RNA Symposium Program Schedule

Friday, March 6, 2026

8:00-9:00 AM	**Registration & Continental Breakfast | Sponsor booths open** ABC Rooms & Atrium, BSRB
MORNING SESSION	
9:00–9:15 AM	**Welcome & Introduction** **Tommy Wang, Dean, University of Michigan Medical School** Kahn Auditorium, BSRB
9:15–10:00 AM	**Epigenetic pathways as targets in human disease** **Keynote Presentation 1: Shelley Berger, University of Pennsylvania** Introduction: Tommy Wang, Dean, University of Michigan Medical School Kahn Auditorium, BSRB
10:00–10:45 AM	**Invited Talks (Selected from Abstracts)** Introduction: Mat Ljungman, University of Michigan Kahn Auditorium, BSRB IT1 (10:00-10:15): "Single-Molecule Nanoscopic Fingerprinting of Individual Lipid Nanoparticles for Quality Control of mRNA Delivery," Pavel Banerjee, University of Michigan IT2 (10:15-10:30): "CRISPR-associated Cyclic Oligoadenylate Signaling in Sigma54-Regulated Transcription," David Bushhouse, Van Andel Institute IT3 (10:30-10:45): "Measuring the functional impact of 5'UTR variants in human disease," Srikar Gopinath, Yale University
10:45–11:15 AM	**Networking Break | Refreshments and Sponsor booths open** ABC Rooms & Atrium, BSRB
11:15 AM–12:00 PM	**Prime Assembly with Linear DNA Donors Enables Large Genomic Insertions** **Keynote Presentation 2: Erik Sontheimer, UMass Chan Medical School** Introduction: Yan Zhang, University of Michigan Kahn Auditorium, BSRB
12:00–1:00 PM	**Lunch & Networking | Sponsor booths open** ABC Rooms & Atrium, BSRB
AFTERNOON SESSION	
1:00–1:45 PM	**Invited Talks (Selected from Abstracts)** Introduction: Jay Brito Querido & Chase Weidmann, University of Michigan Kahn Auditorium, BSRB IT4 (1:00-1:15): "Regulation of RNA condensation by ribosomes," Stephanie Moon, University of Michigan IT5 (1:15-1:30): "Ku limits aberrant mRNA splicing promoted by intronic antisense Alu elements," Jens Schmidt, Michigan State University IT6 (1:30-10:45): "Whole-blood RNA-seq signatures support prediction of ALS case status, survival, and therapeutic prioritization," Yue Zhao, University of Michigan

Friday, March 6, 2026

1:45–2:45 PM	**Invited Talks (Selected from Abstracts)** Jay Brito Querido & Chase Weidmann, University of Michigan Kahn Auditorium, BSRB IT7 (1:45-2:00): “Validation of your RNA studies with multi-omic methods,” Jared Shelerud, illumina presentation IT8 (2:00-2:15): "RBM15-MKL1 fusion protein promotes leukemia via m6A methylation and Wnt pathway activation," Madeline Mayday, Yale University IT9 (2:15-2:30): "microRNA-181a-driven Regulation of Immune Responses in High Grade Ovarian Cancer," Sreeja Sekhar, University of Michigan IT10 (2:30-2:45): NUCLEIC ACID THERAPEUTICS (NATx) CORE at the University of Michigan, Michelle Hastings/Katelyn Lacy, University of Michigan
2:45–3:15 PM	**Networking Break | Refreshments and Sponsor booths open** ABC Rooms & Atrium, BSRB
3:15–4:00 PM	**A splice-switching antisense oligonucleotide approach for pediatric epilepsies** **Keynote Presentation 3: Madeleine Oudin, Tufts University** Introduction: Michelle Hastings, University of Michigan Kahn Auditorium, BSRB
4:00–5:00 PM	**Patient Advocacy Panel** **Victoria Gray, Patient Advocate** **Madeleine Oudin, Tufts University** **Sean Doerr, MI Rare Disease Advisory Council, Patient Advocate** **Grace Meyers, Patient and Family Advisor, Michigan Medicine** Moderators: Beth Ames, Michelle Hastings, University of Michigan Kahn Auditorium, BSRB
5:15–8:00 PM	**Networking Dinner** Great Lakes Room, Palmer Commons

Saturday, March 7, 2026

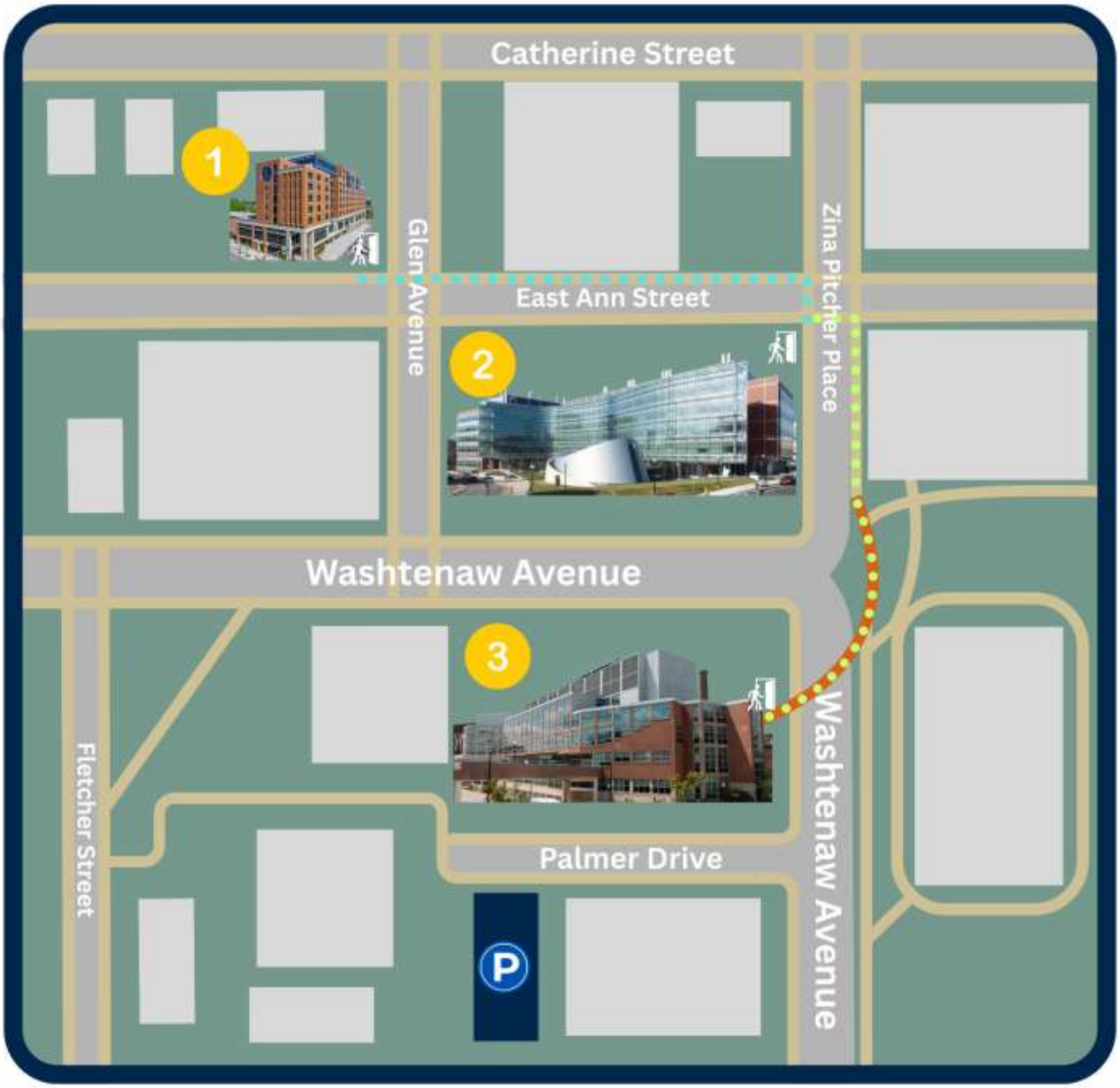
8:00–9:00 AM	**Registration & Continental Breakfast** Sponsor booths open ABC Rooms & Atrium, BSRB
MORNING SESSION	
9:00–9:15 AM	**Welcome & Introduction** **Kelly Sexton, U-M Innovation Partnerships** Kahn Auditorium, BSRB
9:15–10:00 AM	**Slip stick folding of CAG repeat drives aggregation of expanded HTT RNA** **Keynote Presentation 4: Sarah Woodson, Johns Hopkins University** Introduction: Kelly Sexton, U-M Innovation Partnerships Kahn Auditorium, BSRB
10:00–10:45 AM	**Invited Talks (Selected from Abstracts)** Introduction: Lydia Freddolino, University of Michigan Kahn Auditorium, BSRB IT11 (10:00-10:15): "Cell cycle-informed studies of hnRNPC reveal Transcriptome Vulnerabilities of rapidly dividing cells," Daniel Benhalevy, Tel Aviv University IT12 (10:15-10:30): "Using Plasma Cell-Free RNA for Tumor-Naïve Detection and Monitoring," James George, University of Michigan IT13 (10:30-10:45): "An element in the CFTR 5' UTR represses translation," Ben Pockrass, University of Michigan
10:45–11:15 AM	**Networking Break | Refreshments and Sponsor booths open** ABC Rooms & Atrium, BSRB
11:15 AM–12:00 PM	**Co-transcriptional RNA processing yields unexpected versatility in gene regulation** **Keynote Presentation 5: Karla Neugebauer, Yale School of Medicine** Introduction: Rachel Niederer, University of Michigan Kahn Auditorium, BSRB
12:00–1:00 PM	**Lunch & Networking | Sponsor booths open** ABC Rooms & Atrium, BSRB
AFTERNOON SESSION	
1:00–1:45 PM	**Invited Talks (Selected from Abstracts)** Introduction: Jay Brito Querido & Chase Weidmann, University of Michigan Kahn Auditorium, BSRB IT14 (1:00-1:15): "Structural basis for repeat-associated non-AUG translation initiation on C9orf72 mRNA," Ross Kaufhold, University of Michigan IT15 (1:15-1:30): "Programmed ribosomal frameshifting during PLEKHM2 mRNA decoding generates a constitutively active proteoform that supports myocardial function," Yousuf Khan, Stanford University IT16 (1:30-1:45): "Inhibition of HPV16 E6 splicing with antisense oligonucleotides attenuates aggressive invasion in oropharyngeal cancer," Yvonne Xinyi Lim, University of Michigan

Saturday, March 7, 2026

1:45–2:30 PM	**Life in Flux: Dynamic RNA: Protein Complex Assembly Shapes Biomolecular Function** **Keynote Presentation 6: Nils Walter** Introduction: Sarah Kargbo-Hill, University of Michigan Kahn Auditorium, BSRB
2:30–3:00 PM	**Networking Break | Refreshments and Sponsor booths open** ABC Rooms & Atrium, BSRB
3:00–4:00 PM	**Invited Talks (Selected from Abstracts)** Introduction: Jay Brito Querido & Chase Weidmann, University of Michigan Kahn Auditorium, BSRB IT17 (3:00-3:15): "The specific interactions between selenocysteine-decoding tRNA and HIV-1 Gag regulate progeny infectious particle assembly," Rajat Mudgal, University of Michigan IT18 (3:15-3:30): "Timing Transcript Usage Through Regulated Intron Retention in Heart Maturation and Disease," Subhashis Natua, University of Illinois, Urbana-Champaign IT19 (3:30-3:45): "Unlocking RNA Chemical Reactivity through Mass Spectrometry Screens of Electrophiles," Adam Wier, University of Notre Dame IT20 (3:45-4:00): "Alternative Splicing as a Mechanism of Oligodendrocyte Impairments in Spinocerebellar Ataxia Type 3," Jacen Emerson, University of Michigan
4:15–5:00 PM	**Speaker Panel Discussion** **Shelley Berger, University of Pennsylvania** **Karla Neugebauer, Yale University** **Erik Sontheimer, UMass Chan School of Medicine** **Nils Walter, University of Michigan** **Sarah Woodson, Johns Hopkins University** Moderator: Mats Ljungman, University of Michigan Kahn Auditorium, BSRB
5:15–8:00 PM	**Reception & Poster Session** Food & Refreshments Provided ***Sponsored by Our Corporate Partners Listed Below*** 5:15–6:30 – Poster Session 1 6:30–7:45 – Poster Session 2 Great Lakes Ballroom, Palmer Commons

Thank You To Our Sponsors!

2026 RNA Symposium Wayfinding Map



1 Vanguard Hotel

2 Biomedical Science Research Building (BSRB)

3 Palmer Commons

Main Entrances

P **Limited** Pay-to-Park Public Structure

Sidewalk/Footpath

Pedestrian Bridge

Guest Route: Vanguard Hotel to BSRB (1st Floor)

Guest Route: BSRB to Palmer Commons (4th Floor)

Keynote Presentations

Keynote Presentation 1

Shelley Berger, Ph.D., University of Pennsylvania

Title:
“Epigenetic pathways as targets in human disease”

Abstract:
Chromatin regulatory proteins are frequently mutated or overexpressed in human disease. Because they are enzymes, chromatin proteins are outstanding targets for drug development. Our work focuses on elucidation of epigenetic pathways that might be disease drivers, and epigenetic pathways that might augment clinical treatment.

Our work in cancer focuses on epigenetic pathways utilized by the tumor suppressor p53 and oncogene HIF2alpha; we also study epigenetics of cancer immunotherapy pathways of active and exhausted T cells. In addition, we investigate epigenetics of aging and in regulation of mouse and human memory and diseases of memory, including human Alzheimer’s disease. Aspects of these epigenetic regulatory pathways will be discussed.

Keynote Presentation 2

Erik Sontheimer, Ph.D., UMass Chan Medical School

Title:
“Prime Assembly with Linear DNA Donors Enables Large Genomic Insertions”

Abstract:
Many diseases are caused by diverse mutations across various loci, necessitating precise therapeutic strategies. However, current genome editing approaches struggle to insert or replace large DNA fragments. This has made it difficult to develop editing approaches that can address most or all mutations among a diverse patient population. As a result, separate editing strategies must often be pursued allele by allele, which is slow, arduous, and expensive.

Targeted insertion of large DNA fragments holds great promise for overcoming these limitations of CRISPR engineering. Strategies employing recombinases or integrases increase the already substantial complexity and delivery challenges associated with prime editing (PE). We have developed Prime Assembly (PA) for the insertion of large DNAs whose ends overlap with the 3’ flaps generated by PE. We used PA to insert DNA fragments up to 11 kilobase pairs. Either single-stranded or double-stranded DNA donors can support PA. PA relies on DNA templates that are easily produced, and it does not require co-delivery of exogenous DNA-dependent DNA polymerases, recombinases, or integrases. PA proceeds in non-cycling cells, suggesting independence from homology-directed repair (HDR) pathways. In summary, PA can initiate “Gibson-like” assembly in mammalian cells to generate gene insertions without dsDNA breaks, recombinases, or HDR.

Keynote Presentation 3

Madeleine Oudin, Ph.D., Tufts University

Title:
“A splice-switching antisense oligonucleotide approach for pediatric epilepsies”

Abstract:
Variants in ion channel genes are common causes of pediatric epilepsy, often leading to intractable seizures, developmental delay and other comorbidities, which increases risk of death. Pathogenic variants in the SCN8A gene, which encodes a voltage-gated sodium channel critical for action potential generation in the brain, account for ~1% of genetic epilepsies. The voltage sensor in SCN8A domain 1 is encoded by one of two developmentally-regulated mutually exclusive alternative exons, 5N and 5A. We observe that variants in these exons are more likely to cause infantile spasms, a severe seizure type, than variants elsewhere in SCN8A, and that some pathogenic variants affect exon 5 splicing, impacting patient phenotype. Molecular and evolutionary analyses implicate the exon sequences of these and other voltage-gated ion channel alternative exons in splicing regulation. We identified antisense oligonucleotides (ASOs) that shift splicing of SCN8A exon 5N to 5A or vice versa. These ASOs normalize neuronal activity in patient-derived iPSC neurons, and reduce seizures and motor impairment and extend lifespan in a new exon 5N mutant mouse model. Our results demonstrate that splice-switching ASOs can effectively reduce the expression of pathogenic isoforms and rescue both seizure and non-seizure phenotypes. Similar approaches should be applicable to pediatric genetic epilepsies caused by mutations in other ion channel alternative exons.

Keynote Presentation 4

Sarah Woodson, Ph.D., Johns Hopkins University

Title:
“Slip stick folding of CAG repeat drives aggregation of expanded HTT RNA”

Abstract:
In Huntington’s Disease (HD) and related disorders, expansion of CAG trinucleotide repeats produces a toxic gain of function in affected neurons. Expanded huntingtin (expHTT) mRNA forms aggregates that sequester essential RNA binding proteins, dysregulating mRNA processing and translation. The physical basis of RNA aggregation has been difficult to disentangle owing to the heterogeneous structure of the CAG repeats. Here, we probe the folding and unfolding pathways of expHTT mRNA using single-molecule force spectroscopy. Whereas normal HTT mRNAs unfold reversibly and cooperatively, expHTT mRNAs with 20 or 40 CAG repeats slip and unravel non-cooperatively at low tension. Slippage of CAG base pairs is punctuated by concerted rearrangement of adjacent CCG trinucleotides, trapping partially folded structures that readily base pair with another RNA strand. We suggest that the conformational entropy of the CAG repeats, combined with stable CCG base pairs, creates a stick-slip behavior that explains the aggregation propensity of expHTT mRNA.

Keynote Presentation 5

Karla Neugebauer, Ph.D., Yale University

Title:
“Co-transcriptional RNA processing yields unexpected versatility in gene regulation”

Abstract:
Many steps in RNA biogenesis occur during synthesis by RNA polymerases. Co-transcriptional activities are commonplace in prokaryotes, where the lack of membrane barriers allows mixing of all gene expression steps. In the past decade, an extraordinary level of coordination between transcription and RNA processing has emerged in eukaryotes. I will discuss our recent work, highlighting the development of RNA sequencing strategies that detect transient transcription and RNA processing intermediates that have led to discoveries of how constitutive and alternative splicing, 3′ end cleavage, and dynamic RNA folding are coordinated during transcription. For example, long read sequencing reveals transcript-specific behavior whereby intron retention across a nascent transcript suppresses 3′ end cleavage, resulting in readthrough transcription. A hallmark of eukaryotic cellular stress responses, I will show that cancer therapy triggers transcriptional readthrough and the production of RNA chimeras comprised of exons from two adjacent genes. These and other examples show that co-transcriptional RNA processing events can impact gene output as dramatically as DNA-based mechanisms, such as transcription factor control.

Keynote Presentation 6

Nils Walter, Ph.D., University of Michigan

Title:
“Life in Flux: Dynamic RNA:Protein Complex Assembly Shapes Biomolecular Function“

Abstract:
The explosion of cryo-EM structures in recent years has underscored the stepwise assembly of stable biomolecular machines with defined, fixed compositions. In contrast, advances in single-molecule imaging—both in vitro and in live cells—are revealing a very different picture: Many biological complexes are not static but highly dynamic and transient. Instead of persisting as stable entities, their functions emerge from short-lived, fluid assemblies, whose lifetimes and outputs are governed by the kinetics of their components. This chemistry-driven paradigm shift—from rigid machines to kinetically controlled assemblies—offers a powerful framework for understanding gene regulation, proofreading, checkpoint control, and cellular adaptability. This talk will illustrate this evolving view with two case studies: the kinetically programmed exchange behavior of the RNA silencing machinery, and the dynamic assembly of phase-separated RNA-protein structures (RNP granules) in mammalian cells. I will also explore broader implications of this model, including how regulatory signals can fine-tune molecular function by modulating kinetic parameters, rather than altering structure or affinity per se. By reframing molecular cell biology through the lens of kinetic control and spatiotemporal organization, this presentation aims to offer a unifying conceptual foundation across diverse areas of biomolecular science.

Invited Short Talks

IT1

Pavel Banerjee, University of Michigan

Title:
"Single-Molecule Nanoscopic Fingerprinting of Individual Lipid Nanoparticles for Quality Control of mRNA Delivery"

Abstract:
Lipid nanoparticles (LNPs) have emerged as promising delivery vehicles for RNA therapeutics, but assumptions of uniformity in particle population and mRNA loading often obscure critical heterogeneity that can impact therapeutic efficacy. In this study, we present a single-molecule nanoscopy platform that enables high-resolution analysis of individual LNPs in terms of size, morphology, and encapsulated mRNA content. Using super-resolution imaging with Nile red, we confirm that production-line LNPs appear as spherical particles with a narrow size distribution around 90 nm. By optimizing permeabilization with Triton X-100 and employing specific fluorescent probes, we achieve direct access to and quantification of internalized mRNA while preserving nanoparticle integrity. Quantitative single-particle imaging with Ribogreen dye and sequence-specific fluorescent probes, combined with Total Internal Reflection Fluorescence Microscopy (TIRFM), reveals a broad distribution of mRNA copies per LNP, with a typical peak at about two molecules per particle and a notable fraction of sparsely loaded or empty nanoparticlesÃ¢â‚¬â€• variability hidden by bulk methods. Dynamic probe binding further enables sensitive detection and accurate identification of internalized mRNA cargo. This approach establishes a direct correlation between LNP structural features and mRNA payload at the single-particle level, providing foundational insights into how variations in lipid components influence payload distribution. Understanding these heterogeneities is crucial for optimizing biodistribution, mRNA expression kinetics, and ensuring batch-to-batch consistency. Our methodology delivers a robust quality control framework, paving the way for safer, more effective nucleic acid therapeutics.

IT2

David Bushhouse, Van Andel Institute

Title:
"CRISPR-associated Cyclic Oligoadenylate Signaling in Sigma54-Regulated Transcription"

Abstract:
In contrast to the rapid advances in our understanding of bacterial immunity, the regulatory mechanisms governing the expression of immune machineries remain poorly understood. Bacterial enhancer binding proteins (bEBPs) are a diverse class of transcription factors that activate sigma54-dependent transcription from target promoters in response to cellular changes. RtcR proteins are members of bEBPs that are frequently observed in bacterial anti-phage defense islands, but no biochemical connection has been uncovered between RtcR and anti-phage defense regulation. Using biochemical, structural, and functional in vivo characterization, we discover a CRISPR-associated RtcR homolog that triggers sigma54-regulated transcription in response to cyclic oligoadenylate signaling generated by the CRISPR-Cas system upon invader RNA binding, providing a positive feedback loop on protection against phage attack. CryoEM analysis reveals the structural mechanism of how unliganded-state inhibits ATP hydrolysis and sigma54 binding, the first example for this family of bEBPs. These findings shed light on the allosteric activation of RtcR, and link second-messenger defense signaling with transcriptional activation, opening up new pathways for understanding the transcriptional regulation of bacterial anti-phage defense.

