2026
UPSTATE NY
RNA SYMPOSIUM

2026 UPSTATE NY RNA SYMPOSIUM

March 15 - 17, 2026

AGENDA & ABSTRACT BOOKLET

Symposium Venue:
University at Albany
1400 Washington Ave
Albany, NY 12222

Poster Session & Dinner Venue:
New York State Musuem
222 Madison Ave
Albany, NY 12230

Hosted by

CERRT | CENTER OF EXCELLENCE IN RNA RESEARCH & THERAPEUTICS

The Center of Excellence in RNA Research and Therapeutics (CERRT) is a joint venture between the State University of New York at Albany's RNA Institute and University of Rochester Center for RNA Biology.

Website: rna-cerrt.org/rna-symposium

Contact: nyrnacoe@albany.edu

2026 Upstate NY RNA Symposium
Sunday, March 15th 2026

Venue: Campus Center Ballroom, University at Albany

3:00 PM – 3:50 PM	**Registration & Coffee**
Opening Session **Moderator: Kate Leamy, PhD,** Siena University	
3:55 PM – 4:10 PM	**Day 1: Welcome Remarks** **Jeanette Altarriba, Dean, College of Arts and Sciences,** University at Albany **Andy Berglund, PhD, Co-Director CERRT,** University at Albany
4:10 PM – 4:15 PM	**Keynote Introduction: Kate Leamy, PhD,** Siena University
4:15 PM – 5:00 PM	**Keynote Speaker 1: Philip Bevilacqua, PhD,** Penn State University *Computational Approaches to Uncover Insights into RNA Biology*
5:05 PM – 5:20 PM	**Selected Speaker 1: Dylan Ehrbar,** University at Albany *Aging is Characterized by Significant Shifts in tRNA Modifications, Codon Usage Bias, and Altered Translation*
5:20 PM – 5:35 PM	**Selected Speaker 2: Abby Manning,** University of Rochester *Characterization and Function of a Novel Integrator-associated Complex*
5:40 PM – 6:00 PM	**Invited Speaker 1: Zhangli Su, PhD,** University of Alabama at Birmingham *The life and rebirth of tRNA: beyond translational control*
6:05 PM – 6:30 PM	**Poster Lightning Talks (10 x 2 minutes)**
6:30 PM – 8:00 PM	**Reception**

2026 RNA Institute Annual Symposium
Monday, March 16th 2026

Venue: Campus Center Ballroom, University at Albany

8:00 AM – 8:50 AM	**Breakfast & Registration**
Session 1 **Moderator: Marlene Belfort, PhD**, University at Albany	
9:00 AM – 9:05 AM	**Keynote Introduction: Marlene Belfort, PhD,** University at Albany
9:05 AM – 9:50 AM	**Keynote Speaker 2: Brenda Bass, PhD,** University of Utah *Discriminating Self and Nonself dsRNA During Antiviral Defense*
9:50 AM – 10:10 AM	**Invited speaker 2: Hachung Chung, PhD,** Columbia University Medical Center *Self-RNA Sensing by Our Immune System in Health and Disease.*
10:10 AM – 10:30 AM	**Coffee Break**

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2026 Upstate NY RNA Symposium
Monday, March 16th 2026

Venue: Campus Center Ballroom, University at Albany

Session 2 **Moderator: Cara Pager, PhD,** University at Albany	
10:30 AM – 10:50 AM	**Invited Speaker 3: Steve Bonilla, PhD,** The Rockefeller University *Quantitative Dissection of Dynamic RNA 3D Ensembles in Viral Replication*
10:50 AM – 11:10 AM	**Invited Speaker 4: Melinda Larsen, PhD,** University at Albany *Deciphering Secretory Failure: Ribosomal Gene Dynamics as a Putative Driver of Glandular Hypofunction*
11:10 AM – 11:25 AM	**Selected Speaker 3: Andrew Munoz Gamba,** University at Albany *Phenotypic and Genotypic Signatures of Adaptation of Zika Virus After Serial Passaging in Mosquito and Mammalian Hosts*
11:30 AM – 12:00 PM	**Poster Lightning Talks (10 x 2 minutes)**
12:00 PM – 12:15 PM	**Group Picture – Outside by small fountain**
12:15 PM – 1:30 PM	**Lunch**
Session 3 **Moderator: Ken Halvorsen, PhD,** University at Albany	
1:30 PM - 1:50 PM	**Invited Speaker 5: Diana Dou, PhD,** Duke University *A Complex Issue: When 2 X's need to XIST*
1:55 PM - 2:15 PM	**Invited Speaker 6: Michael Breen, PhD** Icahn School of Medicine at Mount Sinai *Dynamic RNA Editing in the Human Brain: Disease, Development, and Therapeutic Opportunity*
2:20 PM – 2:35 PM	**Selected Speaker 4: Jacen Emerson,** University of Michigan *Alternative Splicing as a Mechanism of Oligodendrocyte Impairments in Spinocerebellar Ataxia Type 3*
2:40 PM – 3:00 PM	**Invited Speaker 7: Young Mee Jung, PhD,** Korea Institute of Science and Technology *Deep-UV Resonance Raman and Two-Dimensional Correlation Spectroscopic Analysis of mRNA Encapsulation in Lipid Nanoparticle Therapeutics*
3:00 PM – 4:00 PM	**Transition to Poster Session/Dinner at NY State Museum**
Session 4 **Poster Session and Dinner: New York State Museum**	
4:00 PM – 5:15 PM	Poster Session 1 Location: NY State Museum
5:15 PM - 6:30 PM	Poster Session 2 Location: NY State Museum
4:30 PM - 6:30 PM	Cocktail Hour Location: NY State Museum
6:30 PM – 8:00 PM	Dinner at NY State Museum

