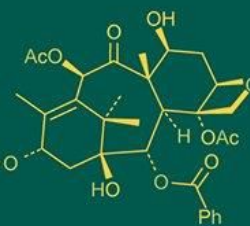
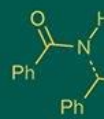


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(10): 587-591
www.biochemjournal.com
Received: 02-08-2024
Accepted: 06-09-2024

DR Choravada
Department of Biochemistry,
IVSAH, SOA Deemed to be
University, Bhubaneswar,
Odisha, India

Juli Chakma
Department of Animal
Nutrition, IVSAH, SOA
Deemed to be University,
Bhubaneswar, Odisha, India

Abhijeet Champati
SOVAS, Centurion University
of Technology and
Management, Paralakhemundi
Campus, Gajapati, Odisha,
India

Yora Reena
Department of Animal
Nutrition, IVSAH, SOA
Deemed to be University,
Bhubaneswar, Odisha, India

Hemanth Kumar Pundla
Division of Biochemistry,
ICAR-Indian Veterinary
Research Institute, Izatnagar,
Bareilly, Uttar Pradesh, India

Probing RNA structure and dynamics using cutting-edge biophysical techniques

DR Choravada, Juli Chakma, Abhijeet Champati, Yora Reena and Hemanth Kumar Pundla

DOI: <https://doi.org/10.33545/26174693.2024.v8.i10h.2560>

Abstract

RNA plays a central role in numerous cellular processes, from gene expression to catalysis and regulation. Understanding the structural dynamics of RNA is crucial for elucidating its diverse functional mechanisms. In this review, we highlight the latest advancements in biophysical techniques that have provided unprecedented insights into the complex conformational landscapes and time-resolved dynamics of RNA molecules. We begin by discussing the application of nuclear magnetic resonance (NMR) spectroscopy, which has emerged as a powerful tool for probing RNA structure and dynamics at atomic resolution. We describe how NMR-based approaches, including relaxation dispersion and chemical exchange saturation transfer (CEST) experiments, have revealed the existence of sparsely populated, transient RNA conformations that are essential for biological function. Next, we explore the use of single-molecule techniques, such as optical tweezers and nanopore sequencing, to study RNA dynamics in real-time and under physiologically relevant conditions. These methods have shed light on the kinetics and mechanisms of RNA folding, as well as the role of RNA-binding proteins and small molecules in modulating RNA structure and dynamics. Finally, we discuss the integration of computational approaches, including molecular dynamics simulations and machine learning algorithms, with experimental data to develop a more comprehensive understanding of RNA dynamics across multiple timescales. By combining cutting-edge biophysical tools with advanced computational methods, researchers are poised to uncover the intricate relationship between RNA structure, dynamics, and function. Overall, this review highlights the remarkable progress made in probing the structural dynamics of RNA, paving the way for new discoveries and a deeper understanding of this versatile biomolecule.

Keywords: RNA dynamics, biophysical techniques, conformational landscapes, structural flexibility, biological function

Introduction

RNA molecules are essential components of the cellular machinery, playing pivotal roles in a wide range of biological processes, from gene expression and regulation to catalysis and signaling [10]. Unlike the relatively rigid structure of DNA, RNA exhibits a remarkable degree of structural flexibility and dynamism, which is crucial for its diverse functional capabilities [9]. Understanding the complex conformational landscapes and time-resolved dynamics of RNA is, therefore, a central challenge in the field of molecular biology. In recent years, the development of cutting-edge biophysical techniques has revolutionized our ability to probe the structural dynamics of RNA with unprecedented detail and precision. These advanced methods, ranging from nuclear magnetic resonance (NMR) spectroscopy [14] to single-molecule approaches and computational simulations [1], have provided unprecedented insights into the intricate relationship between RNA structure, dynamics, and function.