IT3

Srikar Gopinath, Yale University

Title:
"Measuring the functional impact of 5'UTR variants in human disease"

Abstract:
5' Untranslated Regions (UTRs) harbor diverse regulatory elements that strongly influence protein output. Variations in these regions can profoundly affect protein levels, impacting human disease. Despite nearly 95% of disease-associated mutations occurring in non-coding regions, including 5' and 3' UTRs, research has traditionally focused on coding sequence variation, largely due to limited methods for assessing non-coding effects. To address this gap, we adapted a novel massively parallel reporter assay, Nascent Peptide-Translating Ribosome Affinity Purification (NaP-TRAP), to quantify the translational consequence of over one million 5Ã¢â‚¬â„¢UTR variants across ~17,000 genes from UK Biobank and gnomAD. NaP-TRAP is an immunocapture-based method that measures protein output by isolating mRNAs associated with actively translating ribosomes. Using this framework, we uncover widespread and previously uncharacterized effects of variants that alter features such as G-rich motifs, RNA-binding protein motifs, and novel 5'UTR structures, extending beyond canonical regulatory elements including upstream open reading frames and Kozak consensus sequences. We further demonstrate that variants causing strong translation disruptions are under negative selection, underscoring their disease relevance. Importantly, high-impact 5Ã¢â‚¬â„¢UTR variants in cancer driver genes overlap with somatic mutations in tumors catalogued in COSMIC and TCGA and are associated with poor survival outcomes, highlighting a critical role in cancer biology. Finally, leveraging these findings we developed a model to predict the effects of 5'UTR variants on translation. Together, our work identifies novel 5Ã¢â‚¬â„¢UTR regulatory regimes, systematically maps variant effects in several disease-relevant genes, and highlights the importance of considering non-coding regions in the molecular interpretation of human disease.

IT4

Stephanie Moon, University of Michigan

Title:
"Regulation of RNA condensation by ribosomes,"

Abstract:
During the integrated stress response (ISR), translation initiation is suppressed and ribosomes run off mRNAs, driving their condensation into stress granules. Stress-induced genes are preferentially translated via upstream open reading frames (uORFs) to promote cell adaptation to stress. We assessed if ribosome association with stress-induced gene mRNAs inhibits their localization to stress granules using smFISH, chemical genetics, polysome profiling, molecular cloning, and live cell imaging. We observe that inhibiting ribosome association with stress-induced gene mRNAs increases their localization to stress granules. Stabilizing ribosomes on mRNAs inhibits their localization to stress granules. Monosomes and polysomes equally inhibit mRNA condensation, and stabilizing a monosome on all mRNAs inhibits stress granule assembly. Analysis of wild-type and uORF-deficient stress-induced gene mRNA reporters reveals uORFs inhibit mRNA localization to stress granules via ribosome association. We extended these findings to tRNA synthetase inhibitors and amino acid deprivation, which also activate the ISR but inhibit translation elongation. These stressors fail to induce stress granules despite robust ISR activation. Disome and polysome profiling reveals tRNA synthetase inhibition causes ribosome collisions that contribute to ISR activation and are cleared within several hours. However, uncollided ribosomes persist on mRNAs within polysomes for at least 16 hours. These persistent stalled ribosomes activate the ribotoxic stress response and inhibit RNA-protein granule assembly. Our results demonstrate that ribosomes are central regulatory nodes connecting the integrated and ribotoxic stress responses to mRNA compartmentalization during stress.

IT5

Jens Schmidt, Michigan State University

Title:
"Ku limits aberrant mRNA splicing promoted by intronic antisense Alu elements"

Abstract:
Alu elements are short repeats that occupy approximately 10% of the human genome. Saturation of primate genomes with Alu sequences occurred at the prosimian/new-world monkey evolutionary juncture. Alu elements have clearly driven unique aspects of higher primate evolution, but their presence can be detrimental to genomic stability. The expansion of Alu sequences in the genomes of higher primates precisely coincides with a substantial increase in the ubiquitous expression of the three polypeptides of the DNA-dependent protein kinase (DNA-PK), the Ku70/80 heterodimer and DNA-PKcs. Previous work suggests that the elevated levels of Ku70/80 are required to prevent the activation of innate immune signaling pathways triggered by RNA molecules derived from Alu elements. Here we demonstrate that Ku ablation dramatically alters mRNA splicing, by allowing the use of alternative splice sites contained in intronic antisense Alu elements, which are known to directly associate with Ku70/80. Dysregulation of mRNA splicing precedes cell death and preferentially impacts genes involved in essential RNA metabolism processes, including splicing and ribosome biogenesis, likely impacting cell viability. In addition, we demonstrate that cell death after Ku70 depletion cannot be rescued by expression of its prosimian homologue, which suggests that primate Ku70 has evolved specific molecular features to suppress deleterious effects of an Alu element rich genome. We propose a model in which Ku binding of antisense Alu elements in introns of nascent RNAs modulates the use of alternative splice sites to balance beneficial and detrimental contributions of Alu repeats within primate genomes.

IT6

Yue Zhao, University of Michigan

Title:
"Whole-blood RNA-seq signatures support prediction of ALS case status, survival, and therapeutic prioritization"

Abstract:
The lack of disease-specific blood biomarkers for amyotrophic lateral sclerosis (ALS) contributes to diagnostic delays and limited prognostic precision. To address this gap, we performed whole-blood RNA sequencing in a large cohort of individuals with ALS (n=422) and controls (n=272). This dataset captured broad transcriptome features and enabled the development of RNA-based classifiers for ALS diagnosis and survival prediction. Using machine-learning, we trained gene expression models that distinguished ALS from controls with high accuracy and validated their performance in a fully independent external cohort (best AUC=0.894). We also integrated transcriptomic features with clinical variables to develop a survival prediction model that stratified ALS participants into short, intermediate, and long survival groups, improving separation in the external cohort compared to models based on clinical variables alone. Beyond predictions, differential expression and pathway analyses revealed ALS-relevant pathways in blood. We then defined a set of Ã¢â‚¬Å“core genesÃ¢â‚¬Â• shared between blood-based signatures and disease-relevant tissues/models, including iPSC-derived neurons with TDP-43 knockdown and postmortem ALS spinal cord. Using these core genes, connectivity mapÃ¢â‚¬â€œbased drug perturbation analyses prioritized candidate compounds predicted to reverse the ALS transcriptional signature. Collectively, this work demonstrates the feasibility of using whole-blood RNA-sequencing to derive reproducible transcriptomic signatures associated with ALS diagnosis and survival, and provide a framework for therapeutic nomination from RNA-defined ALS disease biology

IT7

Jared Shelerud, illumina

Title:
“Validation of your RNA studies with multi-omic methods”

Abstract:
This presentation outlines Illumina's evolving multi-omics strategy, with a focus on validating RNA studies through integrated spatial transcriptomics and NGS-based proteomics. It highlights how combining genomic, epigenomic, transcriptomic, and proteomic data enables deeper, decision-critical biological insights across discovery research applications.

IT8

Madeline Mayday, Yale University

Title:
"RBM15-MKL1 fusion protein promotes leukemia via m6A methylation and Wnt pathway activation"

Abstract:
The recurrent t(1;22) translocation in acute megakaryoblastic leukemia (AMKL) encodes the RBM15-MKL1 fusion protein. Dysregulation of N6-methyladenosine (m6A) modification affects RNA fate and is linked to oncogenesis. Because RBM15 is critical for bringing the m6A writer complex to specific RNAs, we hypothesized that RM disrupts m6A modification, thereby altering RNA fate to drive leukemogenesis in RM-AMKL. Using a multi-omic approach, we show for the first time, to our knowledge, that RM retains the RNA-binding and m6A-modifying functions of RBM15 while also selectively regulating distinct mRNA targets including Frizzled genes in the Wnt signaling pathway. Treating murine RM-AMKL cells with the methyltransferase 3 (METTL3) inhibitor STM3675, which decreases m6A deposition, induced apoptosis in vitro and prolonged survival in transplanted mice. Frizzled genes were upregulated by RM and downregulated upon METTL3 inhibition, implicating an m6A-dependent mechanism for their dysregulation. Direct Frizzled knockdown reduced RM-AMKL growth in vitro and in vivo, highlighting Wnt signaling as a key oncogenic driver. Elevated Wnt pathway and Frizzled expression in multiple forms of human AMKL underscores the relevance of our findings. Together, our results establish RM-specific m6A modifications and Wnt pathway activation as critical drivers of RM-AMKL, thereby identifying these pathways as potential therapeutic targets.

IT9

Sreeja Sekhar, University of Michigan

Title:
"microRNA-181a-driven Regulation of Immune Responses in High Grade Ovarian Cancer"

Abstract:
Targeting MicroRNAs has become a potential way to treat cancer. Among these, miR-181a has attracted attention for its critical role in driving tumor genesis, development, and metastasis across multiple cancer models. Elevated miR-181a activity in various cancers underscore its central role in cancer initiation, maintenance, dissemination, and recurrence. By regulating critical signaling pathways such as TGFÃŽÂ², Wnt, and innate immune signaling, miR-181a fosters tumor growth and immune evasion, positioning it as a prime therapeutic target. Our work uncovers substantial therapeutic benefits of targeting miR-181a in high-grade serous ovarian cancer (HGSC), the most lethal subtype of ovarian cancer. We demonstrate that inhibiting miR-181a reactivates the STING mediated interferon (IFN) signaling pathway, resulting in robust immune cell activation and significant tumor suppression in vivo. Furthermore, miR-181a lose increased immune cell infiltration and activation at the tumor site which was further accentuated with the addition of the STING agonist, MSA. Specifically, miR181a knockdown with STING activation enhanced the CD8+/CD69 population which displayed an increase in secreted cytokines like granzyme B, IFNG and TNF which are the prime determinants in predicting anti-tumor response. Additionally, studies using athymic mice indicated no significant tumor growth inhibition irrespective of STING activation, further substantiates miR181a knockdown provides the priming of a T-cells dominant immune reactive environment. Thus, these findings not only advance our understanding of the challenges associated with STING agonist-based therapies but also position miR-181a as a critical therapeutic lever for reprogramming the tumor microenvironment.

IT10

Michelle Hastings/Katelyn Lacy, University of Michigan

Title:
NUCLEIC ACID THERAPEUTICS (NATx) CORE at the University of Michigan

Abstract:
The rapid expansion of RNA and other nucleic acid–based tools in both research and therapeutics has created an urgent need for accessible, affordable, high-quality design, synthesis, and functional validation services, needs that can be best-served within academic research environments. To address this need, we have established the Nucleic Acid Therapeutics (NATx) Core, a centralized, service-oriented facility at the University of Michigan dedicated to accelerating basic, translational, and preclinical nucleic acid research. NATx Core aims to lower barriers to entry for nucleic acid–based technologies by minimizing time, cost, and technical overhead associated with procuring and validating synthetic nucleic acids. The Core provides end-to-end support, including strategy development, target identification, oligonucleotide design (incorporating AI-assisted modeling), high-quality synthesis with a broad catalog of innovative chemical modifications, and functional screening to ensure delivery of active, ready-to-use molecules. The Core operates primarily as a programmatic resource, integrating scientific expertise across RNA chemistry, biology, and development to support diverse applications ranging from mechanistic studies to therapeutic proof-of-concept. We will also support oligonucleotide synthesis under a “fee-for-service” model for users. Initial operations are launching in early 2026 in our labs located in the Life Sciences Institute, supported by major institutional investment and serving a rapidly growing internal and external user base spanning neurological, oncologic, and rare disease programs. By centralizing expertise and infrastructure, the NATx Core aims to catalyze innovation, enhance reproducibility, and accelerate the translation of nucleic acid–based discoveries into impactful therapeutics.

IT11

Daniel Benhalevy, Tel Aviv University

Title:
"Cell cycle-informed studies of hnRNPC reveal Transcriptome Vulnerabilities of rapidly dividing cells"

Abstract:
Altered RNA processing has recently emerged as a hallmark of cancer and a promising avenue for therapeutic intervention. Among the key players are RNA-binding proteins (RBPs), whose dysregulation contributes to oncogenesis, progression, and therapy resistance. In this talk, I will present our unpublished findings shedding light on the transcriptomic burden faced particularly by rapidly dividing cells. Using hnRNPC as a model RBP, I will demonstrate the unexpected and significant shifts that RBPs can undergo during cell cycle progression. These changes expose context-dependent vulnerabilities that may be relevant for selective therapeutic targeting. I will discuss how cell cycle-dependent changes in RBP properties can be identified and leveraged as a novel specificity mechanism for targeting RBPs in proliferative cancer cells. This paradigm opens the door to a broader strategy for designing cell cycleÃ¢â‚¬â€œinformed, RBP-targeted research and therapies.

IT12

James George, University of Michigan

Title:
"Using Plasma Cell-Free RNA for Tumor-Naïve Detection and Monitoring"

Abstract:
Current liquid biopsies that are based around circulating tumor DNA (ctDNA) often face sensitivity ceilings of 30-50% in early-stage or minimal residual disease detection and cannot reliably infer tumor cell-of-origin. While plasma cell-free RNA (cfRNA) offers superior tissue-specificity, hematopoietic background noise obscures tumor signals. We hypothesized that targeted capture of tumor-specific transcripts would resolve this, enabling precise, tumor-naÃƒÂ¯ve detection. To achieve this we analyzed bulk RNA-seq data from 59,507 tissue and 23,540 blood samples, and identified genes exhibiting "High-in-Tumor, Low-in-Blood" (HITLIB) expression pattern across the cancer spectrum. We engineered a custom hybrid-capture panel targeting the top ~3,000 HITLIB genes. When coupled with our optimized wet lab protocol, the approach achieved 64XÃ¢â‚¬â€œ128X enrichment of tumor-derived cfRNAs relative to standard whole transcriptome sequencing. In a proof-of-concept study of rare or refractory malignancies, our panel detected pathognomonic cfRNAs absent in benign controls, including steroidogenic enzymes in adrenal cancer, skeletal muscle transcripts in rhabdomyosarcoma, peptide hormones in neuroendocrine prostate cancer, intestinal epithelial keratins in small bowel adenocarcinoma, and mucins in pancreatobiliary cancer. Crucially, unsupervised clustering of our cohort revealed distinct, histology-specific separation. We are currently leveraging these high-dimensional signatures to train AI/ML classifiers for automated disease status and cell-of-origin prediction. This targeted cfRNA platform represents a scalable pan-cancer strategy poised to complement or advance liquid biopsy standards.

IT13

Ben Pockrass, University of Michigan

Title:
"An element in the CFTR 5' UTR represses translation”

Abstract:
The 5' untranslated region (5' UTR) of mRNA contains secondary structures, RNA binding protein sites, and other features which directly regulate translation initiation and overall protein output. These regulatory features can potentially be targeted to modulate translation of therapeutically relevant genes, but the 5' UTR regulatory landscape for individual genes is largely unknown. One such gene is cystic fibrosis transmembrane conductance regulator (CFTR), whose dysfunction causes cystic fibrosis (CF). Although current small molecule therapies improve folding and function of mutant CFTR, they only act on already-synthesized CFTR protein, which is typically lower in CF patients than unaffected individuals. We hypothesize that systematic characterization of the CFTR 5' UTR will reveal regulatory elements that can be targeted to increase overall CFTR expression. To identify 5' UTR regulatory elements in CFTR, we used Direct Analysis of Ribosome Targeting (DART) to measure ribosome recruitment to a pool of systematically mutated CFTR 5' UTRs. This revealed a region of the CFTR 5' UTR that increases ribosome recruitment when mutated compared to the wild type (WT), which we call a putative repressive element (PRE). The PRE not only represses ribosome recruitment but also represses translation of a luciferase reporter both in vitro and in cellulo, consistent with it acting as a repressor. This is not due to differences in mRNA stability, and we are currently performing proteomics and structure probing to explore potential regulatory mechanisms. Our data demonstrate that DART can successfully identify 5' UTR elements that regulate translation and which represent promising therapeutic targets.

IT14

Ross Kaufhold, University of Michigan

Title:
"Structural basis for repeat-associated non-AUG translation initiation on C9orf72 mRNA"

Abstract:
A GGGGCC (G4C2) hexanucleotide repeat expansion in the first intron of the C9orf72 (chromosome 9 open reading frame 72) gene is the most common monogenic cause of both Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). This repeat serves as a template for repeat-associated non-AUG (RAN) translation, a non-canonical form of translation initiation which generates neurotoxic dipeptide repeat (DPR) proteins that aggregate in patient brains and contribute to neurodegeneration in model systems. How G4C2 repeats interact with the ribosomal complex to promote this pathological mechanism of initiation has remained unclear. To address this fundamental question, we used single-particle cryo-electron microscopy (cryo-EM) to determine the structure of a reconstituted human late-stage C9orf72 48S translation initiation complex after start codon selection at 3.2 Ãƒâ€¦ resolution. The structure reveals that RAN translation on C9orf72 mRNA initiates at a near-cognate CUG codon that is located 24 nucleotides upstream of the G4C2 repeat and is flanked by a strong endogenous Kozak sequence. Mutation of either the CUG or the Kozak sequence surrounding it dramatically reduces RAN translation in plated neurons. The structure further reveals a direct interaction between the G4C2 repeat and 18S ribosomal RNA expansion segment 9 (ES9S) on the 40S small ribosomal subunit through a kissing-loop interaction. Preventing this RNA-RNA interaction using complementary antisense oligonucleotides against ES9S markedly reduces RAN translation, indicating that this structural interaction is functionally important. These findings provide mechanistic insights into RAN translation initiation and highlight a potential therapeutic approach to mitigate toxic DPR production in C9orf72-associated neurodegenerative disease.

IT15

Yousuf Khan, Stanford University

Title:
"Programmed ribosomal frameshifting during PLEKHM2 mRNA decoding generates a constitutively active proteoform that supports myocardial function"

Abstract:
Programmed ribosomal frameshifting is a process where a proportion of ribosomes change their reading frame on an mRNA. While frameshifting is commonly used by viruses, very few phylogenetically conserved examples are known in nuclear encoded genes. Here, we report a +1 frameshifting event during decoding of the human gene PLEKHM2 that provides access to a second internally overlapping ORF. The new carboxyl-terminal domain of this frameshift protein forms an ÃŽÂ± helix, which relieves PLEKHM2 from autoinhibition and allows it to move to the tips of cells without activation by ARL8. Reintroducing both the canonically translated and frameshifted protein are necessary to restore normal contractile function of PLEKHM2 knockout cardiomyocytes, demonstrating the necessity of frameshifting for normal cardiac activity.

IT16

Yvonne Xinyi Lim, University of Michigan

Title:
"Inhibition of HPV16 E6 splicing with antisense oligonucleotides attenuates aggressive invasion in oropharyngeal cancer"

Abstract:
The incidence of HPV16-positive oropharyngeal cancer (HPV+ OPC) is rising dramatically and has emerged as the leading HPV-related cancer in the United States, United Kingdom and other developed countries. Although most patients respond well to standard chemoradiation, a significant proportion develop disease recurrence or progression. Biomarkers are needed to distinguish patients at risk of recurrence to personalize treatment. Using bioinformatics approaches in multiple patient cohorts, we discovered that increased splicing of full-length E6 (E6FL) to its shorter isoform, E6*I, correlates with poorer overall and recurrence-free survival. Overexpression of E6*I enhances invasion in vitro and in vivo in HPV+ OPC cells relative to E6FL. These findings suggest that splicing of E6FL to E6*I induces aggressive tumors. To inhibit the splicing of E6FL into E6*I, we designed seven antisense oligonucleotides (ASOs) targeting the splice regulatory regions of E6FL pre-mRNA. ASOs that inhibited splicing of E6FL into E6*I (high E6FL:E6*I) reduced migration in a wound healing assay. In vivo, tumors with high E6FL:E6*I (reduced splicing) generated significantly more tumor islands that invaded less distance compared to low E6FL:E6*I (higher splicing). Invasive islands generated from HPV+ OPC with high E6FL:E6*I had lower membrane:cell E-cadherin compared to OPC with low E6FL:E6*I, consistent with a partial epithelial-mesenchymal transition phenotype. Together, our findings suggest that splicing of E6FL to E6*I drives invasive phenotypes in OPC. E6FL:E6*I may be a promising biomarker to identify aggressive HPV+ OPC. Blocking E6 splicing with ASOs suppressed aggressive phenotypes suggesting the possibility that ASOs have therapeutic potential in HPV+ OPC.

IT17

Rajat Mudgal, University of Michigan

Title:
"The specific interactions between selenocysteine-decoding tRNA and HIV-1 Gag regulate progeny infectious particle assembly"

Abstract:
Beyond their canonical role in translation, tRNAs participate in various stages of replication cycle of viruses including HIV-1. In addition to the well-known role of tRNALys as primer for HIV-1 reverse transcription, accumulating evidence indicates that tRNAs likely regulate binding of the viral structural protein Gag to the plasma membrane, the initial and essential step of viral assembly, through their interactions with the highly basic region of the matrix domain (MA-HBR) in Gag. This interaction is hypothesized to prevent premature or promiscuous membrane binding of Gag, but the specificity of Gag-tRNA binding and its effect on overall virus replication remain to be determined. In the current study, using a modified workflow of cross-linking and immunoprecipitation coupled with tRNA sequencing, we discovered that MA preferentially binds selenocysteine-decoding tRNA (tRNASec) despite its paucity in cells. tRNASec inhibited MA-mediated membrane binding in vitro more efficiently than other tested tRNAs. A comparison between the crystal structure of MA complexed with tRNALys and the AlphaFold 3-predicted structure of the MA-tRNASec complex revealed a binding interface involving a stem-loop unique to tRNASec. Amino acid substitutions in this interface abrogated the binding of MA to tRNASec but not to other tested tRNAs. Importantly, HIV-1 encoding these substitutions showed an altered Gag localization pattern and produced virus particles with a reduced infectivity. Collectively, our results indicate that tRNASec is a specific binding partner for Gag, which likely functions in the formation of infectious HIV-1 particles, and highlight the identified tRNA-protein interface as a potential target of RNA-based therapeutics.

