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COLORADO RNA CLUB LATEST

What's new from our RNA community

LETTER FROM THE EDITOR

"Structure without function is a corpse; function without structure is a ghost." –– Stephen A. Wainwright

Until the 1970s, investigating RNA structure was limited to probing primary and secondary structures via chemical and biochemical methods. With groundbreaking technological advances over the last five decades, we can now study primary, secondary, and tertiary RNA structures with numerous techniques including, SHAPE, X. Ray Crystallography, Cryo-EM, and NMR, as well as with computational methods. Among the most recent breakthroughs is the application of Cryo-EM to determine RNA-only structures, which currently makes up only 1% of the protein data bank.

Harnessing these techniques, scientists have been able to make important discoveries about RNA structure, its associated function, and evolutionary origin. For example, the ribosome structure illustrated that peptide bond formation occurs in an RNA-only environment; riboswitch structures depicted their ligand binding, which is now being used to develop tools and gene therapies; finally, the third kingdom of life, Archae, was found by deducing the secondary structure of ribosomal RNA via sequence alignments.

In the final issue of this year, we wanted to highlight some amazing scientists and their contributions to RNA structural biology: **Rhiju Das**, Ph.D., one of the pioneers of Cryo-EM for RNA-only structures and developer of Eterna, a game that advances medical research by solving RNA structure puzzles; **Steve Bonilla**, Ph.D., who used Cryo-EM to solve the structure of a tRNA mimic in two conformations; **Rob Batey**, Ph.D., who has solved many riboswitch structures and developed a related tool; and **Megan Filbin**, Ph.D., who trains undergraduates to study structure and function of viral RNA elements via SHAPE.

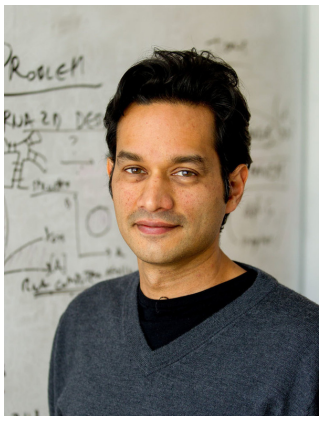
As always, we hope you enjoy reading this issue. If you have thoughts or comments on the topics we cover, we'd love to hear them at ColoradoRNAClub@gmail.com or on social media.

Divya Kolakada
Editor, Colorado RNA Club Latest

The Future of RNA Structure Rivals That of Protein

by: Nicole Moss, PhD candidate at CU Anschutz

"I hope we'll be able to expand the database of known RNA-only structures from about a few hundred to 100,000", says Rhiju Das, Ph.D.



Rhiju Das, PhD. Picture: ssprobe.com

Das, currently an *Associate Professor of Biochemistry at Stanford University School of Medicine* and recently selected as an *HHMI Investigator*, was originally inspired to study RNA structure as a physics student. "I was pulled into a presentation on the first crystal structures of the ribosome in 2001 by a friend and became super fascinated by the physical problem of how an RNA could find such an elaborate structure." Das went on to pursue his PhD with Dr. Dan Herschlag at Stanford and his postdoc with Dr. David Baker at the University of Washington. Throughout his training Das has been captivated by the first RNA structure he made as a graduate student, the Tetrahymena ribozyme, and notes "it's been satisfying to work on a global structure of this paradigmatic RNA, recently with cryo-EM, with colleagues from the cryoEM world".

For so many biomolecules, elucidation of the physical structure has been pivotal for understanding the function and describing the mechanism of action. For example, solving the crystal structure of DNA and visualizing the Cas-9 protein were pivotal in explaining the mechanisms of genetic inheritance and targeting

mechanisms respectively. Over the last several decades there have beensignificant advancements in solving DNA, protein, and RNA-protein structures. However, despite their significant role in biological mechanisms, RNA-only structures have been notably understudied, presumably due to the unique challenges associated with their chemistry.

Today, the Das lab is pioneering methods to model these RNA-only structures. The lab combines high throughput chemical mapping, computational methods, and cryoEM to model RNA and elucidate structures. In a recent publication, Das describes the recent reapplication of cryoEM for studying RNA-only structures at increasingly smaller sizes and generating the coordinates that are later filled in using computational methods. These methods are rapidly expanding the database of known RNA-only structures, and can "reveal the answers to questions about which RNAs are homologous to other ones [and] what does the active site of an RNA enzyme look like". Das envisions applying this knowledge to generate an algorithm similar to the protein AlphaFold. This would offer an exceptionally powerful mechanism to study RNA structure and result in 3D structural databases being dominated by RNA-only structures.

Tetrahymena ribozyme structure. PDB: 6WLS.