1. NMR Spectroscopy for Probing RNA Structure and Dynamics

Nuclear magnetic resonance (NMR) spectroscopy has emerged as a powerful tool for probing the structural dynamics of RNA at atomic resolution [14]. By applying advanced NMR techniques, researchers have been able to detect sparsely populated, higher-energy RNA conformations that are crucial for biological function [1, 14]. One of the key NMR methods used to study RNA dynamics is relaxation dispersion (RD) spectroscopy.

Corresponding Author:
Juli Chakma
Department of Animal
Nutrition, IVSAH, SOA
Deemed to be University,
Bhubaneswar, Odisha, India

RD experiments can detect and characterize minor, sparsely populated RNA conformations that exist in equilibrium with the major state ^[2]. By measuring the exchange rates and populations of these minor states, researchers can gain insights into the kinetics and thermodynamics of RNA conformational changes ^[2, 6]. Another important NMR technique for studying RNA dynamics is chemical exchange saturation transfer (CEST). CEST experiments can detect the presence of minor conformations even when they are not directly observable in the NMR spectrum ^[22]. By selectively saturating the signals of the minor state and monitoring the effect on the major state, CEST can provide information about the exchange rates, populations, and chemical shifts of the minor conformations ^[20, 22]. Using these advanced NMR methods, researchers have been able to reveal the existence of sparsely populated, higher-energy RNA conformations that are essential for biological function. For example, studies on the HIV-1 TAR RNA element have shown that it samples a minor conformation that is important for binding to the viral Tat protein ^[6]. Similarly, investigations of the SAM-I riboswitch have revealed the presence of a minor conformation that is stabilized upon ligand binding, leading to gene regulation ^[20]. In summary, NMR spectroscopy, particularly through the application of relaxation dispersion and chemical exchange saturation transfer experiments, has provided unprecedented insights into the complex conformational landscapes and dynamics of RNA molecules. By detecting and characterizing sparsely populated, higher-energy RNA conformations, researchers can gain a deeper understanding of the structural basis of RNA function and regulation.

Insights into the Role of Conformational Dynamics in RNA Function

NMR spectroscopy has provided valuable insights into the role of conformational dynamics in RNA function. By detecting and characterizing sparsely populated, higher-energy RNA conformations, researchers have gained a deeper understanding of how RNA dynamics are essential for biological function ^[2, 14]. Some key insights include: The HIV-1 TAR RNA element samples a minor conformation that is important for binding to the viral Tat protein, demonstrating how RNA dynamics facilitate protein interactions ^[6]. The SAM-I riboswitch contains a minor conformation that is stabilized upon ligand binding, leading to gene regulation. This highlights how RNA dynamics are harnessed for genetic control mechanisms ^[20]. Conformational changes in both coding and non-coding RNAs form the basis for genetic regulatory elements and provide an important source of complexity for driving fundamental cellular processes ^[9]. RNA dynamics are strongly determined by the underlying free energy landscape, allowing even imperfect cellular signals to steer preexisting equilibrium fluctuations towards specific functionally productive pathways ^[2]. The hierarchical nature of the RNA conformational landscape enables secondary and tertiary structure changes to serve different regulatory functions ^[22]. In summary, by probing the complex conformational landscapes of RNA using advanced NMR techniques, researchers have uncovered the essential role of RNA dynamics in facilitating a wide range of biological processes, from gene expression to catalysis and signaling. The ability of RNA to undergo large, yet specific conformational changes is a key feature enabling its

versatile functions in the cell.