IT18

Subhashis Natua, University of Illinois, Urbana-Champaign

Title:
"Timing Transcript Usage Through Regulated Intron Retention in Heart Maturation and Disease"

Abstract:
The heart matures dynamically after birth, converting fetal cardiomyocytes to adult ones. This cardiac maturation involves metabolic re-programming and isoform switching in sarcomeric proteins to meet the contractile demands of the growing heart. Here, we report a previously unrecognized intron retention (IR) program that coordinates postnatal heart growth and maturity by dynamically shaping the cardiac transcriptome in a stage-specific manner. Both whole-cell and nuclear transcriptome analyses revealed only a modest negative correlation between IR and mRNA abundance, with a high prevalence of stable intron-containing transcripts. This suggests that a large number of retained introns in cardiomyocytes are not degraded via nonsense-mediated decay but accumulate in the nucleus, likely serving regulatory function(s). Strikingly, we discovered that contrary to previous assumptions, the Myh7-to-Myh6 isoform switchÃ¢â‚¬â€• a key feature of murine sarcomere maturationÃ¢â‚¬â€• is not controlled at the transcriptional level. Instead, the myosin-isoform switch is governed by IR-mediated nuclear detention of Myh7 transcripts, which effectively silences MYH7 protein expression in the adult heart. In failing hearts, these retained introns are spliced out, restoring MYH7 protein expression. Mechanistically, we identify SRSF3 as a key regulator of Myh7 intron retention in adult cardiomyocytes. Thus, our findings establish IR as a crucial mechanism that controls timely transcript usage in the developing heart, with direct implications for cardiac disease.

IT19

Adam Wier, University of Notre Dame

Title:
"Unlocking RNA Chemical Reactivity through Mass Spectrometry Screens of Electrophiles"

Abstract:
RNA is decorated with nucleophiles, making differential chemical reactivity a useful readout of higher-order structure (such as SHAPE), small-molecule binding pockets (RBRP), or quaternary interactions with RNA-binding proteins (CLIP). These covalent technologies can react promiscuously or can leverage chemo-selectivity to react with one nucleophile, such as SHAPE reagents for the 2Ã¢â‚¬â„¢-hydroxyl. However, innovation of new chemical reactions with RNA has stalled beyond these, hindering discovery of novel structure/ligand discovery methods. While there are selective chemical reactions for 9 nucleophilic amino acids, chemo-selectivity for each of the four RNA nucleobases has not been demonstrated. To address this gap in knowledge, I have developed liquid chromatography-mass spectrometry (LC-MS) platforms for screening diverse electrophilic fragments against the chemical building blocks of RNA, using individual 1) ribonucleotide monophosphates and 2) methylated nucleobases. These combined assays assess the chemical reactivity of a given electrophile with RNA, discern the reacting nucleophiles, and enable an unbiased census of reactivity under physiological conditions of PBS, pH 7.4. We have validated the chemical selectivity of literature probes such as N-methylisatoic anhydride (NMIA). We have also screened protein-focused electrophiles and discovered novel functional groups that are chemo-selective for multiple nucleobases. These previously unknown reactions pave the way for future covalent technologies for RNA, including structural tools or residue-directed covalent ligand discovery.

IT20

Jacen Emerson, University of Michigan

Title:
"Alternative Splicing as a Mechanism of Oligodendrocyte Impairments in Spinocerebellar Ataxia Type 3"

Abstract:
Dysregulated RNA splicing has recently been implicated in several neurodegenerative disorders, including Spinocerebellar Ataxia Type 3 (SCA3), a debilitating and ultimately fatal repeat expansion disease with no known treatments. Recent work by our lab and others has established that oligodendrocyte maturation impairments, driven by a toxic gain-of-function mutant ATXN3, are among the earliest and most robust pathogenic changes in SCA3 animal models. Our recently published transcriptomic analyses in the SCA3 spinal cord uncovered widespread and progressive gene expression and splicing changes, with particularly pronounced changes in oligodendrocyte signature genes. Here, we build on these findings investigating alternative splicing as a mechanism underlying SCA3 oligodendrocyte dysfunction. We focus on splicing events in oligodendrocyte genes (e.g., Bcas1, and Mag), which appear across vulnerable brain regions in two mouse models of SCA3. We further assess the capacity of genetic and antisense oligonucleotide-mediated mutant ATXN3 knockdown to rescue these splicing abnormalities and interrogate candidate splicing factors responsible for driving these molecular changes. Overall, our research aims to define a novel splicing molecular mechanism that connects mutant ATXN3 to glial pathology, highlighting new molecular targets for biomarker development and therapeutic intervention in SCA3.

Poster Session 1

#	Name	Title	Abstract
1	Audrey Hoelscher	Systematic analysis of ribosome recruitment to trinucleotide repeats	Trinucleotide repeat expansions are associated with a number of neurological disorders such as Huntington's disease, spinocerebellar ataxia, and frontotemporal dementia. Multiple mechanisms can underlie these diseases, including Repeat Associated non-AUG (RAN) translation. RAN translation results in aberrant and aggregation-prone protein products, however the mechanisms used by the repeat elements to recruit translation machinery remain unclear. To investigate the molecular requirements for RAN translation, we used Direct Analysis of Ribosome Targeting (DART) to systematically test ribosome recruitment to UTRs with various trinucleotide repeats. We tested multiple aspects of the repeats by varying the repeat nucleotide identity, position within the 5' UTR, and length. To investigate ribosome recruitment in a relevant context, we performed this DART assay in rat cortical lysates. Overall, we measured ribosome recruitment levels for 24,000 different sequences which revealed interesting trends, such as long stretches of A/U rich repeats being generally repressive regardless of position. Because RAN translation often proceeds via cap-independent mechanisms, we also performed DART on an uncapped version of the pool. Surprisingly, we found a single unique group of transcripts which showed high uncapped ribosome recruitment while capped ribosome recruitment remained low. All transcripts in this subgroup contained the GUC trinucleotide repeat, typically 20 repeats in length and positioned closer to the start codon, indicating that this sequence may be allowing initiation to occur more efficiently than expected in a cap-independent context. These results suggest a novel context where cap-independent initiation may occur that could be relevant to our understanding of repeat expansion disease and translation regulation.
2	Sincere (Yicheng) Kang	Investigating the Effects of CDK7 and MNAT1 Cryptic Splicing Event on Transcription and Cell Viability	Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are two devastating and incurable neurodegenerative diseases. ALS and FTD are highly correlated to TDP-43 proteinopathy, which means TDP-43 is depleted from the nucleus and forms cytosolic aggregates, leading to loss of function as a splicing repressor. This can lead to 'cryptic splicing', which refers to the usage of 'cryptic exons' , unannotated and non-conserved intronic sequences, which often introduce frameshifts and/or premature stop codons, leading to degradation of the RNA target by nonsense-mediated decay (NMD). In iPSC-derived cortical-like neurons, we found that TDP-43 knockdown reduces nascent RNA transcription and neuronal survival. To understand which genetic factors influence this process, we performed a CRISPRi survival screen and identified 2,198 genes essential for neuronal survival, with CDK7 and MNAT1 knockdowns leading to the most significant deficits. Importantly, they both undergo cryptic splicing and show reduced gene expression upon TDP-43 knockdown. CDK7 and MNAT1 are components of the general transcription factor IIH complex (TFIIH) and play essential roles in regulating transcription initiation. To study the effects of CDK7 and MNAT1 cryptic splicing events, I generated cell lines with endogenous deletions of the cryptic exons and successfully restored expression levels of CDK7/MNAT1 upon TDP-43 knockdown. Interestingly, I observed that the CDK7/MNAT1 cryptic exon deletion rescues total RNA levels in TDP-43 knockdown conditions and confers a gain-of-function survival advantage in neurons. Based on my data, I believe that CDK7 and MNAT1 cryptic splicing drive global transcription deficits and contribute to neuron death in TDP-43-related neurodegenerative diseases.
3	Wenzhao Dong	Structural basis of translation initiation in	Apicomplexan parasites, including Plasmodium spp. (causative agent of malaria) and Toxoplasma gondii (causative agent of toxoplasmosis), impose a substantial global health burden, yet the mechanisms underlying their mRNA translation remain poorly

		apicomplexan parasite Toxoplasma gondii	understood. Translation proceeds through initiation, elongation, termination, and ribosome recycling, with initiation being the most tightly regulated stage. Initiation begins when eukaryotic initiation factors (eIFs) assemble on the 40S small ribosomal subunit to form the 43S pre-initiation complex (43S), which is subsequently recruited by the cap-binding complex eIF4F to the 5′ end of mRNA, forming the 48S initiation complex (48S). A defining feature of apicomplexan mRNAs is their unusually long 5′ untranslated regions (5′ UTRs), with median lengths of ~800 nucleotides in T. gondii and ~600 nt in Plasmodium spp., compared with ~200 nt in humans. Here, we present the first high-resolution cryo-electron microscopy (cryo-EM) structure of the 43S translation initiation complex from the apicomplexan parasite T. gondii. The structure reveals a conserved core shared with yeast and human 43S complexes, as well as two parasite-specific components: DDX60 and thioredoxin. Functional knockdown experiments demonstrate that DDX60 is essential for parasite viability, its depletion abolishes plaque formation and markedly impairs replication. Ribo-seq and RNA-seq analyses further show that DDX60 depletion selectively suppresses translation of a defined subset of mRNAs. By establishing the first structural model of the translation initiation complex from any apicomplexan parasite, this work provides key insights into the composition and regulation of protein synthesis in protozoan pathogens and expands our understanding of parasite gene expression regulation.
4	Madeline Mayday	RBM15-MKL1 fusion protein promotes leukemia via m6A methylation and Wnt pathway activation	The recurrent t(1;22) translocation in acute megakaryoblastic leukemia (AMKL) encodes the RBM15-MKL1 fusion protein. Dysregulation of N6-methyladenosine (m6A) modification affects RNA fate and is linked to oncogenesis. Because RBM15 is critical for bringing the m6A writer complex to specific RNAs, we hypothesized that RM disrupts m6A modification, thereby altering RNA fate to drive leukemogenesis in RM-AMKL. Using a multi-omic approach, we show for the first time, to our knowledge, that RM retains the RNA-binding and m6A-modifying functions of RBM15 while also selectively regulating distinct mRNA targets including Frizzled genes in the Wnt signaling pathway. Treating murine RM-AMKL cells with the methyltransferase 3 (METTL3) inhibitor STM3675, which decreases m6A deposition, induced apoptosis in vitro and prolonged survival in transplanted mice. Frizzled genes were upregulated by RM and downregulated upon METTL3 inhibition, implicating an m6A-dependent mechanism for their dysregulation. Direct Frizzled knockdown reduced RM-AMKL growth in vitro and in vivo, highlighting Wnt signaling as a key oncogenic driver. Elevated Wnt pathway and Frizzled expression in multiple forms of human AMKL underscores the relevance of our findings. Together, our results establish RM-specific m6A modifications and Wnt pathway activation as critical drivers of RM-AMKL, thereby identifying these pathways as potential therapeutic targets.
5	Jacob Horn	Analysis of SARS-CoV-2 5’ UTR elements that regulate viral translation	SARS-CoV-2 protein Nsp1 induces a global translation shutdown in host cells upon infection. The viral genome can escape the translational shutdown via secondary structure in its 5’ untranslated region (UTR). The first hairpin structure, stem-loop 1 (SL1), has been identified as necessary and sufficient to evade Nsp1-mediated translation shutdown. Despite proven functional roles within the 5’ UTR, other elements remain understudied. To identify other potentially functional regions within the viral 5’ UTR that might influence translation we used a recently developed method called direct analysis of ribosome targeting (DART), a high throughput method that tests the ribosome recruitment ability of thousands of 5’ UTRs. We generated a diverse pool of sequences that will allow thorough examination of each region of the 5’ UTR and its role in translation and evasion of host shutdown. The pool includes natural mutations from the NCBI virus sequence repository and artificial perturbations to known secondary structure and sequence. This work is novel because analysis of mutations in SARS-CoV-2 is usually restricted to viral proteins, while the mutations to the untranslated regions are left understudied. Our preliminary DART experiment revealed that natural mutations can facilitate up to 10-fold differences in ribosome recruitment scores. For example, some Omicron and Delta variants yielded >8 fold increases in ribosome recruitment compared to the original isolate of the virus. Together, these data will allow us to comprehensively identify translational control elements within the SARS-CoV-2 5’ UTR to find potential therapeutic targets.

6	Suada Leskaj	Structural characterization of translation initiation on HPV-18 E6 mRNA	Human Papillomaviruses (HPVs) are causative agents of nearly all cervical cancers and are implicated in various other human malignancies. It was recently shown that a high-risk strain of HPV (HPV-18) uses mRNA with an extremely short 3 nucleotide ACC 5′-UTR to make the oncogenic protein E6. Canonical models of translation initiation do not readily accommodate ribosomal recruitment to such minimal 5′-UTRs. The precise mechanism by which ribosomes recognize and initiate translation on such minimal 5′-UTRs remains unknown. To investigate translation initiation on a 3-nucleotide 5′-UTR, we in vitro reconstituted human 48S initiation complexes on HPV-18 E6 mRNA and determined the structure by cryo-electron microscopy at 2.9 Å resolution. We captured a late-stage initiation complex in which the initiator tRNA is directly base-paired with the AUG start codon. We observe density 5′ of the start codon that could accommodate the ACC 5′-UTR as well as possible density for the 5′ m7G cap structure. Ongoing refinement of the cap-binding region will clarify whether there is unique mRNA cap–ribosome interactions at play for E6, which may provide further insight into how start site selection and ribosome recruitment are achieved on short 5′-UTRs. These structural insights will advance our understanding of non-canonical translation initiation mechanisms and how they are exploited by viruses.
7	Sonia Ling	A pipeline for Bisulfite Pseudouridine Detection (B-PsiD)	Pseudouridine (Ψ), an isomer of uridine, is a prevalent RNA modification driven by pseudouridine synthase (PUS) enzymes. Dysregulation of one PUS enzyme, PUS7, is implicated in various disorders such as autism and microcephaly. PUS7 modifies the central U within UNUAR motifs of RNA, but only a small fraction is modified within cells, and little is known about how PUS7 chooses which UNUAR substrates to modify. In turn, our laboratory is interested in defining PUS7 substrate specificity for mRNAs, which requires quantification of the number of Ψ sites in cells. Therefore, the Bisulfite Pseudouridine Detection (B-PsiD) pipeline is being developed for integration into our laboratory's workflows. The core of this pipeline extends upon the bisulfite-induced deletion sequencing (BID-Seq) method from Zhang et al., which relies on bisulfite-based conversion of pseudouridines into ribose ring-openings that are then read as deletions during reverse transcription [Nature Protocols, 19, 517–538 (2024)]. B-PsiD adapts BID-Seq using alternative library preparation methods, enabling orthogonal validation and quantification of the ratio of Ψ:U at every UNUAR site. Logistically, B-PsiD mirrors the five main parts of BID-Seq: (i) quality control and merging of the Illumina sequencing reads; (ii) contaminant RNA removal to isolate the mRNA; (iii) alignment to the human genome; (iv) realignment for more accurate deletion-only mapping; and finally, (v) the calculation of real Ψ modification stoichiometry at every UNUAR site. Once all high confidence modified Ψ sites are identified, our laboratory will compare the RNA structure around modified and unmodified sites via chemical probing and mutational profiling.
8	Emily Slobodenyuk	Three genes, one protein: Investigating 3'UTR-driven mRNA localization and protein function of the calmodulin gene family	Calmodulin (CaM) is an essential, highly conserved, ubiquitously expressed calcium-binding protein, and interacts with many proteins to regulate a myriad of cellular functions, including the cell cycle, neurotransmitter release, and the cardiac action potential. Interestingly, in mammals, three divergent CaM genes at separate genomic loci encode an identical calmodulin protein. Notably, the protein-coding regions of Calm1, Calm2, Calm3 mRNA within species are as minimally similar as possible while encoding the same protein sequence. The individual Calm genes behave as though they are functionally distinct. For example, the Calm mRNAs are expressed at different levels in different tissues and exhibit unique subcellular localization patterns in neurons and cardiomyocytes. Identical mutations to the protein-coding sequences induce different disease phenotypes. Further, Calm mRNA 3′UTRs (untranslated regions) are extremely different between the three genes yet are among the most conserved UTR sequences between the species. Since 3'UTRs are critical for regulating mRNA stability and localization, we expect that 3′UTR cis-regulatory elements drive the differential localization of the Calm transcripts and determine the distinct functions of each gene's expressed CaM protein. Through a combination of FISH reporter assays, epitope tagging, and affinity selection-mass spectrometry, we aim to understand how 3′UTR elements control the behavior of Calm mRNAs and the different encoded CaM protein interaction networks and functions. Neuronal cells provide a model for tracking localization of Calm mRNA and encoded protein in distinct cellular locales. I have begun by identifying Calm mRNA

			isoform distributions using short and long-read sequencing, and epitope-tagging the CaM protein products.
9	Yvonne Xinyi Lim	Inhibition of HPV16 E6 splicing with antisense oligonucleotides attenuates aggressive invasion in oropharyngeal cancer	The incidence of HPV16-positive oropharyngeal cancer (HPV+ OPC) is rising dramatically and has emerged as the leading HPV-related cancer in the United States, United Kingdom and other developed countries. Although most patients respond well to standard chemoradiation, a significant proportion develop disease recurrence or progression. Biomarkers are needed to distinguish patients at risk of recurrence to personalize treatment. Using bioinformatics approaches in multiple patient cohorts, we discovered that increased splicing of full-length E6 (E6FL) to its shorter isoform, E6*I, correlates with poorer overall and recurrence-free survival. Overexpression of E6*I enhances invasion in vitro and in vivo in HPV+ OPC cells relative to E6FL. These findings suggest that splicing of E6FL to E6*I induces aggressive tumors. To inhibit the splicing of E6FL into E6*I, we designed seven antisense oligonucleotides (ASOs) targeting the splice regulatory regions of E6FL pre-mRNA. ASOs that inhibited splicing of E6FL into E6*I (high E6FL:E6*I) reduced migration in a wound healing assay. In vivo, tumors with high E6FL:E6*I (reduced splicing) generated significantly more tumor islands that invaded less distance compared to low E6FL:E6*I (higher splicing). Invasive islands generated from HPV+ OPC with high E6FL:E6*I had lower membrane:cell E-cadherin compared to OPC with low E6FL:E6*I, consistent with a partial epithelial-mesenchymal transition phenotype. Together, our findings suggest that splicing of E6FL to E6*I drives invasive phenotypes in OPC. E6FL:E6*I may be a promising biomarker to identify aggressive HPV+ OPC. Blocking E6 splicing with ASOs suppressed aggressive phenotypes suggesting the possibility that ASOs have therapeutic potential in HPV+ OPC.
10	Evrim Yildirim	Drosophila model of CANVAS	Cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a late onset neurodegenerative disease characterized by cerebellar atrophy and peripheral sensory neuronopathy. CANVAS is caused by a biallelic CCCTT/AAGGG repeat expansion in the second intron of replication factor complex subunit 1 (RFC1). The mechanism by which this pentanucleotide repeat drives the pathogenesis remains unclear. Here, using Drosophila Melanogaster as a model, I am evaluating both gain of function and loss of function mechanisms that may contribute to neurodegeneration. CANVAS repeats in flies generate pentapeptide repeats (KGREG) similar to those observed in patient neurons, likely through repeat-associated non-AUG translation. Strong over-expression of CANVAS repeats in fly neurons induced toxicity, while loss of gnf1 (fly homologue of RFC1) led to tissue specific phenotypes. These findings suggest that gain- and loss of function mechanisms might act synergistically in CANVAS, a hypothesis that will be further investigated.
11	Grace McIntyre	Therapeutic inhibition of oncogenic miR-181a processing for cancer therapy	Background: Oncogenic microRNA-181a is a master regulator of tumor pathogenesis, driving initiation, progression, and immune evasion across cancer lineages by suppressing pathways like STING, WNT, and TGF-β. While genetic ablation of miR-181a is potently anti-tumorigenic and well tolerated, no therapies exist targeting miR-181a, presenting a critical therapeutic gap. Targeting miR-181a offers a promising strategy as it regulates multiple pathways, unlike existing single agent therapies. Nucleotide-based miRNA-targeting strategies have made significant advances; yet challenges remain in bioavailability, stability, and effective delivery. Therefore, a pharmacological approach targeting miR-181a processing represents an attractive alternative. Here, we report the discovery and characterization of SMIR-181s (Small Molecule Inhibitors of miR-181a), a novel compound class altering miR-181a biogenesis and suppress cancer properties. Results: Using a high-throughput biosensor-based screening platform in a miR-181a dependent cancer cell line, we identified a class of seven SMIR-181s, that impair miR-181a maturation, resulting in the accumulation of precursor miR-181a and depletion of mature. Mechanistic studies reveal that SMIR-181s mediate this depletion by inducing