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2026 Upstate NY RNA Symposium
Tuesday, March 17th 2026

Venue: Campus Center Ballroom, University at Albany

8:00 AM – 8:50 AM	**Registration & Breakfast**
Session 5 **Moderator: Eric Wagner, PhD, Co-Director CERRT,** University of Rochester	
9:00 AM – 9:05 AM	**Keynote Introduction: Eric Wagner, PhD, Co-Director CERRT,** University of Rochester
09:05 AM – 9:50 AM	**Keynote Speaker 3: Doug Anderson, PhD,** University of Rochester *The Science and Therapeutics of RNA Stitching*
9:55 AM – 10:15 AM	**Invited Speaker 8: Monica Pillon, PhD,** University of Buffalo *Eukaryotic Ribonucleases in Gene Regulation*
10:15 AM – 10:35 AM	**Invited Speaker 9: Nicole Martinez, PhD,** Stanford University *Regulatory Features Driving Pseudouridine Synthase Activity*
10:35 AM – 10:55 AM	**Coffee Break**
Session 6 **Moderator: Gabriele Fuchs, PhD,** University at Albany	
11:00 AM – 11:20 AM	**Invited Speaker 10: Sezen Meydan, PhD,** Vanderbilt University *Landscape of Ribosome Collisions During Chemotherapeutic Stress*
11:20 AM – 11:35 PM	**Selected Speaker 5: Kerry Brown, PhD,** Department of Health, Wadsworth Center *Use your SCULL! Short ORFs Deliver Ribosomes to Downstream Genes in Mycobacteria*
11:35 AM – 11:50 PM	**Selected Speaker 6: Jack Dowling,** University of Pennsylvania *The Adenovirus Splicing Regulator L4-33K Coordinates Viral Splicing With Genome Replication*
12:00 PM – 01:15 PM	**Networking Lunch**
Session 7 **Moderator: Kaalak Reddy, PhD,** University at Albany	
01:20 PM – 01:25 PM	**Keynote Introduction: Kaalak Reddy, PhD,** University at Albany
01:25 PM – 02:10 PM	**Keynote Speaker 4: Samie Jaffrey, PhD,** Weill Cornell Medicine *New modifications of mRNA heads and tails*
02:10 PM – 02:25 PM	**Selected Speaker 7: Jack Schwartz,** University of Rochester *Regulation of Insulin Receptor Translation by a Conserved uORF*
02:25 PM – 02:45 PM	**Invited Speaker 11: Lluis Ribas, PhD,** Institute for Research in Biomedicine (IRB) *Highly Transcribed Human tRNA Genes are Hotspots for Somatic Mutations That Corrupt the Genetic Code in Cancer and Aging*
02:45 PM – 03:05 PM	**Coffee Break**
Session 8 **Moderator: Maksim Royzen, PhD,** University at Albany	
03:05 PM – 03:20 PM	**Selected Speaker 8: Soneya Majumdar, PhD** Department of Health, Wadsworth Center *A Novel Actinobacteria-Specific Ribosome Hibernation Factor in Mycobacterium tuberculosis*
03:20 PM – 03:35 PM	**Selected Speaker 9: Xuhang Liu, PhD,** Roswell Park Comprehensive Cancer Center *Machine Learning-based Small-RNA Liquid Biopsy Enables Accurate Classification of Benign, Malignant, and Metastatic Breast Diseases.*
03:35 PM – 03:55 PM	**Invited Speaker 12: Mehmet Yigit, PhD,** University at Albany *Glucometer for programmable genome sensing: Cell-free in vitro translation approach using RNA switch-CRISPR*
04:00 PM – 4:15 PM	**Awards and Closing Remarks**

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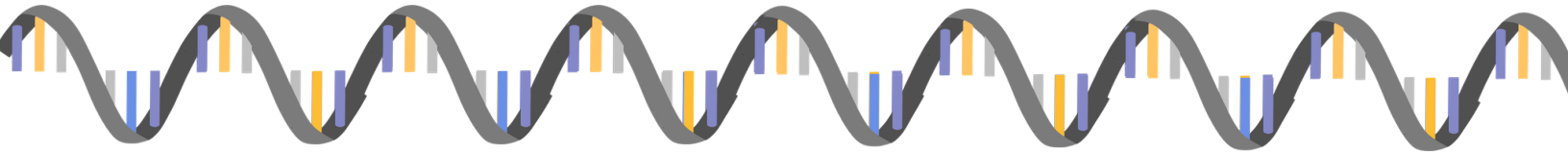