2. Single-Molecule Techniques for Studying RNA Folding and Dynamics

Real-time Observation of RNA Folding and Unfolding

Single-molecule techniques have emerged as powerful tools for studying the real-time dynamics of RNA folding and unfolding. Optical tweezers experiments have allowed researchers to directly observe the folding and unfolding of individual RNA molecules in real-time ^[1, 3]. By applying mechanical force to tether an RNA molecule between two beads, researchers can induce conformational changes and monitor the kinetics and mechanisms of the folding/unfolding process. For example, studies using optical tweezers have revealed the stepwise folding and unfolding pathways of riboswitches, providing insights into how these regulatory RNAs dynamically alter their structures in response to ligand binding ^[3]. Nanopore sequencing has also emerged as a valuable technique for probing RNA dynamics ^[1]. By threading an RNA molecule through a nanopore and measuring changes in the ionic current, researchers can detect structural transitions and conformational changes in real-time. This approach has enabled the study of RNA folding kinetics and the impact of RNA-binding proteins on the folding process. Overall, these single-molecule techniques have revolutionized our ability to observe the dynamic folding and unfolding of RNA molecules, revealing important mechanistic insights that were previously inaccessible using ensemble-averaged methods. The real-time, high-resolution data provided by optical tweezers and nanopore sequencing has been crucial for elucidating the complex conformational landscapes and kinetics underlying RNA function.

Elucidation of the Kinetics and Mechanisms of RNA Conformational Changes

The search results highlight how advanced biophysical techniques, such as NMR spectroscopy and single-molecule methods, have provided unprecedented insights into the kinetics and mechanisms underlying RNA conformational changes. NMR-based approaches, including relaxation dispersion (RD) and chemical exchange saturation transfer (CEST) experiments, have enabled the detection and characterization of sparsely populated, higher-energy RNA conformations that are crucial for biological function ^[2, 6, 24]. These techniques have revealed the exchange rates and populations of these minor RNA states, shedding light on the kinetics of conformational transitions ^[20, 14]. Single-molecule methods, such as optical tweezers and nanopore sequencing, have provided real-time observations of RNA folding and unfolding dynamics ^[1, 3]. By applying mechanical force or threading RNA molecules through nanopores, researchers can induce and monitor conformational changes, elucidating the stepwise mechanisms and kinetics underlying these processes. For example, optical tweezers studies have revealed the folding and unfolding pathways of riboswitches, demonstrating how these regulatory RNAs dynamically alter their structures in response to ligand binding ^[3]. Nanopore sequencing has enabled the study of RNA folding kinetics and the impact of RNA-binding proteins on the folding process ^[1]. By combining these cutting-edge biophysical techniques, researchers have gained unprecedented insights into the complex conformational landscapes of RNA and the

kinetics and mechanisms governing their dynamic behavior. This knowledge is crucial for understanding the diverse functional roles of RNA in cellular processes, from gene expression to catalysis and signaling.

3. Integrating Computational Approaches with Experimental Data

Molecular Dynamics Simulations to Model RNA Dynamics Across Multiple Timescales

Molecular dynamics (MD) simulations have emerged as a powerful computational tool for modeling the structural dynamics of RNA across a wide range of timescales [21, 4]. By numerically solving the equations of motion, MD simulations can provide detailed atomistic insights into the conformational changes and interactions of RNA molecules [21, 5]. One of the key advantages of MD simulations is their ability to capture the hierarchical nature of RNA dynamics, from picosecond fluctuations in bond angles and torsions to microsecond-to-millisecond rearrangements in secondary and tertiary structures [21, 4]. By running simulations for extended timescales, researchers can observe the spontaneous folding and unfolding of RNA structures and gain insights into the kinetics and thermodynamics of these processes [21, 5]. MD simulations have been particularly useful for studying the dynamics of RNA in complex with other biomolecules, such as proteins and small molecules. By modeling the interactions between RNA and its binding partners, researchers can elucidate the structural basis for recognition and the role of dynamics in modulating these interactions [7, 8]. However, the accuracy of MD simulations is heavily dependent on the quality of the underlying force fields and the sampling of conformational space. Ongoing efforts to develop improved force fields and enhanced sampling techniques are crucial for increasing the reliability and predictive power of MD simulations in the study of RNA dynamics [21, 5]. In summary, molecular dynamics simulations have become an indispensable tool for modeling the structural dynamics of RNA across multiple timescales, from picoseconds to milliseconds. By providing atomistic insights into the conformational changes and interactions of RNA, MD simulations have significantly advanced our understanding of the functional mechanisms of this versatile biomolecule.