			degradation of TARBP2, an RNA-binding protein that acts as a rheostat for stress-induced miRNAs such as miR-181a. Importantly, SMIR-181s display broad cytotoxicity across the NCI-60 panel, enhanced efficacy in miR-181a high tumor cells while sparing normal cells. Furthermore, SMIR-181s trigger apoptosis and exhibit a novel mechanism of action distinct from all known agents in the NCI-60 database. Conclusions: Collectively, this work emphasizes miR-181a's potential as a predictive biomarker, establishes proof-of-concept for small molecules targeting miRNA processing, and nominates SMIR-181s as first-in-class candidates for the treatment of miR-181a-driven diseases.
12	Jae Bucknor	Characterizing long non-coding RNA NMRAL2P Impact on the NRF2 Antioxidant Response Pathway in Non-Small Cell Lung Cancer Progression	Non-small cell lung cancer (NSCLC) is the leading cause of cancer deaths worldwide. More than 25% of cases of NSCLC display aberrantly elevated levels of NRF2 protein, a transcription factor that regulates an antioxidant response. NRF2 protein activates expression of cytoprotective genes responsible for increasing detoxification, efflux, and resistance to oxidative stressors. Thus, NRF2 pathway overactivity contributes to cancer cell survival and increased NSCLC therapy resistance through accelerated elimination of drug molecules. Many long non-coding RNAs (lncRNAs) are also expressed with NRF2 activation, and their role in the antioxidant response is not well understood. We found that genes upregulated consistently across different NRF2 activation methods are majority lncRNAs, based on RNA-sequencing. Among the most significantly activated lncRNAs is NMRAL2P, which interacts with NRF2 protein and NRF2 target gene promoters in head and neck cancer cells. I aim to understand whether NMRAL2P supports NRF2 overactivity in NSCLC and contributes to poor prognosis. I have demonstrated that NMRAL2P knockdown using antisense oligonucleotides shows decreased proliferation in cells of multiple NSCLC subtypes. This project will characterize how lncRNA NMRAL2P governs NRF2 pathway overactivity in NSCLC, and how NMRAL2P can be targeted to potentially treat NSCLC or sensitize NSCLC to existing cancer therapies.
13	Regina Lamendella	Paired Metagenomic and Metatranscriptomic Profiling Identifies Fungal-Bacterial Biomarkers in Clostridioides difficile Infection	Clostridioides difficile is a spore-forming, anaerobic bacterium and the leading cause of antibiotic-associated diarrhea and healthcare-acquired infection. CDI severity varies widely, ranging from asymptomatic carriage to life-threatening colitis, and recurrence occurs in up to 35% of cases. Most investigations to date have examined only bacterial community composition, leaving major gaps in understanding how non-bacterial members of the gut microbiome contribute to disease processes. In this study, we applied paired metagenomic (MG) and metatranscriptomic (MT) sequencing to 100 matched diarrheal stool samples from 50 CDI-positive and 50 CDI-negative adults to characterize both bacterial and fungal components of the microbiome and to identify functional biomarkers associated with active disease. Following quality filtering with fastp, taxonomic annotation was performed using Kraken2 with bacterial and fungal reference databases, and gene expression profiles were generated using eggNOG/KEGG Ortholog assignments through Emapper and HUMAnN3. Alpha diversity comparisons revealed that CDI-negative samples exhibited significantly higher community evenness in both MG and MT taxa datasets, while richness differences were modest. In contrast, CDI-positive metatranscriptomes demonstrated significantly greater KO richness, consistent with expanded transcriptional programs under inflammatory, low-oxygen gut conditions. Differential abundance analysis using MaAsLin 3 identified significant enrichment of the fungi Candida albicans and unclassified Saccharomycetaceae in CDI-positive samples. Network analysis with CoNet in Cytoscape further revealed strong co-exclusionary relationships between fungal taxa and key commensal bacterial genera, suggesting altered trans-kingdom ecological structure in CDI. Random forest machine-learning models highlighted evenness-based and mycobiome-derived features as important predictors of CDI status. Together, these results support a multifactorial trans-kingdom model of CDI pathogenesis and emphasize the need to consider fungal activity alongside bacterial dysbiosis in future diagnostic and therapeutic strategies.
14	Isabella Vitanovich	Biochemical characterization of	The eukaryotic initiation factor 4F (eIF4F) is a heterotrimeric cap-binding complex that is involved in translation initiation and assembles at the 5' cap of mRNA. One protein in

		eIF4E during eukaryotic translation initiation	the complex, eIF4E, recognizes and binds the 5' cap. The role of eIF4E in ribosome recruitment is established; however, the mechanism by which eIF4E interacts with the ribosome and is involved with scanning is unclear. Competing models suggest that either eIF4E remains attached to the cap of mRNA throughout the scanning process (the threading model) or that eIF4E detaches from the cap as the ribosome scans the mRNA for a start codon (the slotting model). To address this, we have designed a pull-down assay to capture the native eIF4E-mRNA complex, using HA-tagged eIF4E. We will assess successful complex elution by performing Western Blots and blotting for specific key ribosomal subunits. Additionally, we have synthesized mRNA with a modified photoreactive m7s6G cap via in vitro transcription, using this cap to UV crosslink (360 nm) the mRNA with the HA-tagged eIF4E. Similarly, we will assess complex elution using Western Blots and blot for specific key ribosomal subunits. Using these two systems, we will be able to assess the dependence of ribosome-bound eIF4E for scanning in translation initiation. These experiments will clarify whether eIF4E remains attached to the cap of mRNA or must dissociate before scanning of mRNA can occur and translation can initiate.
15	Brenna Saladin	Structural Basis of Ribosomal Hibernation Across Eukaryotes	The ribosome is an essential piece of cellular machinery responsible for translating messenger RNA (mRNA) to produce proteins. Regulating translation is key for cell survival in response to environmental changes. One point of regulation can be ribosomal hibernation, where the 80S ribosome is held within an inactive state, often with the help of other ribosome-associated protein factors. Ribosomal hibernation has been an important focus of study in bacteria; wherein translational regulation can be enacted during stationary phase as well as other stress conditions. However, ribosomal hibernation within eukaryotes is less understood. Here, we have used cryo-EM to determine a high-resolution structure of hibernating ribosomal states in two different eukaryotic organisms: Homo sapiens and the parasite Toxoplasma gondii. Our high-resolution cryo-EM map enabled us to identify potential factors contributing to ribosomal hibernation: eEF2, tRNA, Ebp1, and Serbp1. Ebp1 is present at the peptide exit tunnel of the 80S ribosome only within the human ribosome. However, both human Serbp1 and eEF2, and their homologous proteins in Toxoplasma gondii, are bound to these inactive ribosomes within both Toxoplasma gondii and humans, indicating potential conservation of hibernation factors across eukaryotic species. Our data provide new insights into ribosome hibernation, revealing conserved features shared between pathogenic eukaryotes and their mammalian hosts.
16	Ishita Purwar	A HiBiT Reporter for Quantifying Endogenous C9orf72 RAN Translation in Human-Derived Neurons	Repeat-associated non-AUG (RAN) translation of the C9orf72 hexanucleotide expansion produces toxic dipeptide repeat proteins (DPRs) that drive amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). However, quantitative measurement of endogenous DPR production in human neurons has remained a major technical challenge. To address this, we used a recently generated genetically engineered poly(GA)–HiBiT reporter iPSC line (DPReporter; generated by the Isaacs laboratory), in which a small HiBiT tag is inserted into the endogenous C9orf72 poly(GA) reading frame, enabling sensitive luminescence-based detection of DPRs without overexpression. DPReporter iPSCs were differentiated into induced neurons (iNeurons) and used to measure poly(GA) levels under basal conditions and following pharmacologic and genetic perturbations. We observed that endogenous poly(GA) levels are significantly higher in iPSCs than in iNeurons, indicating cell-type–specific regulation of RAN translation. Pharmacological inhibition of eIF4E increased the HiBiT signal in iNeurons, consistent with enhanced non-canonical translation under conditions of suppressed cap-dependent initiation. Additionally, antisense oligonucleotides targeting the near-cognate CUG initiation site reduced poly(GA) production, confirming that HiBiT signal reflects RAN translation initiation. To probe post-initiation regulation, we modulated ribosome quality control (RQC) factors using lentiviruses. Knockdown of Vms1 or Ankzf1 decreased poly(GA)–HiBiT signal, whereas overexpression increased it, indicating that RQC components actively regulate DPR production or stability in neurons. Together, these data demonstrate that the HiBiT DPReporter system provides a high-dynamic-range, endogenous readout of RAN translation in neurons, enables mechanistic dissection of RAN translation and offers a powerful tool for therapeutic screening in C9orf72-associated ALS/FTD.

17	Pavel Banerjee	Single-Molecule Nanoscopic Fingerprinting of Individual Lipid Nanoparticles for Quality Control of mRNA Delivery	Lipid nanoparticles (LNPs) have emerged as promising delivery vehicles for RNA therapeutics, but assumptions of uniformity in particle population and mRNA loading often obscure critical heterogeneity that can impact therapeutic efficacy. In this study, we present a single-molecule nanoscopy platform that enables high-resolution analysis of individual LNPs in terms of size, morphology, and encapsulated mRNA content. Using super-resolution imaging with Nile red, we confirm that production-line LNPs appear as spherical particles with a narrow size distribution around 90 nm. By optimizing permeabilization with Triton X-100 and employing specific fluorescent probes, we achieve direct access to and quantification of internalized mRNA while preserving nanoparticle integrity. Quantitative single-particle imaging with Ribogreen dye and sequence-specific fluorescent probes, combined with Total Internal Reflection Fluorescence Microscopy (TIRFM), reveals a broad distribution of mRNA copies per LNP, with a typical peak at about two molecules per particle and a notable fraction of sparsely loaded or empty nanoparticles—variability hidden by bulk methods. Dynamic probe binding further enables sensitive detection and accurate identification of internalized mRNA cargo. This approach establishes a direct correlation between LNP structural features and mRNA payload at the single-particle level, providing foundational insights into how variations in lipid components influence payload distribution. Understanding these heterogeneities is crucial for optimizing biodistribution, mRNA expression kinetics, and ensuring batch-to-batch consistency. Our methodology delivers a robust quality control framework, paving the way for safer, more effective nucleic acid therapeutics.
18	Shahmeer Khan	Effects of Astrocytic Metabolic Dysfunction and Glucose Stress on Gene Expression During Cortical Spreading Depression	Cortical Spreading Depression (CSD) is a propagating wave of neuronal depolarization that has been underlined with migraine aura, epilepsy, and contributes to secondary injury in neurological disorders. Previous studies have shown that CSD severity is influenced by astrocytic ion regulation and metabolic state, and that genes such as Fos, Ptgs2, Hmox1, and Prkcd are altered during CSD events. However, it remains unclear whether gene expression changes associated with CSD are driven primarily by acute depolarization events or by underlying astrocytic metabolic dysfunction. In this study, transcriptomic analyses were performed to compare cortical spreading depression under two specific conditions within rodent populations: astrocyte-specific deletion of the Na+/K+-ATPase subunit Atp1a2 and surgically induced cortical spreading depression under metabolic stress. The analysis revealed that gene expression changes associated with cortical spreading depression differ depending on metabolic context. Astrocyte-specific Atp1a2 deletion resulted in significant alterations in stress and activity-related genes (FosB, Hmox1). Acute CSD induction under glucose stress did not produce comparable transcriptional changes. These results suggest that chronic astrocytic metabolic dysfunction, rather than acute depolarization alone, may prime cortical tissue for altered CSD susceptibility. By emphasizing metabolic and astrocyte-specific mechanisms, this study provides a framework for future efforts towards therapeutic strategies aimed at reducing CSD susceptibility in migraine and other neurological disorders.
19	Marla Gravino	Into the Unknown: Covalently Targeting RNA with Electrophilic Warheads	RNA is widely recognized for its role as a messenger in the central dogma. However, most RNA is non-coding and is largely understudied as structural, functional, and therapeutic characterization remains challenging. Though small molecules are useful tools to probe these properties, RNA is particularly challenging to target with traditional non-covalent ligands due to its dynamic nature. To overcome challenges with RNA dynamics, covalent chemistry can be utilized as RNA nucleotides contain nucleophilic positions that can react with electrophilic warheads, such as the 2’-hydroxyls that react with SHAPE reagents. Reacting RNA nucleotides with electrophiles will allow for a better understanding of reactivity with RNA. However, canonical and commercially available RNA nucleotides (NMPs) do not mimic RNA due to differences in the phosphate charges and the presence of a 3’-hydroxyl group. To address this gap, I designed RNA nucleotide mimetics that model the electronic environment of RNA; they contain ethylated phosphates with a -1 charge on both the 3’- and 5’-positions to mimic the charge state of an oligonucleotide while the ethyl groups mimic the “turn” of an

			additional nucleotide. Once synthesized, I will react these molecules in vitro with a library of diverse electrophiles and measure covalent bond formation by LC/MS. I will then utilize HPLC to quantify percent covalent bond formation of each reaction and NMR to identify where covalent bond formation occurred. I expect this screen to provide insights into RNA nucleotide reactivity, opening the door to capturing RNA selectively and elucidating the functions of this biological enigma.
20	Sasha Rollinson	Investigating the Relationship Between Cellular Stress and TDP-43 Cryptic Splicing	Increased exposure to environmental toxins, including persistent organic pollutants (POPs) and heavy metals have been associated with reduced survivability in clinical studies of Amyotrophic Lateral Sclerosis (ALS). 95% of ALS cases are sporadic, suggesting environmental factors influence disease onset, but their impact on progression remains unknown. A defining hallmark of ALS and the related disorder Frontotemporal Dementia (FTD) is pathology of TDP-43. TDP-43 pathology is defined by nuclear depletion causing a loss of TDP-43 function and cytoplasmic aggregation, causing toxic gain-of-function. TDP-43 functions as a splicing repressor, preventing the incorporation of non-conserved intronic regions, called cryptic exons, into mature mRNA. Loss of TDP-43 results in cryptic splicing—inclusion of hundreds of cryptic exons, some of which induce premature termination, RNA degradation and protein loss. We use a fluorescent splicing reporter (Wilkins et al., 2024) designed to cause premature termination in the presence of TDP-43, but which produces a functional mScarlet protein upon TDP-43 loss. We then expressed this reporter in induced pluripotent stem cell (iPSC)-derived neurons for a fluorescent read out of TDP-43 loss of function. To investigate if environmental toxins associated with ALS can cause TDP-43 loss of function, we screened 22 POPs and heavy metals at 3 different concentrations and measured intensity of our fluorescent reporter and neuronal survival. We found that 9 POPs reduce neuron survival and increase cryptic splicing reporter expression in a dose-dependent manner. These findings support a model where exposure to environmental toxins may contribute to ALS progression by disrupting TDP-43 protein function.
21	**2026 RNA Symposium**	**Poster Session Listings and Location Guide**	
22	Michaela Everly	NUCLEIC ACID THERAPEUTICS (NATx) CORE at the University of Michigan	The rapid expansion of RNA and other nucleic acidâ€“based tools in both research and therapeutics has created an urgent need for accessible, affordable, high-quality design, synthesis, and functional validation services, needs that can be best-served within academic research environments. To address this need, we have established the Nucleic Acid Therapeutics (NATx) Core, a centralized, service-oriented facility at the University of Michigan dedicated to accelerating basic, translational, and preclinical nucleic acid research. NATx Core aims to lower barriers to entry for nucleic acidâ€“based technologies by minimizing time, cost, and technical overhead associated with procuring and validating synthetic nucleic acids. The Core provides end-to-end support, including strategy development, target identification, oligonucleotide design (incorporating AI-assisted modeling), high-quality synthesis with a broad catalog of innovative chemical modifications, and functional screening to ensure delivery of active, ready-to-use molecules. The Core operates primarily as a programmatic resource, integrating scientific expertise across RNA chemistry, biology, and development to support diverse applications ranging from mechanistic studies to therapeutic proof-of-concept. We will also support oligonucleotide synthesis under a â€œfee-for-serviceâ€• model for users. Initial operations are launching in early 2026 in our labs located in the Life Sciences Institute, supported by major institutional investment and serving a rapidly growing internal and external user base spanning neurological, oncologic, and rare disease programs. By centralizing expertise and infrastructure, the NATx Core aims to catalyze innovation, enhance reproducibility, and accelerate the translation of nucleic acidâ€“based discoveries into impactful therapeutics.
23	Logan Peachey	Assessing the Clinical Potential of a Novel Metatranscriptomic Whole-Blood	Sepsis is a life-threatening emergency in which improper pathogen identification limits timely, targeted treatment. Cultures are slow and often negative, while targeted PCR panels detect only predefined organisms or resistance. Metatranscriptomic sequencing enables detection of transcriptionally active pathogens alongside host immune gene

		Pathogen Detection Assay for Unbiased Diagnosis of Sepsis Using the CSI-Dx® Platform	expression. Here, we evaluate CSI-Dx®, a laboratory-developed workflow that integrates RNA extraction, Illumina shotgun sequencing, and the RAPID-Dx® analysis pipeline to generate integrated host–microbe profiles from a single whole-blood sample. Collaborating with Geisinger Medical Center, whole-blood samples (n = 21) from patients with clinically suspected sepsis were collected and processed using automated, contamination-controlled workflows. Matched samples were analyzed with and without CSI-Dx® Proprietary Pathogen Amplification (PPA), a novel approach designed to enhance microbial RNA prior to library preparation. Following bioinformatic removal of human reads, CSI-Dx® results were compared to conventional culture results. Samples from a non-sepsis control population were additionally analyzed to establish preliminary limits of blank (LOB) for baseline microbial signatures. Across 10 paired samples, 514,954,313 raw sequences were generated. All PPA libraries and 9 of 10 non-PPA libraries passed quality control, with ≥5 million post-filtered reads and robust ERCC internal control recovery. CSI-Dx® demonstrated concordance with culture-positive cases, identifying transcriptionally active bacterial pathogens, including Klebsiella, Staphylococcus, Streptococcus, Pseudomonas, and Escherichia coli, across 9 of 10 PPA samples. These findings support CSI-Dx® as an unbiased RNA-based diagnostic for sepsis that integrates viable pathogen detection with host response profiling. Ongoing prospective studies aim to further define diagnostic thresholds and clinical utility in emergency department and ICU settings.
24	Logan Peachey	Clinical Application of the CSI-Dx Metatranscriptomic Platform for Detection of Transcriptionally Active Pathogens in Prosthetic Joint Infection	Accurate and timely pathogen identification is critical for effective management of prosthetic joint infection (PJI). Synovial fluid cultures have limited sensitivity, long turnaround times, and poor taxonomic resolution, particularly following antibiotic exposure. Targeted PCR panels improve speed but are restricted to predefined taxa and cannot distinguish viable from non-viable organisms. Metatranscriptomic (MT) sequencing overcomes these limitations by profiling active microorganisms and expressed antimicrobial resistance (AMR) genes. In this exploratory clinical validation study, 340 deidentified synovial fluid specimens were classified using 2018 International Consensus Meeting (ICM) criteria. Samples were processed using the CSI-Dx® workflow, including automated RNA extraction, Illumina NextSeq sequencing, and a curated k-mer and alignment-based bioinformatics pipeline. Sensitivity and specificity for clinically relevant taxa (CRR) were calculated using limits of blank derived from 152 aseptic arthroplasty controls. Among 43 culture-confirmed infections, CSI-Dx® recaptured the cultured pathogen in 32 cases, yielding a sensitivity of 74.4%. Excluding Staphylococcus epidermidis, consistent with current molecular diagnostics, improved species-level concordance to 87.9%. Specificity across combined native and aseptic arthroplasty controls was 81.5% (95% CI: 76.0–86.3%). Expression of multiple AMR genes was detected across cohorts, providing clinically actionable data not available from culture. Machine-learning models (histogram gradient boosting and random forest) accurately classified infection status, with host immune response markers among the strongest predictors. CSI-Dx® improves pathogen detection relative to culture while providing simultaneous insight into active AMR gene expression and host response. Integration of MT-based diagnostics holds significant promise for improving detection and management of complex musculoskeletal infections and guiding precision antimicrobial therapy.
25	Cirena	High-Yield De-Novo Chemical Synthesis of RNA and Modified RNAs out to 300 Nucleotides in Length for RNA Replacement Therapeutics and Second-Generation Guide RNA Motifs.	With advances in therapeutic RNA and growing understanding of RNA's regulatory roles, researchers now need access to high-quality, long RNA over 100 nucleotides in length that can be modified for improved cellular activity and stability. The study of non-coding RNA functional domains will require producing RNA products of 150 to 400 nucleotides for research and possibly medical use. Previously, these lengths of RNA could only be obtained by transcription methods. Our laboratory has spent the last several decades developing more sophisticated methods for the synthesis, isolation, deprotection, purification, and characterization of long chemically synthesized oligonucleotides. Our methods have focused upon developing RNA synthesis techniques that result in higher integrity native RNA products as well as modified internucleotide bonds that greatly enhance stability to nucleases and persistence in cells and tissues. Using new monomer compositions, we have demonstrated the robust synthesis of RNA product out to 300+ nucleotides in high yield that can be scaled to

			therapeutic quantities. We introduce up-to-date techniques for synthesizing and analyzing RNA, which help further enable the elucidation of RNA's role in cellular metabolism and the development of RNA replacement therapies.
26	New England Biolabs	Efficient co-transcriptional enzymatic capping using an RNA capping enzyme-T7 RNA polymerase fusion protein	The 5' cap structure is essential for the function of synthetic mRNA as vaccines and therapeutics. While complete capping can be accomplished using an all-enzymatic process, optimal efficiency necessitates sequential in vitro transcription and capping unit operations. This requirement for two steps has hindered widespread implementation of this otherwise economical and environmentally sustainable capping solution in manufacturing processes. Here we demonstrate that a novel fusion of Faustovirus Capping Enzyme and T7 RNA Polymerase achieves high levels of 5' capping co-transcriptionally with comparable RNA yield. In vitro transcription reactions were carried out under conditions optimized for both RNA yield and capping efficiency. 1 µg of plasmid linearized by restriction digest was used as template. Inorganic pyrophosphatase (E. coli) and RNase Inhibitor (Murine) were included for optimal yield. Capping was compared using 3 approaches: (1) co-transcriptional capping with FCE::T7 RNAP (2) One-pot capping with FCE added to the IVT reaction (3) standard two-step workflow where capping follows IVT (with or without an intermediate cleanup step). RNA purifications were performed using Monarch RNA Cleanup Columns (500 µg). RNA yields were measured spectrophotometrically and by Qubit™ RNA BR Assay Kit. Capping efficiency was measured using a nuclease protection-based approach utilizing a biotinylated DNA oligo and digestion with RNase 4 to selectively release and enrich a short oligonucleotide fragment from the mRNA 5' end. The enriched 5' fragments were then analyzed by LC-MS/MS to measure cap incorporation. Typically, in vitro transcription reactions supplemented with capping enzymes yield incompletely capped products (e.g., 5' di- and triphosphates, G-cap). We hypothesized that this limitation could be overcome by fusion of a capping enzyme to the polymerase and promoting co-transcriptional capping through proximity to nascent transcripts. To achieve this, we selected the single-subunit capping enzyme from Faustovirus to fuse to the N-terminus of T7 RNA Polymerase via a short linker (FCE::T7 RNAP). FCE::T7 RNAP yields comparable amounts of RNA to T7 RNA Polymerase alone under optimized IVT conditions. Likewise, the fusion provides high levels of 5' capping matching a standard two-step IVT/capping workflow. High performance is generalizable across multiple templates of various sizes, and modified nucleotide incorporation efficiency remains comparable to T7 RNA Polymerase alone. Moreover, co-transcriptional capping with FCE::T7 RNAP more effectively capped RNAs with strong 5' secondary structures that typically impair enzymatic capping. Finally, one-pot co-transcriptional conversion from Cap-0 to Cap-1 is achieved by inclusion of Vaccinia Cap 2'-O-methyltransferase along with FCE::T7RNAP in IVT reactions. The FCE::T7RNAP fusion protein simplifies mRNA synthesis workflows and offers a cost-saving and sustainable alternative to chemically synthesized cap analogs. These attributes can help further advance and democratize research and development of mRNA therapeutics.
27	**2026 RNA Symposium**	**Poster Session Listings and Location Guide**	
28	Mitchell Roth	DNA Methylation-Sensitive Genome Editing with a novel Cas9	Whereas CRISPR-derived tools have been developed to add or remove DNA methylation, no CRISPR-Cas9 has been shown to function in a methylation-sensitive manner, leaving the epigenetic marker unexploited during gene editing. DNA methylation, most commonly 5mCpG, play a tremendous role in various aspects of cell biology and human diseases. The ability to utilize 5mCpG as a selective marker would allow for a safer and more precise approach to manipulate genomes. Here we report the first 5mCpG-sensitive CRISPR-Cas9 in human genome editing. Structural and biochemical characterization show that, in vitro, the nuclease activity of this Cas9 is completely inhibited by a 5mCpG sequence associated with the DNA target, while its unmethylated counterpart enables efficient cleavage. We further demonstrate

