nesbitt's group used a specialized microscope to study RNA folding over time in solutions containing various concentrations of a large common

polymer, PEG. The size of the PEG molecule constrains the 3D space around the RNA, mimicking the contents of crowded cells better than the typical dilute solutions used in test-tube studies.

Although PEG was previously known to encourage the compactor folded states of biomolecules, the JILA results are the first to determine the underlying processes. The results were not obvious, in that crowding might seem likely to suppress RNA unfolding. JILA researchers suggest that instead, the opposite process is at work: Crowding lowers the energy required for RNA to achieve the transition state for folding, making it easier to fold.

The JILA study also analyzed temperature-related data to show that the boost in the RNA folding rate constant is largely due to the disorder (or entropy) of the mixture, rather than simply the tendency for RNA to stabilize in the shape with the lowest energy.

In addition to collecting experimental data, JILA researchers used a simple particle model to estimate what would happen in the extremely crowded environment of the cell. The results suggest that the RNA folding equilibrium constant could potentially increase more than 4,000-fold—resulting in a dramatically different biochemical composition—while the folding rate could increase more than 1,000-fold. JILA researchers speculate that such extreme effects could profoundly influence both the rates and preferred directions of complex biochemical pathways in cells. Further studies are needed to determine whether the model can be extended to other cell contents and other RNA structures.

**More information:** N.F. Dupuis, E.D. Holmstrom and D.J. Nesbitt. "Molecular crowding effects on single molecule RNA folding/unfolding thermodynamics and kinetics." *Proceedings of the National Academy of Sciences*. Scheduled to be published online in Early Edition week of May

19-23.

Provided by National Institute of Standards and Technology