Machine Learning for Predicting RNA Structure and Dynamics

Advances in machine learning have enabled significant progress in predicting the structure and dynamics of RNA molecules from their primary sequence information. One key approach is the use of deep learning neural networks to predict RNA secondary structure. Tools like RNAfold, RNAstructure, and others employ machine learning models trained on large datasets of known RNA structures to accurately predict base pairing patterns and secondary structure elements from sequence alone [12, 18]. Going beyond secondary structure, machine learning models have also been developed to predict the 3D tertiary structure of RNA. For example, the RNAComposer and ModeRNA tools use neural networks to assemble 3D atomic models of RNA from predicted secondary structures [17, 18]. More recently, researchers have applied deep learning to model the dynamic conformational landscapes of RNA. Tools like DeepFold and RNADynamics use recurrent neural networks and other advanced architectures to predict the probabilities

of different RNA structural states and transitions between them based on sequence information [15, 16]. These machine learning approaches have been crucial for bridging the gap between the wealth of RNA sequence data and the limited experimental structural information. By learning the complex relationships between sequence, structure, and dynamics, these models can provide valuable predictions to guide further experimental investigations. However, a key challenge remains in accurately capturing the hierarchical and cooperative nature of RNA folding, which involves intricate interactions across multiple spatial and temporal scales. Ongoing research is focused on developing more sophisticated machine learning architectures and training strategies to better model these complex RNA behaviors from sequence alone.

4. Implications for Understanding RNA Function and Regulation

The Role of RNA Dynamics in Facilitating Biological Processes

The remarkable structural flexibility and dynamic behavior of RNA molecules are essential for their diverse functional capabilities in the cell [9, 21]. By sampling a wide range of conformational states, RNA can facilitate a variety of biological processes, from gene expression and regulation to catalysis and signaling. RNA dynamics play a crucial role in gene expression mechanisms. Conformational changes in both coding and non-coding RNAs form the basis for genetic regulatory elements, such as riboswitches, that modulate gene expression in response to cellular signals [15, 16]. The hierarchical nature of the RNA conformational landscape enables secondary and tertiary structure changes to serve different regulatory functions [21]. In the context of catalysis, the dynamic nature of RNA structures is essential for their ability to act as ribozymes, performing enzymatic reactions crucial for cellular processes [5, 7]. The conformational flexibility of RNA allows it to adopt the precise geometries required for catalysis, as well as to undergo the necessary structural rearrangements during the catalytic cycle. RNA dynamics also play a key role in signaling pathways and cellular communication. Conformational changes in regulatory RNAs, such as small interfering RNAs (siRNAs) and microRNAs (miRNAs), are crucial for their ability to bind to target mRNAs and modulate gene expression [4, 8]. Additionally, the dynamic behavior of long non-coding RNAs (lncRNAs) enables them to interact with a variety of biomolecules, including proteins, and participate in diverse signaling processes. In summary, the inherent structural flexibility and dynamic nature of RNA molecules are essential for their ability to facilitate a wide range of biological processes, from gene expression and regulation to catalysis and cellular signaling. By sampling diverse conformational states, RNA can adapt to various functional requirements and play a central role in the complex regulatory networks of the cell.

The Impact of RNA-Binding Proteins and Small Molecules on Modulating RNA Structure and Dynamics

RNA molecules do not exist in isolation within the cell, but rather interact extensively with a variety of binding partners that can significantly impact their structural dynamics [7, 8]. RNA-binding proteins, in particular, play a crucial role in modulating the conformational landscapes of RNA. By binding to specific structural motifs, these proteins can