			methylation-sensitive editing activity of this Cas9 within human cells including HEK293T, HCT116, MCF-7, and MCF-10A that differ in DNA methylation landscape at the selected loci. Furthermore, optimized delivery and protein-directed engineering significantly improve the editing efficiency without the loss of methylation sensitivity. The discovery and characterization of the methylation-sensitive Cas9 provides the foundation for a novel strategy in epigenetic-based therapeutics and paves the way for an additional layer of cell-type specific editing based solely on 5mCpG marks.
29	Daniel Ben Halevy	Cell cycle-informed studies of hnRNPC reveal Transcriptome Vulnerabilities of rapidly dividing cells	Altered RNA processing has recently emerged as a hallmark of cancer and a promising avenue for therapeutic intervention. Among the key players are RNA-binding proteins (RBPs), whose dysregulation contributes to oncogenesis, progression, and therapy resistance. In this talk, I will present our unpublished findings shedding light on the transcriptomic burden faced particularly by rapidly dividing cells. Using hnRNPC as a model RBP, I will demonstrate the unexpected and significant shifts that RBPs can undergo during cell cycle progression. These changes expose context-dependent vulnerabilities that may be relevant for selective therapeutic targeting. I will discuss how cell cycle-dependent changes in RBP properties can be identified and leveraged as a novel specificity mechanism for targeting RBPs in proliferative cancer cells. This paradigm opens the door to a broader strategy for designing cell cycle–informed, RBP-targeted research and therapies.
30	Subhashis Natua	Timing Transcript Usage Through Regulated Intron Retention in Heart Maturation and Disease	The heart matures dynamically after birth, converting fetal cardiomyocytes to adult ones. This cardiac maturation involves metabolic re-programming and isoform switching in sarcomeric proteins to meet the contractile demands of the growing heart. Here, we report a previously unrecognized intron retention (IR) program that coordinates postnatal heart growth and maturity by dynamically shaping the cardiac transcriptome in a stage-specific manner. Both whole-cell and nuclear transcriptome analyses revealed only a modest negative correlation between IR and mRNA abundance, with a high prevalence of stable intron-containing transcripts. This suggests that a large number of retained introns in cardiomyocytes are not degraded via nonsense-mediated decay but accumulate in the nucleus, likely serving regulatory function(s). Strikingly, we discovered that contrary to previous assumptions, the Myh7-to-Myh6 isoform switch—a key feature of murine sarcomere maturation—is not controlled at the transcriptional level. Instead, the myosin-isoform switch is governed by IR-mediated nuclear detention of Myh7 transcripts, which effectively silences MYH7 protein expression in the adult heart. In failing hearts, these retained introns are spliced out, restoring MYH7 protein expression. Mechanistically, we identify SRSF3 as a key regulator of Myh7 intron retention in adult cardiomyocytes. Thus, our findings establish IR as a crucial mechanism that controls timely transcript usage in the developing heart, with direct implications for cardiac disease.
31	Nour El Osmani	PARP1 Orchestrates RNA Processing by Coupling Chromatin Architecture to Transcriptional Kinetics	PARP1 is an abundant nuclear protein with pleiotropic functions in epigenetics and transcriptional regulation. It plays an integral role in controlling gene expression and transcriptional initiation. However, accumulating evidence now positions PARP1 not merely as a transcriptional regulator, but as a central integrator of chromatin architecture and RNA processing across the RNA life cycle. Here, we show that PARP1 facilitates alternative splicing by associating with nucleosomes at internal exon-intron boundaries, where it functions as a regulatory hub that bridges chromatin, nascent RNA, and splicing factors (e.g., SF3B1) to ensure proper exon recognition. Beyond splicing, PARP1 is essential for mRNA stability and decay; its depletion leads to significantly reduced half-lives of PARP1-target transcripts and activates the nonsense-mediated decay (NMD) pathway to eliminate mis-spliced or aberrant mRNA. Moreover, PARP1 regulates circular RNA biogenesis by controlling back-splicing decisions. Mechanistically, PARP1 modulates the kinetics and pausing of RNA polymerase II (RNAPII) within gene bodies, effectively creating a “window of opportunity” for circularization that depends on gene architecture. Furthermore, PARP1 acts as a direct sensor of R-loops, which are triple-helical structures composed of a stable DNA:RNA hybrid and displaced single-stranded DNA. Consistently, loss of PARP1 is tightly associated with R-loop accumulation, highlighting its critical role in R-loop homeostasis.

			In sum, these findings identify PARP1 as a molecular maestro that orchestrates the transcriptional kinetics, RNA processing, and genome stability to shape the RNA output.
32	Xin Li	Cas9 senses CRISPR RNA abundance to regulate CRISPR spacer acquisition	Prokaryotes create adaptive immune memories by acquiring foreign DNA snippets, known as spacers, into the CRISPR array. In type II CRISPR-Cas systems, the RNA-guided effector Cas9 also assists the acquisition machinery by selecting spacers from the protospacer adjacent motif-flanked DNA. Here we uncovered the first biological role of Cas9 that is independent of its dual RNA partners. Following depletion of CRISPR RNA (crRNA) and/or trans-activating CRISPR RNA, Neisseria apoCas9 stimulates spacer acquisition efficiency. Physiologically, Cas9 senses low concentrations of crRNA in cells with short CRISPR arrays, such as those undergoing array neogenesis or natural array contractions, and dynamically upregulates acquisition to quickly expand the small immune memory banks. As the CRISPR array expands, rising crRNA abundance in turn reduces apoCas9 availability, thereby dampening acquisition to mitigate autoimmunity risks associated with elevated acquisition. Although the nuclease lobe of apoCas9 alone suffices to stimulate acquisition, only full-length Cas9 responds to crRNA concentrations to boost acquisition in cells with low immunity depth. Finally, we showed that this activity is evolutionarily conserved across several type II-C Cas9 orthologues. Altogether, we established an auto-replenishing feedback mechanism in which apoCas9 safeguards CRISPR immunity depth by acting as both a crRNA sensor and a regulator of spacer acquisition.
33	Youcai Xiong	Fully computational design of PAM-relaxed Staphylococcus aureus Cas9 with expanded targeting capability	CRISPR–Cas9 nucleases have transformed genome engineering, yet their application is often constrained by protospacer-adjacent motif (PAM) requirements. Staphylococcus aureus Cas9 (SaCas9) is particularly attractive for in vivo delivery due to its compact size, but its NNGRRT PAM limits targetable genomic sites. Here, we report KRH (E782K/N968R/R1015H), a SaCas9 variant designed entirely through an improved, fully computational protein point-mutation design workflow, UniDesign, without additional experimental optimization. KRH efficiently recognizes the expanded NNNRRT PAM, achieving genome- and base-editing efficiencies comparable to those of the evolution-derived KKH variant across multiple human cell types. Structural and energetic analyses reveal that KRH relaxes PAM specificity by fine-tuning the balance between sequence-specific interactions with PAM bases and nonspecific contacts with the DNA backbone. Beyond its practical utility, KRH demonstrates that computational design can identify a minimal set of mutations sufficient to remodel the PAM interface while preserving high nuclease activity. This approach not only recapitulates evolution-derived performance but also, in some cases, surpasses it, offering a scalable strategy for high-throughput Cas9 variant development. Overall, KRH establishes a blueprint for rationally engineered, PAM-relaxed nucleases and underscores the potential of computational protein design to accelerate next-generation genome editing, complementing traditional molecular evolution approaches.
34	Phillip J. McCown	Single nucleus RNA-sequencing on dissected human kidneys reveals key hypoxic and osmotic differences between cell types that are found in both the cortex and medulla	The inner layer of the human kidney, the medulla, is rarely studied, compared to the outer layer of the kidney, the cortex, due to surgical risks associated with medullary biopsy. The medulla manages drastic osmolarity gradients, is exposed to chronically low oxygen tension, and may serve as a model system to study hypoxia, a common form of injury to the kidney and other organs. Therefore, improved characterization of the medulla could be beneficial to advance physiological and pharmaceutical understanding. We aim to identify unique molecular markers of medullary cells while elucidating differences in transcriptional features of hypoxic injury and osmolarity between cortex and medulla. Using single nuclear RNA-sequencing on matched cortex and medulla samples from noncancerous segments from human kidneys, we differentiated specific cell types within both the cortex and medulla which are often ambiguous to annotate. In the cortex, we identified molecular markers of the mesangium and macula densa, frequently underrepresented in traditional kidney biopsy specimens. Similarly, in the medulla, we discovered markers of the descending and thin ascending limb of the loop of Henle (LOH) which are often mistaken for other kidney cell types. We identified fibroblasts in the kidney medulla, allowing us to compare changes to the kidney interstitium in the cortex and medulla. Finally, we observed transcriptional differences in osmotic- and hypoxic-response genes among cell types found in both regions. These findings highlight key anatomical, biochemical, and molecular

			differences between the kidney cortex and medulla that influence basic kidney functions and injury.
35	Jun Zhou	Efficient and precise correction of CFTR-N1303K mutation by a novel C-to-G base editor mi-zCGBE in cystic fibrosis patient derived bronchial epithelial cells	Base editors enable precise correction of pathogenic single-nucleotide variants (SNVs); however, current cytosine and adenine base editors are largely restricted to transition mutations. Cytosine-to-guanine base editors (CGBEs) are required to correct disease-causing transversion mutations, including CFTR-N1303K, a common cystic fibrosis (CF) mutation. Existing CGBEs suffer from limited efficiency and unfavorable safety profiles, including high indel formation and off-target activity. Here, we report the development of a novel and optimized CGBE, termed mi-zCGBE, which achieves efficient and precise C•G-to-G•C editing. mi-zCGBE was generated by introducing three point mutations (R26G/E27A/V28G) into the TadA deaminase domain and fusing the HDR-promoting peptide Brex27 to the C-terminus of spCas9. In HEK293 cells, mi-zCGBE exhibited enhanced on-target C-to-G conversion while markedly reducing Cas9-independent off-target editing and on-target indel formation. We next applied mi-zCGBE to correct the CFTR-N1303K mutation in cystic fibrosis patient–derived human bronchial epithelial cells. Deep sequencing revealed correction efficiencies of up to 41% with near-zero indel formation and minimal off-target effects. Importantly, genetic correction restored mature CFTR protein expression, as confirmed by Western blot, and rescued chloride channel function, as demonstrated by Ussing chamber assays. Together, these results establish mi-zCGBE as a highly efficient and safe base-editing platform with strong therapeutic potential for correcting CFTR-N1303K and other pathogenic transversion mutations.
36	Dresden Wilson	Single-Cell RNA Sequencing of Circulating Tumor Cells from Metastatic Breast Cancer Patients Reveals Heterogenous Expression of Cancer Driving Genes	Single-cell DNA sequencing of circulating tumor cells (CTCs) from cancer patient blood has demonstrated heterogeneous genomic alterations that evolve with therapy. Gene expression in single CTCs has been less studied. Here we present RNA expression data in single CTCs from breast cancer patients, demonstrating CTC identification by scRNA-seq. Our analytical approach was developed using cultured human cancer cell lines spiked into normal donor blood to mimic CTCs. Patient whole blood (WB) samples were enriched for CTCs using the Parsortix system. 7.5 ml of fixed WB was enriched, and harvested cells were affixed to a microscope slide for cell identification and enumeration by immunofluorescent imaging (IF). Concurrently, 20 ml of non-fixed WB from the same patient was enriched and harvested. If CTCs were identified by IF in the fixed sample, an aliquot of live cells from the nonfixed sample harvest were submitted for 10x 3'scRNA-seq. Populations of white blood cells (WBCs), cell lines, and CTCs were accurately identified by gene expression profiles. Among patient CTCs, presence and heterogeneity of expression in epithelial genes and oncogenes were observed, but not in WBCs. Specific molecular features in patients' tumor tissue were identified in the matched single-CTC transcriptome. Genes associated with metastasis and aggressiveness were observed in patient CTCs, as was intra-patient, inter-CTC expression heterogeneity in ESR1 and ERBB2 (HER2), two breast cancer therapeutic targets. Combining expression data with clinical information and genomic analysis of CTCs can help monitor tumor heterogeneity and evolution non-invasively with potential clinical implications for precision oncology.
37	James George	Using Plasma Cell-Free RNA for Tumor-Naïve Detection and Monitoring	Current liquid biopsies that are based around circulating tumor DNA (ctDNA) often face sensitivity ceilings of 30-50% in early-stage or minimal residual disease detection and cannot reliably infer tumor cell-of-origin. While plasma cell-free RNA (cfRNA) offers superior tissue-specificity, hematopoietic background noise obscures tumor signals. We hypothesized that targeted capture of tumor-specific transcripts would resolve this, enabling precise, tumor-naïve detection.

			To achieve this we analyzed bulk RNA-seq data from 59,507 tissue and 23,540 blood samples, and identified genes exhibiting "High-in-Tumor, Low-in-Blood" (HITLIB) expression pattern across the cancer spectrum. We engineered a custom hybrid-capture panel targeting the top ~3,000 HITLIB genes. When coupled with our optimized wet lab protocol, the approach achieved 64X–128X enrichment of tumor-derived cfRNAs relative to standard whole transcriptome sequencing. In a proof-of-concept study of rare or refractory malignancies, our panel detected pathognomonic cfRNAs absent in benign controls, including steroidogenic enzymes in adrenal cancer, skeletal muscle transcripts in rhabdomyosarcoma, peptide hormones in neuroendocrine prostate cancer, intestinal epithelial keratins in small bowel adenocarcinoma, and mucins in pancreatobiliary cancer. Crucially, unsupervised clustering of our cohort revealed distinct, histology-specific separation. We are currently leveraging these high-dimensional signatures to train AI/ML classifiers for automated disease status and cell-of-origin prediction. This targeted cfRNA platform represents a scalable pan-cancer strategy poised to complement or advance liquid biopsy standards.
38	Nya Huff	Expanding RNA Chemistry: Characterizing Covalent Warhead Reactions with Oxidized Nucleobases	Through its dynamic conformations, RNA enables diverse biological functions, creating both opportunities and challenges for small molecule targeting. Covalent chemistry offers a solution as RNA contains nucleophilic positions that can react with electrophilic warheads. Beyond its complex tertiary structures, its canonical nucleotides have over 150 post-transcriptional modifications (PTMs) that present opportunities for selective electrophilic reactivity. Among these PTMs, oxidative modifications are prevalent in many neurodegenerative diseases and cancers. These oxidized nucleobases present additional chemical handles for selective reactivity over canonical nucleobases, such as sp2-hybridized alcohols as nucleophilic sites. In understanding the mechanisms and consequences of RNA oxidation, novel avenues for detection and selective small molecule targeting can be explored for diagnostic tools and therapeutic strategies, respectively. However, there are limited modified nucleotides and/or nucleobases that are commercially available. To address this gap, I am synthesizing oxidized nucleobases that are methylated at the site of ribose connectivity in RNA. By LC/MS, the oxidized nucleobases will be screened with a library of over 50 electrophiles, and their reactivity will be compared to canonical nucleotides to observe selective reactions. I expect this screen will provide insights into the potential of covalent chemistry to selectively target oxidized nucleobases, elucidating the biological functions of RNA modifications in disease.
39	Rodolfo Murguia	Structural and functional characterization of MYC mRNA translation initiation	In eukaryotes, canonical translation initiation involves the recruitment of the ribosome by the cap-binding complex eIF4F. However, some mRNAs use complex secondary structures to recruit ribosomes independently of eIF4F. One example of such mRNA is the proto-oncogene MYC. The MYC 5′UTR is a highly structured regulatory region that contains two alternative translation start sites, an AUG and an upstream near-cognate CUG, enabling the production of two protein isoforms. Despite extensive research highlighting the regulatory role of the MYC 5′UTR in mRNA translation, the molecular mechanisms by which this region recruits the ribosome and facilitates initiation at alternative start codons remain unclear. Here, we investigate the contribution of the MYC 5′UTR in regulating translation initiation. We have designed and purified mRNA constructs containing the MYC 5′UTR linked to a NanoLuc luciferase reporter, capped either with ARCA to enable eIF4F-dependent translation or with an A-cap to assess eIF4F independence. Translation assays in cell-free extracts confirm the critical role of the MYC 5′UTR in regulating translation independently of the cap-binding protein–eIF4E. Furthermore, we test small molecule inhibitors designed to target the MYC 5′UTR and demonstrate their effective inhibition of MYC translation. Our next steps include determining which of these inhibitors can stall the translational machinery within MYC's 5′UTR, allowing for structural characterization via cryo-electron microscopy to elucidate the structural and regulatory elements that regulates ribosome recruitment and start codon selection during MYC translation initiation.
40	Kira Holton	Substrate Selection and Functional	Human RNAs are heavily modified post-transcription, yet the functions of many modifications remain unclear. The enzyme PUS7 is responsible for a large fraction of

		Consequences by the mRNA Modifying Enzyme PUS7	one such RNA modification, pseudouridylation, where uridine is isomerized to pseudouridine (Ψ). Altered PUS7 activity is implicated in several diseases, but the mechanisms of PUS7-dependent substrate modification and how Ψ contributes to disease pathogenesis are unknown. While PUS7 modifies almost any UNUAR sequence in vitro, only a tiny fraction of these sites are modified inside cells. Without understanding the cellular contexts that direct PUS7 target selection, we cannot predict the functional consequences of PUS7-dependent Ψ in RNA. We hypothesize that distinct RNA structural contexts and protein-RNA interactions drive selection of Ψ sites by PUS7 in cells. We are employing live-cell chemical probing and sequencing technologies to identify these cellular contexts. Protein interaction network probing (RNP-MaP) in human cells finds that Ψ occurs in RNA regions with limited protein binding compared to unmodified UNUAR sites. We are examining whether protein occupancy prevents modification or whether Ψ itself alters protein binding by profiling PUS7-deficient cells. We are similarly probing whether RNA structural motifs are conserved at PUS7-modified sites (by SHAPE-MaP). Additionally, we are developing a luciferase reporter system to measure mRNA expression, processing, and stability in the presence and absence of Ψ. Preliminary results suggest that Ψ-dependent regulation is mRNA-specific. We anticipate that this research will allow us to predict novel PUS7-dependent Ψ sites and may be suggestive of a novel gene regulatory mechanism based on RNA modifications.
41	Jacen Emerson	Alternative Splicing as a Mechanism of Oligodendrocyte Impairments in Spinocerebellar Ataxia Type 3	Dysregulated RNA splicing has recently been implicated in several neurodegenerative disorders, including Spinocerebellar Ataxia Type 3 (SCA3), a debilitating and ultimately fatal repeat expansion disease with no known treatments. Recent work by our lab and others has established that oligodendrocyte maturation impairments, driven by a toxic gain-of-function mutant ATXN3, are among the earliest and most robust pathogenic changes in SCA3 animal models. Our recently published transcriptomic analyses in the SCA3 spinal cord uncovered widespread and progressive gene expression and splicing changes, with particularly pronounced changes in oligodendrocyte signature genes. Here, we build on these findings investigating alternative splicing as a mechanism underlying SCA3 oligodendrocyte dysfunction. We focus on splicing events in oligodendrocyte genes (e.g., Bcas1, and Mag), which appear across vulnerable brain regions in two mouse models of SCA3. We further assess the capacity of genetic and antisense oligonucleotide-mediated mutant ATXN3 knockdown to rescue these splicing abnormalities and interrogate candidate splicing factors responsible for driving these molecular changes. Overall, our research aims to define a novel splicing molecular mechanism that connects mutant ATXN3 to glial pathology, highlighting new molecular targets for biomarker development and therapeutic intervention in SCA3.
42	Ross Kaufhold	Structural basis for repeat-associated non-AUG translation initiation on C9orf72 mRNA	A GGGGCC (G4C2) hexanucleotide repeat expansion in the first intron of the C9orf72 (chromosome 9 open reading frame 72) gene is the most common monogenic cause of both Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). This repeat serves as a template for repeat-associated non-AUG (RAN) translation, a non-canonical form of translation initiation which generates neurotoxic dipeptide repeat (DPR) proteins that aggregate in patient brains and contribute to neurodegeneration in model systems. How G4C2 repeats interact with the ribosomal complex to promote this pathological mechanism of initiation has remained unclear. To address this fundamental question, we used single-particle cryo-electron microscopy (cryo-EM) to determine the structure of a reconstituted human late-stage C9orf72 48S translation initiation complex after start codon selection at 3.2 Å resolution. The structure reveals that RAN translation on C9orf72 mRNA initiates at a near-cognate CUG codon that is located 24 nucleotides upstream of the G4C2 repeat and is flanked by a strong endogenous Kozak sequence. Mutation of either the CUG or the Kozak sequence surrounding it dramatically reduces RAN translation in plated neurons. The structure further reveals a direct interaction between the G4C2 repeat and 18S ribosomal RNA expansion segment 9 (ES9S) on the 40S small ribosomal subunit through a kissing-loop interaction. Preventing this RNA-RNA interaction using complementary antisense oligonucleotides against ES9S markedly reduces RAN translation, indicating that this structural interaction is functionally important. These findings provide mechanistic insights into RAN translation initiation and highlight a potential therapeutic approach to mitigate toxic DPR production in C9orf72-associated neurodegenerative disease.