The lab is primarily focused on methods development in predominantly unexplored areas with the appreciation that often knowledge of RNA structure is indispensable for deciphering the function and mechanism of action. In addition to the methodological and computational advancements, the Das lab has also leveraged citizen scientists through an interactive RNA folding game. EteRNA is a game that allows players to design RNA structures by solving puzzles. Each puzzle has a distinct set of goals and players are challenged to find the optimal solution based on the unique parameters. The lab has noted that in some cases, human responses have surpassed supercomputers challenged with the same puzzles. The data collected from the game is then validated by careful experimentation. "Working together, players and researchers can find novel solutions, invent new medicines, and unravel the secrets of RNA."

When asked where he sees the future of RNA structural biology, Das imagines that one day we will have the knowledge of RNA structure that rivals that of proteins. The expansion of known RNA-only structures will "provide a wealth of insights into how RNA evolves, functions, and how its ultimate design could contribute to novel functions."

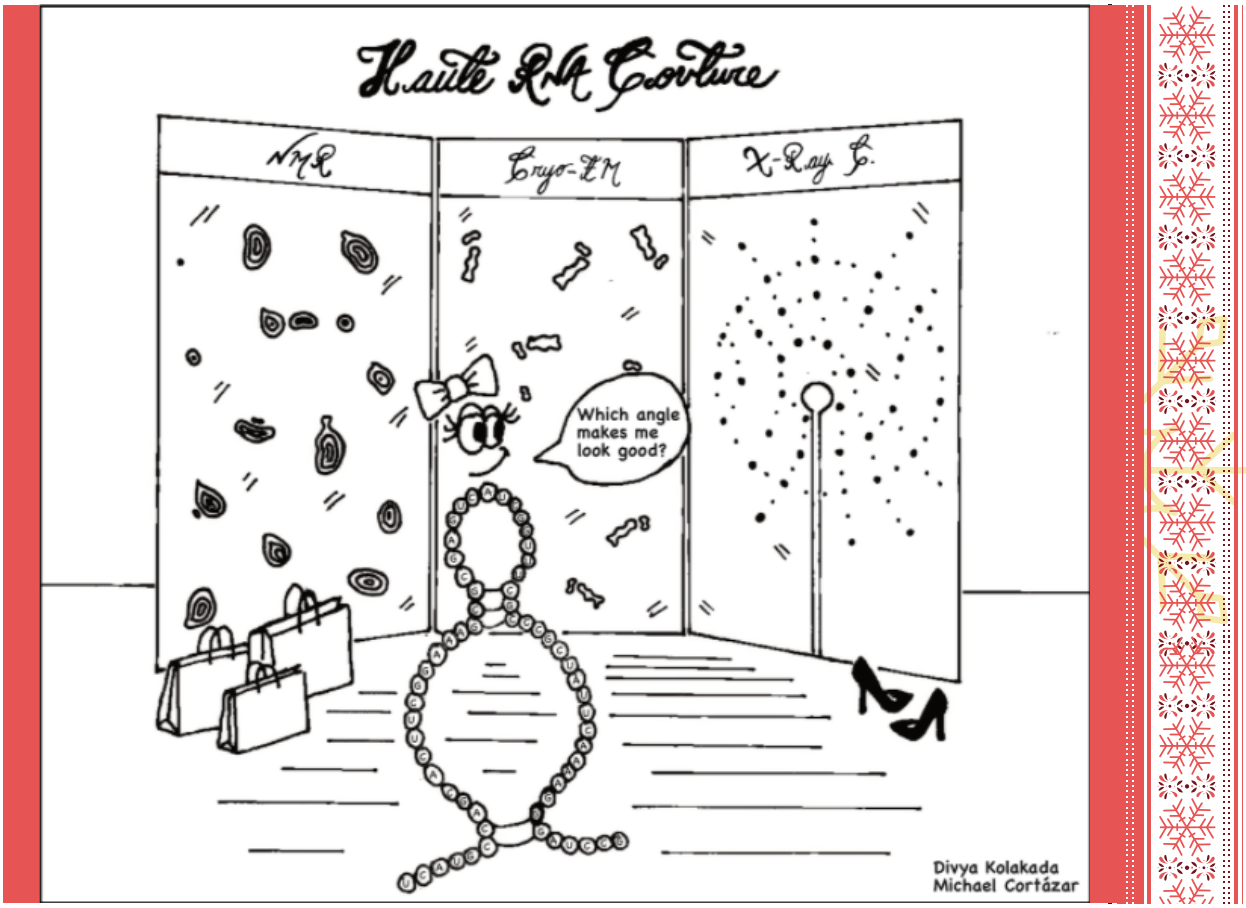
Visualizing RNA Structural Changes Using Cryo-EM