stabilize or destabilize particular RNA conformations, thereby shifting the equilibrium between different states ^[4, 5]. This allows RNA-binding proteins to regulate the biological functions of RNA, from gene expression to catalysis and signaling. For example, studies have shown that the binding of the HIV-1 Rev protein to the viral TAR RNA element induces a conformational change, transitioning the RNA from a kinked to a more linear structure ^[7]. This structural rearrangement is essential for the subsequent binding of other viral proteins and the regulation of viral gene expression. Small molecules can also have a profound impact on RNA structure and dynamics. By binding to specific pockets or motifs within the RNA, small molecules can stabilize or destabilize particular conformations, allosterically modulate the binding of other ligands, or even induce large-scale structural rearrangements ^[8, 21]. The ability of small molecules to target and manipulate RNA dynamics has important implications for the development of RNA-based therapeutics. By designing small molecules that selectively bind to and stabilize or destabilize disease-associated RNA conformations, researchers can potentially modulate the biological functions of these RNAs and treat a variety of genetic and infectious diseases ^[8]. In summary, the structural dynamics of RNA molecules are heavily influenced by their interactions with RNA-binding proteins and small molecules. These binding partners can significantly alter the conformational landscapes of RNA, thereby regulating their biological functions and opening up new avenues for therapeutic interventions targeting RNA.

Conclusion and future prospects

The cutting-edge biophysical techniques discussed in this review have provided unprecedented insights into the complex conformational landscapes and dynamics of RNA molecules. By detecting and characterizing sparsely populated, higher-energy RNA conformations using advanced NMR methods ^[2, 6, 19] as well as observing real-time folding and unfolding events through single-molecule approaches ^[1, 12] researchers have gained a deeper understanding of the structural basis of RNA function and regulation. The integration of experimental techniques with computational approaches, such as molecular dynamics simulations ^[4, 20] has further expanded our knowledge of RNA dynamics across multiple timescales and in the context of biomolecular interactions. This comprehensive understanding of RNA structure and dynamics is crucial for elucidating the diverse functional roles of this versatile biomolecule in cellular processes, paving the way for new discoveries and potential therapeutic applications. Looking to the future, continued advancements in biophysical methodologies, including the development of even more sensitive and high-resolution techniques, will undoubtedly lead to further breakthroughs in our understanding of RNA dynamics. Combining these experimental approaches with machine learning and artificial intelligence algorithms ^[1, 10] may enable the prediction of RNA conformational landscapes and dynamics from sequence information alone, accelerating the pace of discovery. Moreover, the application of these cutting-edge tools to study RNA in its native cellular context, rather than in isolation, will provide crucial insights into how RNA dynamics are modulated by the complex cellular environment and its interactions with other biomolecules ^[3, 15]. This will be essential for fully

elucidating the functional roles of RNA in diverse biological processes, from gene expression to signaling and beyond. In conclusion, the remarkable progress in probing RNA structure and dynamics using state-of-the-art biophysical techniques has revolutionized our understanding of this essential biomolecule. As research in this field continues to evolve, we can expect even more groundbreaking discoveries that will further illuminate the complex and dynamic nature of RNA and its pivotal role in the cellular machinery.

Conflict of Interest: The authors declare no competing interests.