43	Rajat Mudgal	The specific interactions between selenocysteine-decoding tRNA and HIV-1 Gag regulate progeny infectious particle assembly	Beyond their canonical role in translation, tRNAs participate in various stages of replication cycle of viruses including HIV-1. In addition to the well-known role of tRNALys as primer for HIV-1 reverse transcription, accumulating evidence indicates that tRNAs likely regulate binding of the viral structural protein Gag to the plasma membrane, the initial and essential step of viral assembly, through their interactions with the highly basic region of the matrix domain (MA-HBR) in Gag. This interaction is hypothesized to prevent premature or promiscuous membrane binding of Gag, but the specificity of Gag-tRNA binding and its effect on overall virus replication remain to be determined. In the current study, using a modified workflow of cross-linking and immunoprecipitation coupled with tRNA sequencing, we discovered that MA preferentially binds selenocysteine-decoding tRNA (tRNASec) despite its paucity in cells. tRNASec inhibited MA-mediated membrane binding in vitro more efficiently than other tested tRNAs. A comparison between the crystal structure of MA complexed with tRNALys and the AlphaFold 3-predicted structure of the MA-tRNASec complex revealed a binding interface involving a stem-loop unique to tRNASec. Amino acid substitutions in this interface abrogated the binding of MA to tRNASec but not to other tested tRNAs. Importantly, HIV-1 encoding these substitutions showed an altered Gag localization pattern and produced virus particles with a reduced infectivity. Collectively, our results indicate that tRNASec is a specific binding partner for Gag, which likely functions in the formation of infectious HIV-1 particles, and highlight the identified tRNA-protein interface as a potential target of RNA-based therapeutics.
44	Sreeja Sekhar	microRNA-181a - driven Regulation of Immune Responses in High Grade Ovarian Cancer	Targeting MicroRNAs has become a potential way to treat cancer. Among these, miR-181a has attracted attention for its critical role in driving tumor genesis, development, and metastasis across multiple cancer models. Elevated miR-181a activity in various cancers underscore its central role in cancer initiation, maintenance, dissemination, and recurrence. By regulating critical signaling pathways such as TGFβ, Wnt, and innate immune signaling, miR-181a fosters tumor growth and immune evasion, positioning it as a prime therapeutic target. Our work uncovers substantial therapeutic benefits of targeting miR-181a in high-grade serous ovarian cancer (HGSC), the most lethal subtype of ovarian cancer. We demonstrate that inhibiting miR-181a reactivates the STING mediated interferon (IFN) signaling pathway, resulting in robust immune cell activation and significant tumor suppression in vivo. Furthermore, miR-181a lose increased immune cell infiltration and activation at the tumor site which was further accentuated with the addition of the STING agonist, MSA. Specifically, miR181a knockdown with STING activation enhanced the CD8+/CD69 population which displayed an increase in secreted cytokines like granzyme B, IFNG and TNF which are the prime determinants in predicting anti-tumor response. Additionally, studies using athymic mice indicated no significant tumor growth inhibition irrespective of STING activation, further substantiates miR181a knockdown provides the priming of a T-cells dominant immune reactive environment. Thus, these findings not only advance our understanding of the challenges associated with STING agonist-based therapies but also position miR-181a as a critical therapeutic lever for reprogramming the tumor microenvironment.

Poster Session 2

#	Name	Title	Abstract
1	Noah Helton	uORFs inhibit stress-induced gene mRNA condensation	The integrated stress response (ISR) is critical for cellular adaptation to stress and is implicated in neurodegeneration, neurodevelopmental disorders, and cancers. During the ISR, translation is repressed, stress-induced genes are translated, and translationally repressed mRNAs condense into stress granules. The relationship between stress-induced gene expression and stress granules is poorly understood. To begin to address this gap, we asked if and how stress-induced gene mRNAs localize to stress granules. We measured endogenous stress-induced gene mRNA localization to stress granules using single-molecule fluorescence in situ hybridization in the presence or absence of small-molecule translation inhibitors that modulate ribosome occupancy. We observed that reducing ribosome occupancy increases the localization of stress-induced gene mRNAs to stress granules, while increasing ribosome association inhibits localization to stress granules. The presence of an upstream open reading frame (uORF) in mRNA reporters reduces mRNA localization to stress granules in a ribosome-dependent manner. We also observed that stabilizing a single ribosome globally in the cell inhibits the formation of stress granules. Together, our results suggest that uORF-mediated ribosome association inhibits stress-induced gene mRNA localization to stress granules. Our results imply a novel role of uORFs beyond their known translational regulation, as regulators of mRNA localization, and highlight that ribosome-bound mRNAs are incompatible with condensation.
2	Li-kUnag Tsai	Overexpression of ZSCAN4 and its variants increases CRISPR/Cas9-mediated knock-in efficiency in human cells	CRISPR/Cas9-mediated gene editing is broadly used in research and clinical applications. However, large-size knock-in has an unsatisfied outcome due to the low efficiency of homology-directed recombination (HDR). We previously showed that overexpression of ZSCAN4 reduces the endogenous DNA double-strand breaks in mouse embryonic stem cells, suggesting its functions beyond telomere maintenance. To evaluate the HDR efficiency, we transfected a spCas9 coding plasmid, which also contains a mCherry coding sequence, along with a knock-in donor plasmid that possesses a promoter-less GFP coding sequence flanked by homologous arm sequences, into human 293 cells by lipofectamine. HDR-mediated knock-in events were quantified by flow cytometry as mCherry and GFP double-positive cells. By co-transfection of a ZSCAN4-expressing plasmid with spCas9 and donor plasmid, the knock-in rate was increased by ~2 fold across different loci (AAVS1, ROSA26, GAPDH, and ACTB) in human 293 cells. Next, by functional domains prediction, we constructed truncated ZSCAN4s of smaller sizes to form ZSCAN4-mid (Δ222-338) and ZSCAN4-mini (Δ169-391). The results show that the ZSCAN4-mid had a comparable knock-in rate improvement to full-length ZSCAN4. However, the ZSCAN4-mini lost the beneficial effect on knock-in rate. In summary, our study identified ZSCAN4 and ZSCAN4-mid as factors that can be used to enhance Cas9-mediated knock-in efficiency.
3	Aiden Saunders	Steric Blocking Antisense Oligonucleotides to Inhibit Precursor MicroRNA Processing	MicroRNAs (miRNAs or miRs) are a type of non-coding RNA that, when incorporated into an Argonaute protein, forms an RNA-induced silencing complex (RISC), which degrades messenger RNA and ultimately downregulates protein expression. Antisense oligonucleotides (ASOs) are short, synthetic nucleic acids that bind to RNAs through Watson-Crick base pairing. ASOs can be used to promote target RNA cleavage, sterically block mRNA splicing, or sequester the mature miRNA within the RISC. Our work uses ASOs to target a structural element, a junction region, that ~20% precursor-miRNAs contain, preventing Dicer/TRBP processing. Electrophoretic mobility shift assays (EMSAs) of junction-containing and non-junction-containing miRNAs were performed with their respective ASOs to verify binding. To assess the effect of ASOs in cells, reverse transcriptase quantitative PCR (RT-qPCR) was performed on all three miRNAs. The ASOs target the apical loop region of precursor miRNAs, which are also present in primary miRNAs. To determine at which step the ASOs prevent processing, RT-qPCR was used to measure levels of primary miRNA with and without ASO

			treatment. Overall, our results indicate that ASOs significantly reduce mature miRNA levels containing junctions but have no effect on miRNAs that lack junctions. Our work provides an alternative approach by inhibiting the biogenesis of miRNA, paving a path towards structure-informed therapeutic design.
4	Alice Youle	Systematic Identification of Functional 5′UTR Structures in Translation Regulation	The 5′ untranslated region (UTR) of an mRNA is a critical feature in regulating translational output. The 5′UTR mediates the loading and scanning of the preinitiation complex, and subsequent recognition of the start codon. Several features within the 5′UTR are known to affect translation, such as secondary structure. Secondary structure is often repressive of translation initiation, by impeding scanning of the ribosome or recruiting regulatory RNA-binding proteins. However, there is well-documented evidence that specific secondary structures can also stimulate translation. This context-dependent behavior of secondary structure has limited our ability to predict translational output. Thus, systematic identification and characterization of 5′UTR structural elements is needed to uncover new mechanisms and to be able to predict their functional impact. Here, we describe a novel high-throughput strategy that combines SHAPE-MaP and DART to determine the secondary structure of 5′UTRs and their effect on translational output. Using a pool of disease-relevant RNAs and by introducing structure-disrupting and compensatory mutations, we directly test the role of secondary structure in translation initiation. Preliminary analysis has identified 5′UTRs in which ribosome recruitment is either stimulated or repressed in a structure-dependent manner. Of note, these include genes whose 5′UTRs are largely uncharacterized, and are involved a wide range of disease such as hypercholesteremia, tumorigenesis, and immune response. This approach offers a powerful strategy to systematically identify and functionally characterize secondary structures that regulate translation, and may uncover potentially novel avenues for therapeutics.
5	Andrews Afrifa	RNA Processing in Brain Health and Disease: Alternative Splicing, RNA-Binding Proteins, and Therapeutic Transcript Modulation in Neurology	The brain exhibits transcriptomic complexity driven by extensive alternative splicing, long 3′UTR regulation, and neuron-specific RNA localization.These RNA processing programs are essential for synaptic plasticity, neuronal survival, and neural network plasticity. However, they also create vulnerability to dysregulated splicing and RNA-binding protein (RBP) dysfunction. In neurodegenerative and neurodevelopmental disorders, disruptions in RBPs such as TDP-43 and FUS alter pre-mRNA processing, promote cryptic exon inclusion and intron retention, and shift isoform expression toward toxic or nonfunctional products. Stress-induced RNA remodeling also interfaces with innate immune signaling pathways, providing a mechanistic bridge between cellular RNA dysregulation and neuroinflammation. This literature review integrates mechanistic insights across three interconnected themes: (1) neuronal alternative splicing as a determinant of protein isoform output and neuronal phenotype, (2) RBP-mediated regulation of transcript fate including splicing, stability, and transport as a central axis in neurodegeneration, and (3) translation of these mechanisms into RNA-directed therapies. We highlight how antisense oligonucleotide (ASO) platforms enable direct manipulation of brain transcripts through splice-switching or RNase H–mediated knockdown, and we compare these approaches with siRNA strategies and emerging RNA editing systems designed to correct pathogenic transcripts without modifying DNA. Finally, we discuss major translational challenges including CNS distribution, endosomal escape, durability-to-toxicity tradeoffs, and biomarker validation and propose a “processing-aware” framework for designing next-generation RNA therapeutics in neurology. Together, these advances demonstrate how fundamental RNA mechanisms in the nervous system are increasingly precise and scalable therapies for brain disease.
6	Medhasri Jasti	miR181a and Myc Drive Purine Pathway Activation via IMPDH2 to Fuel Metastasis and Chemoresistance in	High-grade serous ovarian cancer (HGSC) is the most common and lethal subtype of ovarian cancer. Often diagnosed at advanced stages and marked by its high genomic instability, HGSC exhibits aggressive metastatic potential with high chemoresistance and limited treatment options. As a result, exploring the molecular pathways driving this malignancy is essential for advancing therapeutic strategies. We have found that elevated levels of miR181a drive early cell transformation, leading to poor patient

		High-Grade Serous Ovarian Cancer	outcomes. However, in-vivo experiments with miR181a high expressing fallopian tube cell (FTE) alone did not metastasize, suggesting that additional genetic alterations contributed to the aggressive phenotype of HGSC. Remarkably, we found that the addition of the oncogene Myc which alone does not lead to tumor formation, resulted in robust tumor formation and metastasis in-vivo. Further, cells co-expressing Myc and miR181a levels indicate resistance to platinum-based chemotherapy prior to any exposure. We found that this increased tumorgenicity was mediated through the up-regulation of purine metabolism, specifically the metabolic enzyme IMPDH2, which is critical for nucleotide biosynthesis. Additionally, FTE cells with high miR181a and Myc expression exhibited increased sensitivity to the IMPDH inhibitor (MPA), highlighting IMPDH2 as a metabolic vulnerability. Importantly, IMPDH2 knockdown decreased tumor burden and progression in vivo. Overall, these findings suggest that the combined up-regulation of miR181a and Myc enhances metastatic potential through purine metabolic pathways. Targeting IMPDH2 may provide new therapeutic strategies for platinum-resistant HGSC patients.
7	Kinsey Van Deynze	Human transposable element derived STRs exhibit differential evolution and disease-associated instability	Transposable elements, particularly Alu and SVA sequences, are increasingly associated with short tandem repeat expansion disorders. It remains unclear whether the elevated mutability of these loci extends to all transposable-element–associated STRs and what mechanisms drive their increased instability relative to other STRs in the genome. Using 100 PacBio HiFi genomes, we characterize STRs associated with transposable elements genome-wide and compare them to non–transposable-element–associated STRs. We find that both disease-associated STRs and those associated with Alu and SVA elements show the greatest length variation, with 3′ poly(A) tail–derived Alu-STRs accounting for 47% of all length and variance outliers. Evolutionary divergence analysis reveals that Alu-poly(A) homopolymers rapidly decline as Alu-poly(A) tetramers increase over evolutionary time, with a strong preference for AT-rich STRs, contradicting genome-wide expectations toward transversions and STR period distributions. While on average Alu poly(A)-STRs shorten and become less polymorphic over time, a subset of unstable Alu-STRs located at the beginning of the poly(A) tail maintain longer and more variable repeat tracts than expected for their age and motif class. Together with known Alu retrotransposition mechanisms and activity levels, we propose a model in which long Alu poly(A)-STRs facilitate retention of retrotransposition activity while promoting increased genome stability relative to long homopolymer poly(A) tails. Our findings indicate that a subset of mobile element insertions disproportionately contribute to some of the most mutable STRs in the human genome and represent promising candidates for novel disease-associated expansion loci.
8	Madison Uyemura	Engineered Circular RNAs as Novel Therapeutics for miR-181a Degradation in High- Grade Serous Cancer	MicroRNA-181a (miR-181a) is a highly conserved oncogenic microRNA that plays a role in many hallmarks of malignancy, including tumor initiation, enhanced proliferation, immune evasion, and metastasis by modulating key pathways such as WNT, STING, and PTEN. miR-181a is overexpressed in a wide variety of cancers, including colorectal, gastric, and ovarian cancers, and this overexpression is correlated with reduced survival and increased recurrence. Downregulation of miR-181a has been shown to have anti-tumorigenic effects with minimal off-target toxicity, highlighting its value as a therapeutic target. Circular RNAs (circRNAs), with their intrinsic stability and capacity to act as microRNA sponges and degraders, present a promising RNA-based therapeutic platform. Here, we investigate engineered circRNAs, both rationally designed and AI-generated circRNAs from the first artificial intelligence to predict circular RNA structures (NovaEngine™). Using a validated miR-181a biosensor, we demonstrate that exogenously delivered circRNAs effectively suppress miR-181a activity in high-grade serous cancer. This inhibition results in reduced mature miR-181a levels and upregulation of key downstream targets, including STING, RB1, and SMAD7. Our findings showcase the efficacy of engineered circRNAs, including those designed by generative AI, in modulating oncogenic microRNAs and potentially restoring tumor suppressor pathways. This strategy supports the therapeutic capabilities of circRNAs as targeted interventions in high-grade serous ovarian cancer.

9	Adam Wier	Unlocking RNA Chemical Reactivity through Mass Spectrometry Screens of Electrophiles	RNA is decorated with nucleophiles, making differential chemical reactivity a useful readout of higher-order structure (such as SHAPE), small-molecule binding pockets (RBRP), or quaternary interactions with RNA-binding proteins (CLIP). These covalent technologies can react promiscuously or can leverage chemo-selectivity to react with one nucleophile, such as SHAPE reagents for the 2'-hydroxyl. However, innovation of new chemical reactions with RNA has stalled beyond these, hindering discovery of novel structure/ligand discovery methods. While there are selective chemical reactions for 9 nucleophilic amino acids, chemo-selectivity for each of the four RNA nucleobases has not been demonstrated. To address this gap in knowledge, I have developed liquid chromatography-mass spectrometry (LC-MS) platforms for screening diverse electrophilic fragments against the chemical building blocks of RNA, using individual 1) ribonucleotide monophosphates and 2) methylated nucleobases. These combined assays assess the chemical reactivity of a given electrophile with RNA, discern the reacting nucleophiles, and enable an unbiased census of reactivity under physiological conditions of PBS, pH 7.4. We have validated the chemical selectivity of literature probes such as N-methylisatoic anhydride (NMIA). We have also screened protein-focused electrophiles and discovered novel functional groups that are chemo-selective for multiple nucleobases. These previously unknown reactions pave the way for future covalent technologies for RNA, including structural tools or residue-directed covalent ligand discovery.
10	Grace Eramo	Splice-switching in the 5'UTR with antisense oligonucleotides to modulate mRNA translation	Precision genetic medicine needs additional strategies to selectively upregulate endogenous gene expression. Traditional gene therapy and transcriptional activation approaches face limitations due to gene size, complexity, or risks from global gene activation. An alternative strategy to boost protein output is through amplifying protein production from endogenous gene transcripts by modifying mRNA regulatory content. Sequence features in the 5' untranslated region (5'UTR) of mRNA play a critical role in translational control, including RNA-binding protein motifs, secondary structures, and upstream start codons and open reading frames. Therefore, we hypothesize alternative splicing events in this region can dramatically affect translation initiation and protein levels. We propose leveraging splice-switching antisense oligonucleotides (ASOs) to modulate alternative splicing in 5'UTRs and thereby upregulate protein production of target genes as a novel therapeutic approach to accomplish this. Using the high-throughput ribosome recruitment assay, DART (Direct Analysis of Ribosome Targeting), we have identified 957 genes where 5'UTR splice isoforms drive greater than a 2-fold difference in ribosome recruitment. Luciferase reporters are being used in in vitro and in cellulo translation assays to validate these results. Further, we utilize targeted long-read sequencing to precisely map and quantify 5'UTR isoforms in relevant tissues and cell lines. This strategy could unlock new therapeutic options for diseases requiring increased protein expression, with special utility for genetic diseases associated with haploinsufficiency.
11	Jarrett Wilson	Mapping Sm Protein Binding Sites on snRNA Using RNP-MaP	RNA–protein interactions are abundant in nearly every aspect of gene regulation, governing RNA transcription, processing, localization, stability, and function, and ultimately shaping cell identity and fate. However, defining the organization and function of RNA–protein interaction networks remain a major challenge, particularly because many RNAs lack strong primary sequence conservation or well-defined structural annotation. Traditional approaches to study RNA-protein interactions – for example RNA immunoprecipitation or reconstituted binding assays – often miss the dynamic and cooperative nature of Ribonucleoprotein (RNP) assemblies inside cells and are limited to the interactions of only a few proteins at a time. Ribonucleoprotein Networks Analyzed by Mutational Profiling (RNP-MaP), in contrast, identifies protein binding sites and protein interaction networks on RNAs of interest inside cells. Here, we aim to develop RNP-MaP to look across the transcriptome and identify global patterns of multi-protein interaction networks on RNA, and ideally simultaneously identify RNPs from their interaction signatures. As proof-of-principle, here we investigate interactions of a shared set of RNA-binding proteins and small nuclear RNAs (snRNA), which are central to spliceosomal function. Sm-associated snRNPs assemble at a structurally conserved Sm binding site, forming well-defined complexes that provide an ideal system for learning patterns of RNP network formation. Our analyses reveal similarities

			in mapped profiles around the Sm binding site, providing a template for mapping similar interactions transcriptome-wide. This work represents a first step toward mapping a comprehensive, cell-wide network of Sm complex interactions, and lays the groundwork for profiling all RNP networks transcriptome-wide.
12	Tina Li	Integrative DNA Methylation and Transcriptomic Analyses Reveal Effects of Harmful Algal Blooms(HAB) on ALS Survival	Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by substantial clinical heterogeneity. We previously reported associations between harmful Algal bloom(HAB) exposure and worse survival in ALS patients; however, the molecular mechanisms involved in these associations remain poorly understood. This study aims to investigate how HAB exposure may worsen ALS survival through molecular alterations in gene expression and DNA methylation (DNAm). In 428 ALS cases, we collected whole blood samples and performed RNA-seq and EPIC BeadChip DNAm profiling. We first identified differential expressed genes (DEGs) and differential methylated CpGs (DMCs) associated with ALS survival by Cox proportional hazards models. We then identified DEGs and DMCs between patients stratified by high versus low HAB exposure, adjusting for age, sex, race, batch, and unknown technical variance. We assessed the overlap between survival DEGs/DMCs/pathways and HAB DEGs/DMCs/pathways, highlighting potential exposure-mediated mechanisms influencing ALS survival. Cis-expression quantitative trait methylation (cis-eQTM) analyses identified which gene expression changes of HAB exposure and ALS survival were regulated by DNA methylation. Our results demonstrate that HAB exposure is associated with distinct transcriptomic and epigenetic signatures in ALS patients. These HAB-related molecular signatures are not only enriched in ALS survival-related molecular signatures but show regulating direction consistent with the previous findings. Notably, DEGs associated with HAB exposure were highly enriched in processes related to neurodegeneration, regulation of actin and oxidative phosphorylation. Together, these findings provide multi-omics evidence that HAB exposure may exacerbate ALS progression. IRB Approval: IRBMED HUM28826 Funding: This study was supported by the NINDS (R01NS127188, R01NS120926); National ALS Registry/CDC/ATSDR (1R01TS000289, R01TS000327); NIEHS (P30ES017885, R01ES030049); National Center for Advancing Translational Sciences at the National Institutes of Health (UL1TR002240); Intramural Research Program of the National Institute on Aging (ZIA-000933); ALS Association (20-IIA-532); the Coleman Therapeutic Discovery Fund, the Scott L. Pranger ALS Clinic Fund, the Dr. Randall W. Whitcomb Fund for ALS Genetics, the Richard Stravitz Foundation, the Stanford Morris ALS Research Fund, James and Margaret Hiller and Linda and Eric Novak, and the NeuroNetwork for Emerging Therapies, University of Michigan. This project was also supported by the Michigan Diabetes Research Center, NIDDK grant number P30DK020572.
13	Amelia Cochran	Comparison of eukaryotic and bacterial Pus4 homologs reveals origin of an expanded eukaryotic substrate scope	Pseudouridine is an abundant post-transcriptional modification important to RNA structure and function. The isomerization of uridine (U) to pseudouridine (Ψ) is catalyzed by members of the pseudouridine synthase (Pus) family throughout all domains of life. All Pus enzymes modify non-coding RNAs, and a subset also pseudouridylate protein-coding messenger RNAs (mRNAs). Although the precise role of Ψ in mRNAs remains to be firmly established, emerging evidence suggests that it might contribute to the post-transcriptional control of gene expression. Furthermore, how individual Pus enzymes select their mRNA targets remains to be defined. The bacterial Pus enzyme TruB has been well characterized and primarily modifies tRNA at position U55 within T-loops. In addition to modifying U55 in most tRNAs, the eukaryotic homolog Pus4 also pseudouridylates mRNAs. While it has been proposed that Pus4 catalyzes mRNA Ψ sites that mimic its tRNA targets in sequence and secondary structure, only a fraction of such sites are pseudouridylated in cells. Here, we demonstrate that Saccharomyces cerevisiae Pus4 binds and modifies RNAs that differ in secondary structure from its established tRNA substrates. Comparison of Pus4 and TruB activities on structurally diverse substrates reveals that while both enzymes can modify a variety of substrates, TruB is less efficient than Pus4. We also find that the bacterial-specific PUA domain modulates TruB substrate selection. These findings are consistent with reports demonstrating that other mRNA-modifying Pus enzymes are