by: Jillian Ramos, PhD, Postdoctoral Scholar at CU Anschutz

Between the twitter buzz that his recent article generated and the catchy caption on the cover of the November issue of Science, Steve L. Bonilla, Ph.D., is causing commotion. Bonilla is a Hanna H. Gray postdoctoral scholar in the Kieft lab at the University of Colorado, Anschutz Medical Campus. In his research article, Bonilla used cryogenic electron microscopy (cryo-EM) to solve the structure of a tRNA mimic, termed tRNA-Like Structure (TLS), found at the 3' end of specific positive-sense RNA viruses. By solving the structure of the RNA both in isolation and bound to a host cell tRNA synthetase, he identified that this dynamic RNA molecule changed shape to hijack the host biochemical machinery. Remarkably, the viral RNA did not assume the classic L-shape of tRNA. Instead, it used a distinct geometry for synthetase recognition. I sat down with Bonilla to discuss his recent paper and the future of using cryo-EM to solve the structure of small, dynamic RNAs and RNA-protein complexes.

Steve Bonilla, PhD. Personal photo collection.

It is uncommon to hear of people solving RNA-only molecules using cryo-EM, so my first question to Bonilla was if he was one of the first. His answer was, "yes and no". He described older studies combining cryo-EM, NMR, and computer simulations to look at relatively simple RNAs. But at that time, cryo-EM was not powerful enough to give a lot of structural details. When Bonilla began his postdoc, however, work pioneered by Rhiju Das, Wah Chiu, and colleagues had shown that cryo-EM could be used for RNA-only structures. Inspired by this work, Bonilla decided to apply these advances to the study of structured, multi-functional viral RNA elements. The task did not come without its own challenges. Due to the dynamic nature of the RNA, ample optimization of both sample preparation and imaging was necessary. Initially he thought his data was unusable. Only after continuous repeated processing of aligning and reclassifying particles did he start to see promising results.



by: Divya Kolakada, PhD candidate at CU Anschutz & Michael Cortázar, PhD, Postdoctoral Scholar at CU Anschutz

"Just like functional things in our every-day lives (think a ladder or a chair) require a shape that is compatible with their function, the function of molecules is determined by their shape. Thus, de-termining how the structure of RNA dictates function is important to understanding biolo-gical processes", Bonilla asserts, continuing, "our understanding of structure-function relationships in viral RNAs is still in its infancy, partly because these RNAs are structurally dynamic and hard to characterize". Although he believes that cryo-EM opens the opportunity to solve RNA mysteries that have been elusive to structural biology for decades, he maintains that the scientific question still dictates the method used, whether that be old-school biochemistry, x-ray crystallography, single molecule methods, etc. In the future, Bonilla anticipates a revolution in RNA determination, with cryo-EM of RNA-only molecules at its center. From this he foresees signi-ficant advances in 3D structure prediction based on RNA sequence, very similar to what has recently been developed for proteins, like Alphafold.

Crystalizing the Function of RNA Sensors

by: Giulia Corbet, PhD candidate at CU Boulder

For over 20 years, Professor Robert Batey, has been studying the structure of RNAs using X-ray crystallography. Now the Associate Chair for Graduate Affairs and Full Professor in the Biochemistry Department at CU Boulder, Batey's research focuses on studying small molecule-RNA interactions,

Robert Batey, PhD. Personal photo collection.

specifically in the context of riboswitches.

Riboswitches are RNAs that bind to small molecules such as metabolites or ions, and this binding serves to regulate gene expression. Batey has long been captivated by how RNA is structured to create binding pockets whose ability to recognize small molecules rivals that of protein, in both affinity and specificity. The Batey lab has resolved the structures of numerous riboswitches using X-ray crystallography, including the guanine riboswitch and cobalamin riboswitch. These structures have provided valuable insight into how riboswitches bind their ligands and regulate expression. Furthermore, the cobalamin riboswitch structure helped the Batey lab to adapt the riboswitch into a tool for imaging single RNA molecules in live eukaryotic cells.

For the Batey lab, X-ray crystallography serves as the starting point for understanding RNA-small molecule interactions. The structure helps to develop hypotheses regarding structure-function relationships and determine which questions to ask using other biochemical and biophysical approaches. As to why crystallography is the lab's structural technique of choice, Batey stated: "it is the most robust means of being able to generate atomic-level models of interactions between small molecules and any macromolecule...we're looking for the details of things like hydrogen bonding, base stacking, and orientation of small molecules."

The wealth of knowledge contributed by studying the structure of riboswitches has far-reaching implications. Numerous pharmaceutical companies are working to develop small molecule drugs which target RNAs as therapeutics. Examples of these include drugs for RNA repeat expansion diseases, such as the G4C2 repeat expansion found in some ALS patients, as well as for gene therapies. For companies looking to control the expression of gene therapies, using riboswitches that can be controlled with an orally bioavailable small molecule represents a promising avenue for such efforts. "Riboswitches really present themselves as a model system for thinking about how RNA can be recognized by small molecules and targeted by small molecules in a high-affinity, high-specificity fashion", Batey says.