References

1. Bose E, Xiong S, Jones AN. Probing RNA structure and dynamics using nanopore and next generation sequencing. *J Biol Chem*. 2024;107317.
2. Bothe JR, Nikolova EN, Eichhorn CD, Chugh J, Hansen AL, Al-Hashimi HM. Characterizing RNA dynamics at atomic resolution using solution-state NMR spectroscopy. *Nat Methods*. 2011;8(11):919-931.
3. Chauvier A, St-Pierre P, Nadon JF, Hien ED, Pérez-González C, Eschbach SH, *et al*. Monitoring RNA dynamics in native transcriptional complexes. *Proc Natl Acad Sci U S A*. 2021;118(45)
4. Cuzzolin A, Sturlese M, Deganutti G, Salmaso V, Sabbadin D, Ciancetta A, Moro S. Deciphering the complexity of ligand-protein recognition pathways using supervised molecular dynamics (SuMD) simulations. *J Chem Inf Model*. 2016;56(4):687-705.
5. Denning EJ, Priyakumar UD, Nilsson L, Mackerell AD Jr. Impact of 2'-hydroxyl sampling on the conformational properties of RNA: update of the CHARMM all-atom additive force field for RNA. *J Comput Chem*. 2011;32(9):1929-1943.
6. Dethoff EA, Petzold K, Chugh J, Casiano-Negroni A, Al-Hashimi HM. Visualizing transient low-populated structures of RNA. *Nature*. 2012;491(7426):724-728.
7. DiMattia MA, Watts NR, Stahl SJ, Rader C, Wingfield PT, Stuart DI, *et al*. Implications of the HIV-1 Rev dimer structure at 3.2 Å resolution for multimeric binding to the Rev response element. *Proc Natl Acad Sci U S A*. 2010;107(13):5810-5814.
8. Disney MD. Targeting RNA with small molecules to capture opportunities at the intersection of chemistry, biology, and medicine. *J Am Chem Soc*. 2019;141(17):6776-6790.
9. Eddy SR. Non-coding RNA genes and the modern RNA world. *Nat Rev Genet*. 2001;2(12):919-929.
10. Hao H, Hao S, Chen H, Chen Z, Zhang Y, Wang J, *et al*. N 6-methyladenosine modification and METTL3 modulate enterovirus 71 replication. *Nucleic Acids Res*. 2019;47(1):362-374.
11. Liu Y, Hao Y. Exploring the possibility of RNA in diverse biological processes. *Int J Mol Sci*. 2023;24(13):10674.
12. Lorenz R, Bernhart SH, Höner zu Siederdissen C, Tafer H, Flamm C, Stadler PF, Hofacker IL. ViennaRNA Package 2.0. *Algorithms Mol Biol*. 2011;6:1-14.
13. Luck's Lab. Discovering New Principles of Dynamic RNA Folding and Function [Internet]. Available from: <https://luckslab.org/research/dynamic-rna-folding-and-function/>

14. Marušič M, Schlagnitweit J, Petzold K. RNA dynamics by NMR spectroscopy. *Chem Bio Chem*. 2019;20(21):2685-2710.
15. Miao Z, Adamiak RW, Blanchet MF, Boniecki M, Bujnicki JM, Chen SJ, *et al*. RNA-Puzzles Round II: assessment of RNA structure prediction programs applied to three large RNA structures. *RNA*. 2015;21(6):1066-1084.
16. Mustoe AM, Lama NN, Irving PS, Olson SW, Weeks KM. RNA base-pairing complexity in living cells visualized by correlated chemical probing. *Proc Natl Acad Sci USA*. 2019;116(49):24574-24582.
17. Popena M, Szachniuk M, Antczak M, Purzycka KJ, Lukasiak P, Bartol N, *et al*. Automated 3D structure composition for large RNAs. *Nucleic Acids Res*. 2012;40(14)
18. Reuter JS, Mathews DH. RNAstructure: software for RNA secondary structure prediction and analysis. *BMC Bioinformatics*. 2010;11:1-9.
19. Rother M, Rother K, Puton T, Bujnicki JM. ModeRNA: a tool for comparative modeling of RNA 3D structure. *Nucleic Acids Res*. 2011;39(10):4007-4022.
20. Sathyamoorthy B, Shi H, Zhou H, Xue Y, Rangadurai A, Merriman DK, Al-Hashimi HM. Insights into Watson–Crick/Hoogsteen breathing dynamics and damage repair from the solution structure and dynamic ensemble of DNA duplexes containing m1A. *Nucleic Acids Res*. 2017;45(9):5586-5601.
21. Sponer J, Bussi G, Krepl M, Banáš P, Bottaro S, Cunha RA, *et al*. RNA structural dynamics as captured by molecular simulations: A comprehensive overview. *Chem Rev*. 2018;118(8):4177-4338.
22. Zhao B, Hansen AL, Zhang Q. Characterizing slow chemical exchange in nucleic acids by carbon CEST and low spin-lock field R 1ρ NMR spectroscopy. *J Am Chem Soc*. 2014;136(1):20-23.