Editorial

Beyond Static Structures of RNA by NMR: Folding, Refolding, and Dynamics at Atomic Resolution

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These are exciting times for those interested in RNA dynamics. Over the past decade, there has been an explosion in the number of cellular roles ascribed to RNA and many more will undoubtedly be uncovered in the near future. RNA can store and relay genetic information based on its primary sequence as well as fold into complex three-dimensional structures that can surgically recognize proteins and small molecules or catalyze a wide range of highly sophisticated chemical reactions.[1–5] RNA catalyzes the synthesis of proteins, regulates their expression at both the transcriptional and translational level, and is involved in their cellular transport.[2,6–8] An entirely new genetic regulatory network is also being uncovered that is operated by a universe of micro-[9,10] and interfering RNAs.[11,12] The search for these noncoding RNAs has only just begun and unlimited applications in basic research, technology development, and drug discovery are on the horizon.

But why the excitement about RNA dynamics in particular? That RNA is involved in such a variety of functions is surprising not least because one can only have limited expectations for the complexity of structures that can be achieved by a polyanionic biopolymer composed of only four chemically similar building block nucleotides. Although the structural coverage of "naked" RNA is likely to be significantly more limited than of its protein counterpart with its 20 more diverse amino acids, RNA is remarkable at "borrowing" molecular cofactors from the cellular toolkit, including proteins, small molecules, and metals. This ability provides the opportunity for formation of more complex structures and action as a molecular switch that turns on and off as cofactors come into play. Indeed, much of RNA's functional diversity appears to derive from dramatic conformational changes that can be triggered by binding to cofactors or even changes in temperature[1,3,13,14] and RNA synthesis itself.[15] The lifetime of a noncoding RNA will feature several conformational transitions, from initial folding to refolding into molecular complexes, and many more conformational changes during functional cycles, such as those involved in substrate binding, catalysis, and product release. Clearly, the conventional view that one RNA sequence folds into one structure has to be dispelled for a more dynamic view that is captivating in its complexity.

Many biomolecules including proteins undergo conformational changes as part of their function. What is unique in the case of RNA is not only the diversity of cellular signals that can trigger these conformational transitions, but also the magnitude of these structural transformations that can involve rearrangements at the secondary, tertiary, and quaternary structure level. This dynamic view of RNA calls for the development and application of new techniques that tell us more about RNA structures; how do they fold, what are their internal motions, and how do they refold in response to cellular signals.

Internal motions in biomolecules consist of many motional modes each having distinct amplitudes (sub-angstroms to many angstroms), frequencies (10^{-12} to 10^{2} s), directions (correlated versus anticorrelated), and involving different chemical moieties (single bonds, residues, entire domains). All of these motions come together to form a functional biomolecule much like many instruments come together harmoniously in an orchestra. The power of NMR spectroscopy is that it uniquely provides insight into all of these motional properties at a per nucleus or bond basis—or in the analogy to the orchestra—identifies each instrument working together to create the music. It is therefore not surprising that NMR spectroscopy has contributed immensely to our basic understanding of protein dynamics and its role in function.[16–18]

What is surprising, however, is that until recently, there have been far fewer applications of solution NMR in the study of dynamics in nucleic acids. Part of the reason is that the techniques developed to probe protein dynamics cannot easily be applied to study nucleic acids because of a whole

host of challenges having to do with their unique spin physics and hydrodynamic properties. Current developments in NMR methodology have begun to address many of these limitations. These developments includes advances in the interpretation of relaxation data,[19–21] application of novel measurements such as residual dipolar couplings (RDCs),[22–25] and the innovative design of fast photo-triggers of RNA folding and refolding coupled with ultrafast NMR experiments for real-time analysis of dynamic trajectories.[26,27] Thus, as new noncoding RNAs continued to be uncovered in the last decade, developments in NMR spectroscopy kept apace and RNA structures are finally coming under the dynamic scrutiny of this powerful technique. This issue of *Biopolymers* features three review articles that highlight these recent developments in NMR methods for the characterization of dynamic processes in RNA.