RNA Structure: A Scaffold for Undergraduate Initiatives

by: Pablo Maldonado, PhD candidate at CSU Fort Collins

At a glance, Metropolitan State University of Denver (MSU Denver) may not present itself as a prime RNA-structure research hub. MSU Denver selectively offers masters degrees unrelated to molecular biology, thus, you might not expect to find MSU Denver's finest chipping away at questions regarding viral RNA structure. Introducing the Filbin Lab: MSU Denver's very own seedbed for undergraduates interested in RNA biology. Led by Megan Filbin, Ph.D, Associate Professor in the Department of Chemistry and Biochemistry at MSU Denver, the Filbin Lab focuses on fundamental questions regarding non-coding RNA structure and function. Drawing upon her graduate work at CU Anschutz on the internal ribosome entry site in the Hepatitis C Virus, Filbin aims to understand how RNA structure of plant viruses regulates protein synthesis. She explains that non-coding RNAs (ncRNAs) including viral ncRNAs are still poorly classified, noting that we've only recently been able to interpret the functions of their unique motifs. Our advancements in whole genome sequencing and transcriptomics have made it brutally apparent that "junk DNA" is not in fact "junk" but it encodes ncRNA with unknown functions. However, the Filbin Lab embraces the unknown for its learning opportunities, parsing through these non-coding sequences in search of functions related to protein synthesis.

Most impressively, the Filbin Lab relies heavily on the capacity of its undergraduate students to decipher how the structure of the 3' UTR of nepovirus RNA initiates translation in the absence of a 5' cap, in what is recognized as cap-independent translation enhancer (CITE). To achieve this, the lab studies

nepovirus CITE RNA structure using Selective 2′ Hydroxyl Acylation analyzed by Primer Extension (SHAPE). SHAPE involves acetylating the exposed 2′ ribose hydroxyl groups in single-stranded/flexible regions of the CITE RNA in a sequence-independent estimate of viral RNA secondary structure. The adducts are then identified via reverse transcription and sequencing to build an RNA secondary structure. "My students are doing some pretty advanced science and independently drive these projects … they are far better prepared than I ever was when doing undergrad research" explains Filbin, noting that her undergraduates are also organically synthesizing 2-aminopyridine-3-carboxylic acid imidazolide and 2-methylnicotinic acid imidazolide probes in house for these RNA probing experiments. "These are fundamental techniques that allow for impactful research at a low cost", Filbin clarifies. The lab further mutates these viral RNAs before SHAPE and ties this into functional luciferase reporter assays to understand the CITE ability to direct translation initiation.

The Filbin Lab. Personal photo collection.

It's quite obvious that Megan Filbin places tremendous trust in her undergraduate team. Using COVID-19 as the prime example, Filbin explains that the next generation of scientists are embarking on an era of pandemics and highlights our need to comprehend how viral RNAs work. Thus, she desires to make RNA biology fun and interesting to students who are routinely left behind if not attending a top research institute. "I'm extremely passionate about teaching and creating opportunities for students who lack the resources in STEM". Filbin proudly expects her six undergraduates to be ready for the next level in academic research, "We have goals to publish and build more collaborations", she proclaims. With four institutional undergraduate mini-grants including two for underrepresented minorities, two institutional faculty grants, and a NIH R15 for a separate, but related protein synthesis project, the Filbin Lab continues to carve an undergraduate RNA niche into MSU Denver's community.

A LOOK BACK AT 2021

In the midst of many challenges and uncertainties, 2021 was also a year of recommencement. With the return to in-person events, we were once again able to gather with our fellow scientists to engage in stimulating talks and discussions.

This year, the Colorado RNA Club proudly hosted **9 Evenings with RNA**, with a total of **27 speakers**, between students and faculty. Together with the Project Bridge, we also held a successful **RNA Day celebration** on AUGust 1st and an **Undergraduate Outreach Event** on October 20th. We further published **4 editions of our Newsletter**, with the latest RNA research updates in our community and beyond.

We are immensely grateful to our amazing team of volunteers who made all this possible!
The Colorado RNA Club Latest organizing committee wishes everyone Happy Holidays and a Happy New Year!

See you in 2022!

By: Giovana Breda Veronezi, PhD student at CU Anschutz & Divya Kolakada, PhD candidate at CU Anschutz

For announcements of recent RNA-relevant publications, job openings, events or awards from your lab, e-mail us at ColoradoRNAClub@gmail.com

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