			more promiscuous in vitro than in cells. Together, our results suggest that Pus4 substrate selection might rely on cellular factors beyond protein-RNA recognition.
14	Dana Beseiso	Investigating alternative Transcription Start Site (TSS) usage in breast cancer	The expression of multiple messenger RNA (mRNA) isoforms by a singular gene is essential for promoting cellular diversity and adapting to stress. Alternative transcription start site (TSS) usage results in mRNAs that differ in their 5′-ends and, as a result, their 5′ untranslated regions (5′ UTRs). 5′ UTRs harbor translational control elements that modulate protein output, but it remains poorly understood whether alternative TSS usage is broadly employed as a mechanism to modulate protein abundance, especially throughout disease progression. We hypothesize that widespread changes in TSS usage drive translational reprogramming which promotes the cellular and morphological changes necessary for cancer progression. We show that the annotated 5′ UTR isoforms of the Breast Cancer Resistance Protein (BCRP) and the metastasis-associated proteins, NODAL, NANOG, and SNAIL, are differentially translated in vitro. Furthermore, we generated translationally competent lysates from epithelial MCF10A and tumorigenic MCF10CA1h cells and performed Direct Analysis of Ribosome Targeting (DART) on a comprehensive list of annotated human 5′ UTR isoforms. DART revealed extract-dependent variation in ribosome recruitment of the isoforms. Identifying novel translational control elements in 5′ UTRs will elucidate their role in modulating gene expression and inform novel anticancer therapeutic strategies.
15	Marc Dean	PHOTOAFFINITY PROFILING TO IDENTIFY NOVEL TARGETS AFFECTING AN ABERRANTLY SPLICED UNDRUGGABLE ONCOGENE	Aberrant RNA splicing is a major contributor to human disease, including rare genetic diseases, neurological disease, and cancers. Thought to be undruggable, AR-V7 is a constitutively active splice variant of the androgen receptor often found in castration-resistant prostate cancer (CORC) and accompanied by poor prognosis. We performed a phenotypic split luciferase HTS screen against AR-V7 in 22Rv1 cells with an RNA-focused library of 12,055 compounds. Two hit compound showed specific loss of AR-V7 protein and mRNA levels over WT AR, and anti-proliferative activity in relevant cancer cell lines. Our preliminary data suggests that these compounds function via unique mechanisms compared to other AR-V7 targeting compounds. To identify these potentially novel mechanisms, photoaffinity-based proteomic profiling was performed. Details regarding our screen and follow-up mechanistic studies will be described.
16	Hideyuki Komori	N6-methyladenosine modification promotes exit from stemness in fly neural stem cell progeny by facilitating decay of NSC gene transcripts	N6-methyladenosine (m6A) plays important roles in multiple mRNA regulations including translation and degradation. Although abundant transcripts possess m6A modification in a cell, only a subset of m6A transcripts is regulated during developmental transitions. Therefore, the developmental cues specifically select certain m6A transcripts to determine their fate, but the mechanisms remain unclear. In the fly larval brain, neural stem cell (NSC) asymmetrically divides to give rise a progeny in which RNA-binding protein, Brain tumor (Brat) robustly induces decay of NSC transcripts to initiate differentiation. We hypothesized if m6A modification facilitates decay of Brat target transcripts in NSC progeny. We found that loss of mettl3 or ythdf function enhanced brain tumor phenotype in brat hypomorphic mutant brains. Next, we investigated the correlation of m6A and Brat-mediated mRNA decay by profiling m6A modification and identifying genes downregulated by Brat in the fly larval brain and then identified 120 Brat target m6A transcripts. Interestingly, Brat target transcripts possessing m6A modification in their 5'UTR showed relatively faster decay in NSC progenies compared to other Brat target transcripts. Our results suggest a possibility that YTHDF binds to m6A on 5'UTR of Brat target transcripts and associates with Brat when Brat binds to 3'UTR in neuroblast progeny, facilitating decay of specific neuroblast gene transcripts. Our study provides a new model explaining how m6A modification tightly link to mRNA decay triggered by developmental transition cues.
17	Jiayi Zhou	High-resolution spatial transcriptomics uncovers tumor microenvironment dynamics in prostate cancer progression	Prostate cancer exhibits diverse cellular composition, and interactions between epithelial cells and the tumor microenvironment (TME) critically drive disease progression. However, technical limitations have hindered systematic characterization of microenvironmental contributions to prostate cancer progression. We generated high-resolution spatial transcriptomics profiles from 13 high-grade (grade group ≥2) and 7 low-grade (grade group 1) primary prostate tumors using the Visium HD platform. A large-scale assembled single-cell transcriptomic dataset comprising

			756,000 cells collected from publicly available datasets was used as a reference for robust cell type decomposition (RCTD)-based annotation of the spatial data. This approach identified 15 major TME cell types and revealed dynamic microenvironmental changes from benign to low- and high-grade lesions. Inflammatory fibroblasts exhibited activation of “TNF-α signaling via NF-κB” pathway and were more prevalent in benign and low-grade microenvironments but reduced in high-grade TMEs. Conversely, myofibroblasts showed activation of the “extracellular matrix organization” pathway and were enriched in high-grade TMEs. Within the smooth muscle compartment, the THY1-high population, characterized by a more proliferative and migratory synthetic phenotype and high activation of “MET promoted cell motility” pathway, is increased in high-grade TMEs, while the contractile type (RERGL-high) was enriched in benign and low-grade microenvironments. Among endothelial cells, the abundant pro-angiogenesis subpopulation (EDNRB-high) was noted in high-grade TMEs, while the preferential enrichment of anti-angiogenesis subpopulation (RGS16-high) was observed in benign and low-grade microenvironments. Our findings delineate heterogeneity within the tumor microenvironment and highlight potential cell populations that may contribute to prostate cancer progression, offering insights for future therapeutic exploration.
18	Keyana Blake	Investigating the link between Cryptic Splicing and Autophagy in ALS/FTD	Dysfunctional autophagy, a crucial cellular degradation pathway, is linked to neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). These diseases also share TDP-43-related pathology, including neuronal cytoplasmic aggregates and TDP-43 nuclear depletion. TDP-43 is essential for RNA metabolism; its nuclear depletion leads to the inclusion of intronic sequences or “cryptic exons” in hundreds of transcripts. To model patient TDP-43 loss of function, we induced TDP-43 depletion using CRISPR interference in human iPSC-derived glutamatergic neurons (iNeurons). This results in a cryptic exon in ATG4B (autophagy-related 4B cysteine peptidase) between exons 10 and 11, predicted to cause premature termination, as evidenced by reduced mRNA and protein levels. ATG4B is crucial for autophagosome formation, yet the consequences of ATG4B cryptic splicing in neurons remain unknown. We find that TDP-43-depleted neurons exhibit reduced LC3B-GFP-labeled autophagosomes, suggesting a defect in autophagy affecting both LC3B and GABARAP autophagosome-family proteins. To test how the loss of ATG4B contributes to dysfunctional autophagy, we used CRISPR interference to deplete ATG4B alone. Both TDP-43 and ATG4B depletion result in decreased levels of GABARAP and GABARAPL2, proteins critical for autophagosome genesis. Additionally, we show that GABARAP decrease is a direct consequence of ATG4B loss, demonstrated by rescue experiments. These results suggest that dysfunctional autophagy in TDP-43-depleted neurons is a consequence of ATG4B loss. Our study will gain insight into how TDP-43 nuclear depletion can lead to defective autophagy in ALS/FTD, aiding therapeutic strategies to rescue autophagy and neuronal survival.
19	Minghua Li	TDP-43-Linked Alternative Splicing Signatures in Whole Blood of ALS Patients	Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with limited treatment options and a median survival of 2–4 years. Aberrant alternative splicing (AS) has been increasingly implicated in ALS pathogenesis, particularly in relation to dysfunction of the RNA-binding protein TAR DNA-binding protein 43 (TDP-43), a key regulator of RNA processing whose nuclear depletion and cytoplasmic aggregation are hallmarks of ALS. Although TDP-43–associated splicing defects have been extensively studied in neuronal systems, it remains unclear whether such abnormalities can be robustly detected in peripheral blood, a clinically accessible tissue. Here, we analyzed poly(A)-selected whole-blood RNA sequencing data from a large cohort of individuals with ALS (n = 161) and controls (n = 146) to identify differential splicing events while adjusting for age, sex, batch effects, and cell-type composition. We identified 358 differentially spliced introns derived from 125 intron clusters spanning 109 genes. Pathway enrichment analysis revealed significant over-representation of pathways related to RNA binding and immunological synapse function. Notably, the differentially spliced genes showed significant overlap with previously reported TDP-43 target genes. Furthermore, motif enrichment analysis revealed a significant enrichment of TDP-43 binding motifs in the intronic sequences flanking differentially spliced junctions, with a pronounced positional bias toward donor splice sites compared to acceptor sites.

			Together, our results demonstrate widespread and TDP-43–associated splicing alterations in whole blood from ALS patients, suggesting that peripheral splicing signatures may provide novel insights into ALS pathogenesis and highlight the potential of blood-based splicing signatures as accessible biomarkers for ALS. IRB Approval: IRBMED HUM28826 Funding: This study was supported by the NINDS (R01NS127188, R01NS120926); National ALS Registry/CDC/ATSDR (1R01TS000289, R01TS000327); NIEHS (P30ES017885, R01ES030049); National Center for Advancing Translational Sciences at the National Institutes of Health (UL1TR002240); Intramural Research Program of the National Institute on Aging (ZIA-000933); ALS Association (20-IIA-532); the Coleman Therapeutic Discovery Fund, the Scott L. Pranger ALS Clinic Fund, the Dr. Randall W. Whitcomb Fund for ALS Genetics, the Richard Stravitz Foundation, the Stanford Morris ALS Research Fund, James and Margaret Hiller and Linda and Eric Novak, and the NeuroNetwork for Emerging Therapies, University of Michigan. This project was also supported by the Michigan Diabetes Research Center, NIDDK grant number P30DK020572.
20	Henry Arthur	A phage-encoded protein inhibitor evades CRISPR-Cas9 immunity by PAM mimicry	CRISPR-Cas systems are bacterial defenses against phages that have revolutionized biomedical research by enabling RNA-guided genome editing. To fully realize the therapeutic potential of CRISPR-Cas9, effective and mechanistically defined off-switches that enable precise control over editing duration are critically needed. Type II-C Cas9 enzymes have attracted broad interest due to their compact gene sizes that favor in vivo therapeutic delivery; however, well-defined and potent anti-CRISPR inhibitors for these systems remain limited. Here we identify and characterize AcrX7, a potent inhibitor encoded by Neisseria filamentous phages to evade anti-phage immunity mediated by the type II-C Cas9 enzyme in Neisseria meningitidis (NmeCas9). Biochemically, AcrX7 binds the NmeCas9-crRNA ribonucleoprotein complex, but not apoCas9, and blocks target DNA binding and cleavage by Cas9. High resolution cryo-EM structures reveal that AcrX7 mimics the N4GATT PAM DNA and directly occupies the PAM-interacting (PI) domain of NmeCas9. In contrast to previously reported PAM-mimicking anti-CRISPR inhibitors of Streptococcus pyogenes Cas9, AcrX7 adopts an extended architecture that mimics an ~11-nt DNA segment extending beyond the PAM into the seed-binding pocket, resulting in robust inhibition of II-C Cas9s that use longer PAMs. Additionally, we found that AcrX7 inhibition is avoided by the naturally occurring Nme2Cas9 variant, which differs from NmeCas9 only in the PI-domain, underscoring AcrX7's role in driving Cas9 diversification. Finally, we show that AcrX7 robustly inhibits NmeCas9-mediated genome editing in human cells. These findings provide new insights into phage biology and pave the way towards safer gene therapy using NmeCas9.
21	**2026 RNA Symposium**	**Poster Session Listings and Location Guide**	
22	Michaela Everly	NUCLEIC ACID THERAPEUTICS (NATx) CORE at the University of Michigan	The rapid expansion of RNA and other nucleic acidâ€“based tools in both research and therapeutics has created an urgent need for accessible, affordable, high-quality design, synthesis, and functional validation services, needs that can be best-served within academic research environments. To address this need, we have established the Nucleic Acid Therapeutics (NATx) Core, a centralized, service-oriented facility at the University of Michigan dedicated to accelerating basic, translational, and preclinical nucleic acid research. NATx Core aims to lower barriers to entry for nucleic acidâ€“based technologies by minimizing time, cost, and technical overhead associated with procuring and validating synthetic nucleic acids. The Core provides end-to-end support, including strategy development, target identification, oligonucleotide design (incorporating AI-assisted modeling), high-quality synthesis with a broad catalog of innovative chemical modifications, and functional screening to ensure delivery of active, ready-to-use molecules. The Core operates primarily as a programmatic resource, integrating scientific expertise across RNA chemistry, biology, and development to support diverse applications ranging from mechanistic studies to therapeutic proof-of-concept. We will also support oligonucleotide synthesis under a â€œfee-for-serviceâ€•

			model for users. Initial operations are launching in early 2026 in our labs located in the Life Sciences Institute, supported by major institutional investment and serving a rapidly growing internal and external user base spanning neurological, oncologic, and rare disease programs. By centralizing expertise and infrastructure, the NATx Core aims to catalyze innovation, enhance reproducibility, and accelerate the translation of nucleic acidâ€“based discoveries into impactful therapeutics.
23	Dustan Bonnin	A Two-day, Femtomolar-Scale, In-vitro Pharmacodynamic Benchmarking Assay for siRNA Screening	In spite of the promise held by siRNA therapeutics, there remains a need for a practical, field-deployable in vitro benchmarking assay that enables siRNA screening within the context of clinically feasible delivery system. Here, we describe a two-day, femtomolar-scale in vitro pharmacodynamic (PD) assay, in which RNA is delivered by clinically-compatible, magnesium-integrated iron oxide nanoparticles, that can be used for siRNA screening. Magnesium-integrated iron oxide nanoparticles (~45 nm hydrodynamic diameter) were synthesized via a single-pot, aqueous process. Particles were post-synthetically functionalized with ~160 iron-binding maleimide ligands. Thiolated siPLK1 or scrambled control oligonucleotides were reduced using a solid-phase activator without the need for purification and self-assembled with the particles and complex formation confirmed by dynamic light scattering. PLK1-sensitive HT29 and SW480 colorectal adenocarcinoma cells were plated at 20,000 cells per well and treated with 2, 20, or 80 fmol siPLK1, scrambled oligo, nanoparticle alone, or media control. Viability and morphology were assessed at two days with standard imaging-compatible dye sets. Two days following exposure to PLK1 siRNA oligonucleotide, robust, selective PLK1 inhibition was observed. However, counter to expectation, PLK1 siRNA-treated cells retained viability, despite exhibiting a phenotype characteristic of PLK1 inhibition. Viability effects extended beyond transfected cells, suggesting a population-level response. Bipolar “barbell” morphologies were observed, even with 82 fmol oligonucleotide. Furthermore, cells treated with the particle alone exhibited increased viability, suggesting a particle-driven effect. Collectively, these behaviors are not readily reconciled with expectations around dose, timing, PD effect, and toxicity constraints derived from existing, FDA-approved siRNA delivery systems.
24	Neal D. Amin	The Human Single Cell RNA Splicing Atlas v1.0	Splicing plays critical roles in cell identity and disease but remains underexplored in single-cell datasets due to limited transcript coverage and computational constraints. Using our proprietary Bio-Orthogonal Barcode sequencing (BOB-seq) and Patchwork platforms, we integrated RNA splicing events across diverse human tissues spanning development to adulthood, including immortalized cell lines, stem cell–derived populations, and healthy and disease states, to construct the first single-cell–resolution human RNA splicing atlas. Key findings include a distinct RNA processing signature in spinal motor neurons not observed in other neural cell types; validation of stem cell–derived organoid splicing patterns against stage- and organ-matched in vivo populations; and modules of co-regulated splicing events that regulate biological functional properties. Together, this atlas reveals recurrent regulatory programs and cell type–specific isoform switches, uncovering disease-relevant pathways and therapeutic targets not detectable through conventional gene-level analyses.
25	Cirena	High-Yield De-Novo Chemical Synthesis of RNA and Modified RNAs out to 300 Nucleotides in Length for RNA Replacement Therapeutics and Second-Generation Guide RNA Motifs.	With advances in therapeutic RNA and growing understanding of RNA's regulatory roles, researchers now need access to high-quality, long RNA over 100 nucleotides in length that can be modified for improved cellular activity and stability. The study of non-coding RNA functional domains will require producing RNA products of 150 to 400 nucleotides for research and possibly medical use. Previously, these lengths of RNA could only be obtained by transcription methods. Our laboratory has spent the last several decades developing more sophisticated methods for the synthesis, isolation, deprotection, purification, and characterization of long chemically synthesized oligonucleotides. Our methods have focused upon developing RNA synthesis techniques that result in higher integrity native RNA products as well as modified internucleotide bonds that greatly enhance stability to nucleases and persistence in cells and tissues. Using new monomer compositions, we have demonstrated the robust synthesis of RNA product out to 300+ nucleotides in high yield that can be scaled to

			therapeutic quantities. We introduce up-to-date techniques for synthesizing and analyzing RNA, which help further enable the elucidation of RNA's role in cellular metabolism and the development of RNA replacement therapies.
26	New England Biolabs	Efficient co-transcriptional enzymatic capping using an RNA capping enzyme-T7 RNA polymerase fusion protein	The 5' cap structure is essential for the function of synthetic mRNA as vaccines and therapeutics. While complete capping can be accomplished using an all-enzymatic process, optimal efficiency necessitates sequential in vitro transcription and capping unit operations. This requirement for two steps has hindered widespread implementation of this otherwise economical and environmentally sustainable capping solution in manufacturing processes. Here we demonstrate that a novel fusion of Faustovirus Capping Enzyme and T7 RNA Polymerase achieves high levels of 5' capping co-transcriptionally with comparable RNA yield. In vitro transcription reactions were carried out under conditions optimized for both RNA yield and capping efficiency. 1 µg of plasmid linearized by restriction digest was used as template. Inorganic pyrophosphatase (E. coli) and RNase Inhibitor (Murine) were included for optimal yield. Capping was compared using 3 approaches: (1) co-transcriptional capping with FCE::T7 RNAP (2) One-pot capping with FCE added to the IVT reaction (3) standard two-step workflow where capping follows IVT (with or without an intermediate cleanup step). RNA purifications were performed using Monarch RNA Cleanup Columns (500 µg). RNA yields were measured spectrophotometrically and by Qubit™ RNA BR Assay Kit. Capping efficiency was measured using a nuclease protection-based approach utilizing a biotinylated DNA oligo and digestion with RNase 4 to selectively release and enrich a short oligonucleotide fragment from the mRNA 5' end. The enriched 5' fragments were then analyzed by LC-MS/MS to measure cap incorporation. Typically, in vitro transcription reactions supplemented with capping enzymes yield incompletely capped products (e.g., 5' di- and triphosphates, G-cap). We hypothesized that this limitation could be overcome by fusion of a capping enzyme to the polymerase and promoting co-transcriptional capping through proximity to nascent transcripts. To achieve this, we selected the single-subunit capping enzyme from Faustovirus to fuse to the N-terminus of T7 RNA Polymerase via a short linker (FCE::T7 RNAP). FCE::T7 RNAP yields comparable amounts of RNA to T7 RNA Polymerase alone under optimized IVT conditions. Likewise, the fusion provides high levels of 5' capping matching a standard two-step IVT/capping workflow. High performance is generalizable across multiple templates of various sizes, and modified nucleotide incorporation efficiency remains comparable to T7 RNA Polymerase alone. Moreover, co-transcriptional capping with FCE::T7 RNAP more effectively capped RNAs with strong 5' secondary structures that typically impair enzymatic capping. Finally, one-pot co-transcriptional conversion from Cap-0 to Cap-1 is achieved by inclusion of Vaccinia Cap 2'-O-methyltransferase along with FCE::T7RNAP in IVT reactions. The FCE::T7RNAP fusion protein simplifies mRNA synthesis workflows and offers a cost-saving and sustainable alternative to chemically synthesized cap analogs. These attributes can help further advance and democratize research and development of mRNA therapeutics.
27	**2026 RNA Symposium**	**Poster Session Listings and Location Guide**	
28	Zhengde Liu	Identification of CELF5 as a novel interacting partner and effector of TDP-43	Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are incurable neurodegenerative disorders that lack effective treatments. TDP-43 is an RNA-binding protein that plays critical roles in RNA transcription, RNA splicing, RNA transport, and RNA stability. Loss of nuclear TDP-43 is observed in brain tissues of ALS and FTD patients, highlighting its contribution to disease progression. One function of TDP-43 is to act as a splicing repressor. Loss of nuclear TDP-43 induces cryptic splicing, in which a normally repressed intronic sequence or splice junction is retained in mature RNA, often causing premature termination and degradation of the transcript. Previous work identified a cryptic exon in CELF5 that encodes a premature termination codon upon