Varani and coworkers review spin relaxation techniques that can be used to probe dynamics at ps–ns timescales and chemical exchange measurements that can be used to probe slower motional processes occurring at μs–ms timescales. The review focuses on recent developments in measuring and interpreting ^{13}C relaxation data, which allow visualization of internal motions in both base and sugar moieties. These methods overcome traditional restrictions posed by the paucity of suitable imino nitrogens probes for ^{15}N relaxation measurements. Some of the key developments have to do with data interpretation such as the recent solution determination of chemical shift anisotropy tensors for carbon spins[19,28,29] and introduction of domain elongation methods for separating internal and overall motional contributions.[30,31]

Al-Hashimi and coworkers review the application of the relatively new RDC methodology in studies of RNA structural plasticity. These techniques are providing insight into the amplitudes and in favorable cases directionalities of internal motions ranging from collective motions of helical domains to local motions of noncanonical residues occurring at sub-ms timescales. They are also allowing characterization of how the structure and dynamics of RNA changes in response to cellular signals, including recognition, metal binding, and various chemical modifications.

Finally, Schwalbe and coworkers provide a comprehensive review of RNA folding and refolding together with biophysical methods for its characterization. New time-resolved NMR methods are described that combine rapid photo-triggers tailored uniquely for nucleic acid applications with ultrafast NMR spectroscopic experiments to literally visualize RNA folding/refolding under nonequilibrium conditions and at timescales as fast as 10^{-1} s. These NMR methods uniquely afford site resolved kinetic information paving the way for uncovering conformational pathways at atomic resolution—namely how an RNA structure switches from one form to another.

Together, these three reviews emphasize how innovations in NMR methods provide a platform not only to answer existing questions, but also for making new discoveries and for formulating future questions. For example, as highlighted by Varani, flexibility is often observed at sites that undergo conformational changes during the RNA conformational transition. Recent NMR studies have provided direct site-specific evidence that small molecules may bind to dynamically existing RNA conformations rather than induce new ones. RNA can therefore act as a self-switch that settles into one structure in the presence of external cofactors. Many aspects of the uncovered motions are not well understood. For example, subtle mutations in an RNA sequence cause dramatic changes in structural dynamics by mechanisms that are poorly understood. How do RNA sequences code for internal motions? The functional significance of motions is also often not obvious. Some of the difficulties arise because RNA elements are often removed from their native larger context making it difficult to interpret the relevance of the dynamics seen. As noted by Varani in his concluding remarks, extension of the NMR methods to take on larger and larger RNAs is a critical goal for the future.

Some of the exciting developments are the integration and combination of these different NMR methods in studies of nucleic acids. As highlighted by Varani and Al-Hashimi, spin relaxation, chemical exchange, and RDC methods are increasingly used in concert to achieve a more complete spatial and temporal description of the dynamics. Schwalbe concludes with a glimpse into the future featuring the marriage of RDC methodology with time-resolved NMR methods—i.e. time-resolved NMR structures.

Only 2 years ago, I concluded a review[25] on NMR studies of RNA dynamics by noting that, "To achieve the greatest impact, existing methodological limitations in studies of dynamics in nucleic acids need to be critically addressed and a consensus built around well-defined protocols that can help streamline such NMR investigations." The three reviews in this issue of Biopolymers shows that ample progress has been made in addressing NMR limitations. From the limited studies thus far, we know that the folding and refolding dynamics of RNA is complex, important to function, and worthy of studying with the same atomic resolution that we have grown accustomed to in charactering the static structures of biomolecules. To continue this progress, we need to streamline applications so that many in the community can apply these NMR techniques. Only then will a large repertoire of RNA dynamics begin to emerge that allows detailed relationships

and trends to be established with respect to sequence, structure, and function.

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