			TDP-43 depletion in human neurons. I confirmed downregulation of CELF5 RNA and protein in TDP-43-depleted human iPSC-derived neurons using RT-qPCR and Western blot. CELF5 belongs to the CUGBP, Elav-like family, an RNA-binding protein family known to regulate RNAs involved in synaptic function and axonal development. Interestingly, published data reveal that CELF5 and TDP-43 share the identical UG-rich binding motif. When we perform RNA-seq in TDP-43 and CELF5 knockdown neurons, it reveals over 600 shared differentially expressed genes enriched in neurite-localizing transcripts. To test whether CELF5 and TDP-43 interact in the cytoplasm, I performed co-localization and co-immunoprecipitation experiments and observed cytoplasmic co-localization and a partially RNA-dependent interaction between the two proteins. Our findings support a model that CELF5 acts as a cytoplasmic partner of TDP-43 for transcript co-regulation, implicating the mechanism behind RNA mis-regulation in TDP-43-related neurodegenerative diseases.
29	Jacob Diehl	Leveraging evolutionary relationships to determine how RNA binding proteins selectively bind their targets	RNA binding proteins (RBPs) regulate most aspects of RNA metabolism and function through binding to targets with sequence specificity and affinity. The repertoire of RNA targets recognized by RBPs is expansive, necessitating that RBPs be as diverse as the RNAs they bind. This diversity is partially reflected in various types of highly conserved RNA Binding domains (RBDs), but analysis of various RBD-RNA structures suggests many RBP families, like the heteronuclear Ribonucleoproteins (hnRNPs), contain loop regions interfacing with the RNA target. Despite their functional importance, these loops are poorly conserved. One explanation for this discrepancy is that they have specialized in recognizing their specific RNA targets and/or other proteins. We hypothesize that if these loops are functionally important, then grouping RBPs by similar functional characteristics will reveal higher levels of sequence conservation in these loop regions. Goodson et al. (1999)'s 'relative rate of change' approach compares the divergence of a sequence region against the background neutral mutation rate identifying functional but non-conserved sequences. This comparison is facilitated by categorizing protein sequences as orthologs (proteins that diverge by speciation) and paralogs (proteins that diverge by gene duplication). We developed PODiv (Paralog-Ortholog Divergence) a Python-based framework that leverages the 'relative rate of change' concept and automates paralog-ortholog divergence comparisons. Here, we expand its application to the hnRNP protein family. Developing PODiv and applying it to RBPs will advance our understanding of how evolution specializes proteins for specialized tasks as well as contribute to fundamental research on how RBPs function and recognize RNA targets.
30	Martin O'Steen	Great Expectations from Single Molecules: Defining the prfA RNAT Unfolding Mechanism	~~RNA thermometers (RNATs) are thermosensitive regulatory structures found in the 5′ untranslated regions (UTRs) of some bacterial messenger RNA (mRNA) transcripts. Operating at the translational level, RNATs undergo temperature-dependent structural changes to regulate translation of the accompanying transcript. While typically associated with temperature-related genes, RNATs also regulate some virulence gene transcripts, such as the prfA RNAT found in Listeria monocytogenes (Lm). PrfA (positive regulatory factor A) is the master virulence gene activator in Lm. PrfA translation is regulated by the prfA RNAT, which suppresses translation at temperatures below 37 °C. The prfA RNAT shares no homology with known classes of RNATs, and the structural underpinnings of its regulation remain unknown.~~ ~~Here, we apply analytical ultracentrifugation (AUC) and single-molecule kinetic analysis of RNA transient structure (SiM-KARTS) to determine the unfolding mechanism of the prfA RNAT. Sedimentation velocity AUC (SV-AUC) experiments were used to determine the $Mg^{2+}{}_{1/2}$ of prfA RNAT folding, leading to methodological improvements in SV-AUC analysis of RNA folding. Temperature-dependent SiM-KARTS analysis demonstrated that the prfA RNAT principally unfolds at the ribosome binding site (RBS), while the upper portion of the hairpin remains well folded. The measured increase in RBS binding kinetics mirrored increases in temperature-dependent translation, providing a functional correlation to our kinetic analysis. Further mutational analysis of the upper helix revealed that this portion of the RNAT is crucial to thermal regulation of translation. Here, we propose that the prfA RNAT unfolds first at the RBS, with the upper helical elements participating in thermal regulation.~~

31	Alec Garasimowicz	Probing the Binding Mechanism of sreA and prfA in Listeria Monocytogenes	Non-coding RNA (ncRNA) serve important gene regulatory functions in the cell. Enriched in the 5' untranslated region of bacterial mRNA, these dynamic elements exert genetic regulation by changing structure in response to stimuli, such as small molecules (riboswitches) or temperature (RNA thermosensors). RNAT-based regulation is often tied to environmental related genes. However, this regulatory mechanism is also found in virulence gene transcripts, such as the prfA RNAT found in the 5' UTR of Positive Regulatory Factor A transcript in Listeria Monocytogenes (LM). The prfA RNAT is a central regulator of virulence factors in LM, only allowing translation of the prfA transcript at host temperatures. prfA was found to be involved in a trans interaction with sreA, an S-adenosyl methionine (SAM) class I riboswitch, which inhibits translation of prfA even at elevated temperatures. The original model for this interaction incorporated the P3 helical domain of sreA as central to the RNA:RNA interface, however alternative structural predictions suggest that this domain is not involved in the interaction. Through the use of site-directed mutagenesis, we probed the functional importance of the sreA P3 domain. In vitro translation and electromobility shift assays (EMSA) showed that P3 is dispensable for both binding and inhibition. Analytical ultracentrifugation (AUC) demonstrated that the prfA:sreA complex is insensitive to mutations in this region. Our results indicate that the P3 domain has no contribution to the prfA:sreA interaction. Further research is needed to determine the contributions of the other helical domains in this interaction.
32	Amanda Bekkala	Unraveling the Role of miR-181a in T Cell Exhaustion and Therapeutic Resistance in High Grade Serous Cancer	High-grade serous ovarian cancer (HGSC) is among the deadliest gynecologic malignancies, with poor five-year survival rates (20–40%) and limited responsiveness to immunotherapy, largely due to an immunosuppressive "immune-cold" tumor microenvironment (TME). Our lab has identified microRNA, miR-181a, as a potent oncogene frequently overexpressed in HGSC, correlating with chemotherapy resistance, reduced survival, and immune-cold tumors. miR-181a regulates pro-inflammatory signaling genes, including STING, and was initially recognized for its role in T cell activation and thymocyte development. Preliminary data show that high miR-181a levels within the HGSC TME elevate miR-181a in circulating effector T cells, triggering T cell overactivation followed by chronic exhaustion—characterized by impaired anti-tumor responses and immunotherapy resistance. Using a fallopian tube-specific, inducible Cre-loxP model, we drove miR-181a overexpression and validated tissue specificity through PCR and organ-wide expression analysis. Sustained miR-181a elevation in the fallopian tubes led to higher miR-181a in circulating T cells and spleen, not reporter-driven, indicating uptake from circulation. Resulting T cells-maintained viability yet showed an exhausted phenotype (high PD-1, inhibited DUSP6). Based on our current findings, we hypothesize that miR-181a is secreted from fallopian tube epithelial cells via exosomes, transferred to T cells, and establishes a feedback loop that exacerbates exhaustion in response to excessive tumor-derived miR-181a. Our ongoing studies aim to delineate this mechanism and its impact on T cell exhaustion in HGSC. Ultimately, strategies to lower miR-181a in effector T cells may help prevent exhaustion and enhance anti-tumor immunity in HGSC and other miRNA-driven cancers.
33	Brandon Klein	Development of unbiased affinity profiling of bioactive RNA-Ligand interactions in live-cells	Affinity profiling of ligandable RNAs promises a means of unlocking the druggable transcriptome and revealing unknown mechanisms of RNA biology. To address the limitations of current RNA-affinity profiling techniques, this work sets out to solve the problems of feature detection and feature selection by developing an RNA-unique signal for affinity profiling and then leverage those improvements to map ON and OFF targets of splicing modulators in multiple cell types. We will integrate our RNA-unique signal into a multi-parameter hit-calling paradigm, which will integrate RNA-RBP interactions from ENCORE to prioritize likely functional loci over non-specific interactions. Upon completion, we will have established a set of robust data standards and best practices for affinity-based RNA profiling, serving our long-term goal of expanding the Encyclopedia of RNA Elements (ENCORE) and ushering in the era of the druggable transcriptome through mapping the ligandable faces of RNAs and their binding partners in live cells.

34	Kayla Lenshoek	Uncovering how lncRNA MALAT1 controls gene expression in cancer metastasis	Long noncoding RNA Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) is linked to the progression of multiple cancer types. Upregulation of MALAT1 is often correlated with aggressive metastasis, and depletion of MALAT1 in cancer cells decreases metastatic potential. Interestingly, in healthy cells and wild-type mouse models, loss of MALAT1 has no effect on viability. The mechanisms by which MALAT1 promotes metastatic activity exclusively in cancer cells is not well understood, and MALAT1's function in healthy cells is also unknown. Here we aim to uncover the mechanisms by which MALAT1 alters gene regulatory programs to promote metastasis in a lung cancer cell model. We create and validate A549 lung adenocarcinoma cell lines where CRISPR-based gene deletions and insertions alter MALAT1 expression or destabilize the MALAT1 transcript. We find that MALAT1 accumulates at high levels in nuclear speckles, but loss of MALAT1 does not strongly alter nuclear speckle formation. Increased MALAT1 expression promotes a mesenchymal phenotype, whereas reduction in MALAT1 expression promotes a more epithelial cell state. Further, we find that MALAT1 expression is inversely correlated with levels of EZH2 protein, the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2). Consequently, levels of the PRC2 epigenetic mark, Histone H3 Lysine 27 tri-methylation (H3K27me3) are inversely correlated with MALAT1 levels. In line with MALAT1's pro-metastatic activity, we observe an upregulation in genes and pathways associated with cell adhesion, mobility, and mesenchymal cell identity in MALAT1 overexpression lines. Further, we establish human bronchial epithelial (HBE) cell lines with inducible exogenous MALAT1 expression. We confirm the pro-metastatic phenotype in A549 MALAT1 overexpression is unique to cancer cells, finding no changes to key markers when MALAT1 is overexpressed in HBEs. These results are consistent with a model wherein MALAT1 reprograms specifically cancer cells through reorganization of epigenetic marks to promote metastasis through mesenchymal gene programs.
35	Xiaomeng Du	HPV integration into the human genome may lead to alternative splicing events in HNSCC	Background: Head and neck squamous cell carcinomas (HNSCCs) are the 6th most common cancer. Approximately 50% cases are associated with a DNA virus, Human papillomavirus (HPV), that can integrate into the genome. Recent studies have suggested alternative mechanisms of oncogenesis in addition to the loss-E2-overexpress-E6/7 pathway. Here, we aim to test the hypothesis that HPV integrations can lead to alternative splicing events, resulting in chimeric/new transcripts of genes that may drive oncogenesis. Methods: We obtained samples from HPV16+ cell lines and patients and linked them to categories of integration sites complexity we labeled as type 1 (single copy), type 2 (localized genomic rearrangements) or type 0 (no integration) using HPV-targeted-capture DNA-sequencing data. We then statistically assessed the overlap between fusion/splicing sites at the integration locations. We performed Oxford Nanopore DNA and RNA sequencing to HPV16+ cell line samples. We aligned the reads and compared detected mRNA sequences to known HPV16 isoforms. Results: We observed a statistical difference in the on-HPV-off-Human-splice-site (Wilcox-rank-test P-value=0.04) events across the type 1&2 samples and in the off-HPV-off-Human-splice-site events (P-value=0.0006). Similar differences are observed between type 0&2. The RNA sequencing data of type 2 sample SCC-090 supported 7/16 known HPV16 mRNA isoforms strongly and 4 isoforms absent. Conclusion: HPV integrations into human genome may introduce chimeric transcripts and alter abundancy of isoforms. Further, we plan to perform multiple alignment to separate supporting reads for each HPV isoforms and analyze long read samples of type 1 and 0 to seek preponderant isoforms. This will help us 1) refine isoform references; 2) identify major chimeric isoforms; 3) explore how these integrations may alter local gene expressions that link to HNSCC carcinogenesis.
36	Leidy Johana Vanegas Cano	Investigating the role of ribosomal RNA pseudouridylation in regulating translation	Pseudouridine (Ψ) is the most ubiquitous modification across eukaryotic ribosomes. Dyskerin (DKC1), the primary pseudouridine synthase responsible for rRNA pseudouridylation, utilizes small nucleolar RNA (snoRNA) as a guide to direct the specific positioning of uridine at its active site, where the enzyme subsequently modifies the rRNA1. In mammalian cells, DKC1 deficiency shows compromised translation initiation and fidelity2,3. Despite evidence linking rRNA pseudouridylation to ribosome function, how these modifications are regulated and their role in selective

			mRNA translation remains unclear. To investigate this, we examined whether DKC1 expression levels influence ribosome pseudouridylation and mRNA translation. Using the Genotype-Tissue Expression (GTEx) Portal, we selected three cell lines with varying DKC1 gene expression, ranging from low to high abundance: SKOV3 (Ovarian adenocarcinoma), 549 (lung adenocarcinoma), and Hep G2 (hepatocellular carcinoma). Western blot and RT-qPCR analysis showed DKC1 expression differed between cell lines at both the RNA and protein level, which suggests that its regulation depends on the cell type. In vitro and in vivo translation assays revealed significantly higher translation activity in SKOV3 than in A549 and Hep G2, suggesting that intrinsic factors in SKOV3 cells may enhance translation efficiency, possibly through ribosome modifications or associated protein interactions. Additionally, Purified ribosomes from A549 and SKOV3 are active and can restore translation in ribosome-depleted extracts.
37	Yousuf Khan	yakRNA: A multimodal RNA designer	RNA is a molecule that can adopt a variety of folds and therefore functions. Like proteins, RNAs have been a target for exploitation to generate synthetic molecules that can adopt enhanced and novel functions. High-throughput assays, combined with meticulous biochemistry, has led to the generation of some artificial RNAs but the ability to program RNAs with an intended function remains difficult. While generative models have revolutionized protein design, RNA design remains challenging due to the dearth of 3D RNA structures. Here we present You Always Know RNA (yakRNA), a frontier language model that can reason over sequence, biological function, consensus sequence, and secondary structure to generate sequences of the user's specification. yakRNA is not trained on any 3D structural information but rather a corpus of semantically labeled sequences. yakRNA is able to design sequences based off any combination of multimodal inputs. We were able to prompt yakRNA to generate complex Programmed Ribosomal Frameshift (PRF) signal RNAs that were able to change the reading frame of an elongating ribosome at a rate higher or comparable to most other PRF signals. Additionally, these RNAs had no BLAST homology to any known sequence, demonstrating yakRNA's ability to generate complex and novel RNAs.
38	Helen SaTsu	Development of Gallium-68 Labelled Antisense Oligonucleotides to Enable Biodistribution Studies	Antisense Oligonucleotides (ASOs) are synthetic nucleic acids that are designed to base-pair to a target mRNA and modulate its expression. ASOs are being used as disease-modifying therapies, with many FDA-approved medicines in the market. As they continue to gain traction as therapeutics, there is an increasing demand to understand and optimize their biodistribution and pharmacokinetics (PK). Positron emission tomography (PET) is one non-invasive approach that can facilitate ASO preclinical development by imaging radiolabeled ASOs to quantify in vivo PK. We have established an ASO radiolabeling method using gallium-68. An ASO targeting mouse MALAT-1 lncRNA with a hexylamine modification was conjugated to the NOTA chelator (precursor). Gallium-68 was eluted from a 68Ge/68Ga generator, concentrated, and reacted with the precursor ([68Ga]Ga-NOTA-ASO). The apparent specific activity of [68Ga]Ga-NOTA-ASO was 2.05 mCi/μg. To study biodistribution of radiolabeled ASOs delivered systemically, nu/nu mice were injected with [68Ga]Ga-NOTA-ASO. ASO distribution in the mice was subsequently evaluated by dynamic PET/CT scans. [68Ga]Ga-NOTA-ASO was observed to have rapid clearance and primarily accumulated in the kidneys (6.33 %ID/g) and adrenal glands (1.75 %ID/g). Biodistribution was markedly different compared to free [68Ga]GaCl3 and [68Ga]Ga-NOTA. This work introduces a straightforward method to radiolabel ASOs and study their biodistribution in vivo using preclinical models and PET imaging. This technique addresses an unmet need for rapid, non-invasive evaluation of in vivo ASO delivery.
39	Julia Haas	Covalently Targeting Conserved Cysteines in RNA Binding Domains	RNA Binding Proteins (RBPs) control many cellular processes including transcription, translation, and alternative splicing. RBPs have the most disease annotated mutations of any protein class, but these proteins have historically been considered "unligandable" due to their i) highly dynamic and disordered nature and ii) high level of conservation within their RNA binding domains (RBDs). We hypothesized that differences in protein dynamics could be exploited to enable covalent ligands to selectively target conserved cysteines within RBDs. To investigate this, we screened a library of cysteine-reactive covalent ligands against homologous RBPs with differences in their protein dynamics: heterogeneous nuclear ribonucleoproteins H and F (hnRNP H

			and F). hnRNPs H and F are an excellent model system for RBP conservation because they have > 85% sequence conservation, three parallel quasi-RNA recognition motifs (qRRMS), and three conserved cysteines (C22, C34, & C122) within their RBDs. Despite this conservation, we observed differences in the covalent labeling of hnRNP H and F during in vitro screenings. Notably, we found one small molecule with a ten-fold difference in selectivity for hnRNP H over hnRNP F and we consistently observed greater covalent labeling of cysteines in hnRNP H. This work demonstrates that covalent ligands can be used to differentially engage conserved cysteines. Moreover, our findings suggest that the unique dynamics of hnRNP H, specifically slower conformational exchange, may contribute to greater covalent labeling. By understanding how protein dynamics contribute to covalent selectivity, we can cultivate a powerful new approach for targeting RBPs and other dynamic and disordered proteins.
40	Maddy Zamecnik	Title: Incorporating common ASO modifications into mRNAs impacts translation speed	RNA therapeutics require chemical modifications to their RNAs to extend their lifespans in the cell. Nucleobase modifications like N1-methylpseudouridine have recently been shown to increase the effectiveness of mRNA therapeutics, but modifications including 2'-fluoro RNA (2'F) and locked nucleic acids (LNA) have been used for decades in ASO therapeutics to improve binding affinities, decrease immunogenicity, and slow RNA degradation. However, these modifications have not been explored for their potential applicability to mRNA therapeutics. 2'F and LNA are ideal candidates for further exploration in emerging mRNA therapeutics because they are safe for humans, compatible with existing delivery techniques, and commercially available for use in synthetic oligos. Given this knowledge gap, we employed an in vitro, reconstituted, E. coli translation system to assess how a set of modifications commonly used in ASOs influences the kinetics of translation elongation. Our findings indicate that the effects of the modifications on peptide bond formation are context dependent, varying with position of the modification in the codon. Singly or doubly modified 2'F or LNA modified codons showed either no or mild (4-fold) changes in amino acid addition rates relative to unmodified codons. Furthermore, these results held for multiple repeated amino acid additions. We expanded on these results using molecular dynamics simulations, which identified the mechanisms affecting the translation of modified mRNAs, and now presents a path for further optimization. These results suggest that the suite of RNA modifications already developed for ASOs may provide a viable avenue to expand the chemical toolbox for emerging mRNA therapeutics.
41	Vanessa Quevedo Barrios	Lipid Nanoparticles for the Delivery of KLIPP, a Precision Cancer Genome Targeting Therapeutic	Advances in whole-genome sequencing and CRISPR technologies have enabled the identification of sequence-specific features that are unique to an individual cancer genome, creating new opportunities for precision therapeutics. In the Ljungman lab, we have developed KLIPP, a CRISPR-based therapeutic strategy that selectively targets cancer-specific structural variant junctions (SVJs) while sparing normal cells. KLIPP employs a split-enzyme design in which a catalytically dead Cas9 (dCas9) is fused to the Fok1 endonuclease. Paired single-guide RNAs (sgRNAs) are designed to flank SVJs, such that FokI dimerization occurs only when the two Fok1 subunits bind adjacently at the cancer specific junction; leading to an induced DNA double-strand break. KLIPP is delivered as mRNA, which necessitates a drug delivery system to penetrate cells. To address this challenge, KLIPP mRNA is encapsulated in lipid nanoparticles (LNPs). Our work focuses on optimizing KLIPP delivery by evaluating the delivery of the KLIPP components in pre-formed and loaded LNP formulations to maximize functional mRNA delivery and therapeutic efficacy. In parallel, we are developing strategies to functionalize LNP surfaces for targeted delivery, with the goal of directing KLIPP mRNA and sgRNAs to tissues and organs that are traditionally difficult to access with LNPs. Together, this work integrates whole genome sequencing, CRISPR precision targeting, and mRNA delivery to advance a personalized precision cancer therapeutic platform.
42	Angelica Previero	THE MOLECULAR IMPLICATIONS OF	Polyploidy is a highly widespread phenomenon, appearing in processes such as evolution, cancer, regeneration, and development, sometimes in all cells which are part of a tissue, while other times in only a subset. However, even though polyploidy is

		TETRAPLOIDY ON GENE EXPRESSION	clearly a fundamental phenomenon across life, the gene expression differences between diploid and polyploid cells are not clear. One of the few organisms that can be fully turned tetraploid is the roundworm Caenorhabditis elegans. Tetraploid C. elegans develop more slowly, are less reproductively fit, age faster, and have an overall shorter lifespan. Besides physiological differences, RNAseq data also shows differential gene expression in tetraploid C. elegans with respect to their diploid counterparts. Our hypothesis is that the lower surface-to-volume ratio affects the interactions between the DNA and the nuclear lamina, consequently affecting gene expression. Physiological and sequencing data about otherwise wild type diploid and tetraploid worms as well as diploid and tetraploid cec-4 mutants and diploid lon-2 mutants elucidate the implications of tetraploidy and the molecular basis of such consequences. Furthermore, this data highlights the effects of multiple copies of DNA on specific genes and starts to elucidate why this not always advantageous phenomenon is so conserved across different organisms and stages of life.
43	Neil White	RNA and the Elements of Life	RNA is an essential molecule when thinking about how life biochemically came to be and of great importance in the biology of the modern era. RNA devices that regulate gene expression, termed Riboswitches, both exemplify RNA Relics of the ancient RNA World and recent evolution. We have recently validated the nhaA-I, nhaA-II and DUF1646 motif RNAs as riboswitch classes that selectively bind Na+ or Li+. The Na+ riboswitch is only the second device, in all domains of life, known to bind Na+ and regulate gene expression. The Li+ riboswitch represents the first biological aptamer for Li+. Thus, RNA plays a major role in elemental ion biology which is just starting to be appreciated. We are seeking to address the fundamental nature of how RNA interacts with monovalent ions in a specific manner. We are also pursuing an understanding of the fundamental biology of Li+ in bacteria, which we hypothesize will have important implications for human mental health.

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