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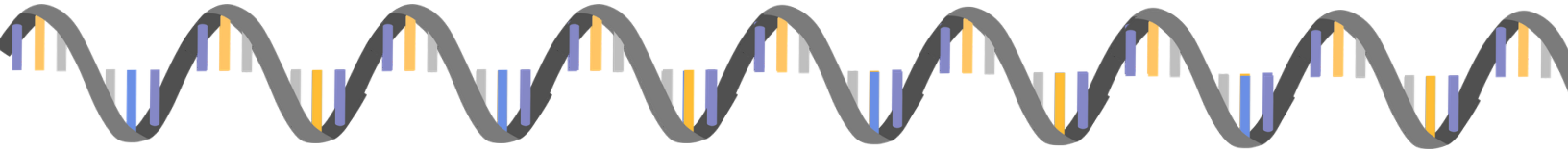

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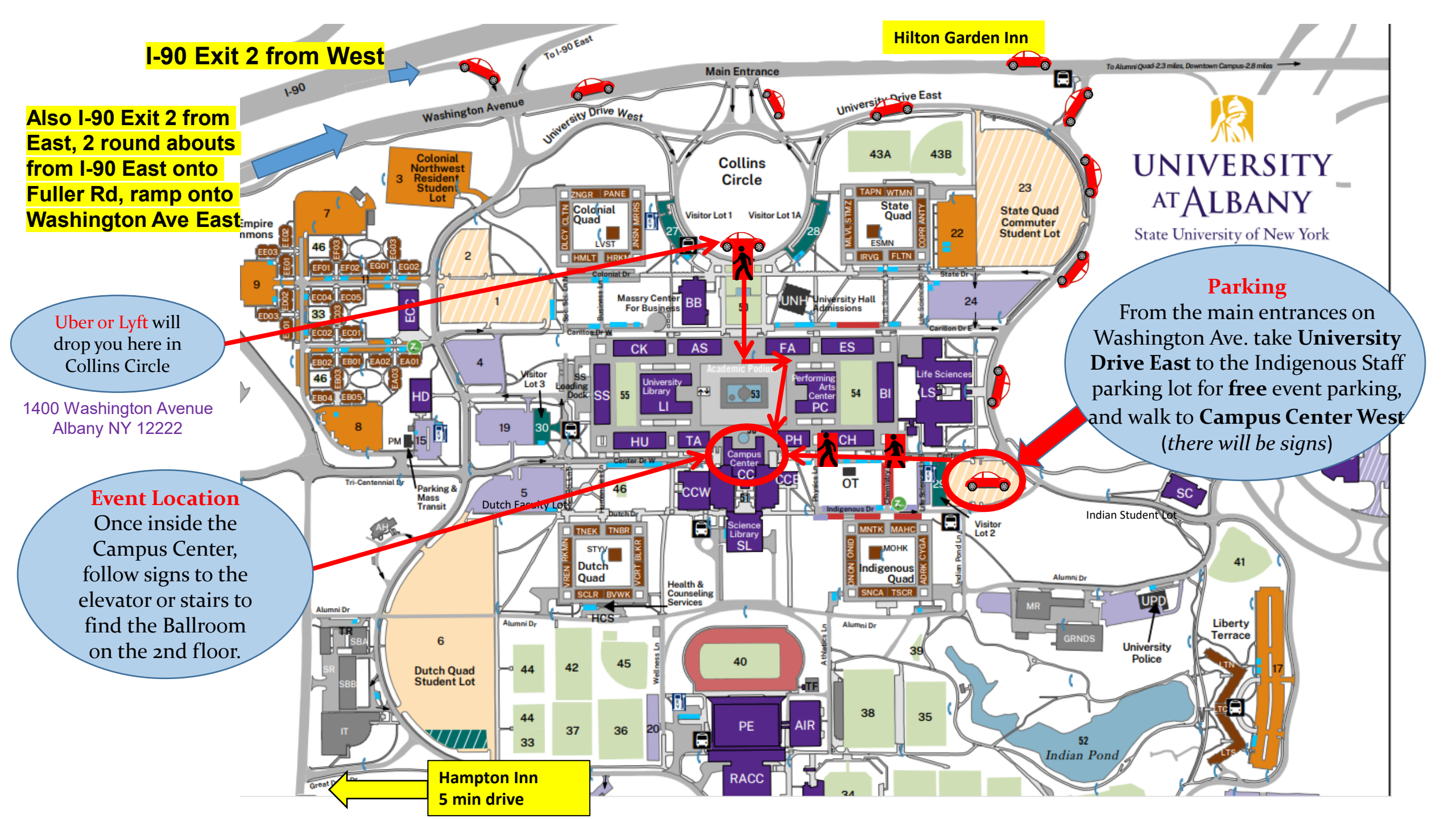
I-90 Exit 2 from West
Also I-90 Exit 2 from East, 2 round abouts from I-90 East onto Fuller Rd, ramp onto Washington Ave East
Hilton Garden Inn
UNIVERSITY AT ALBANY
State University of New York
Uber or Lyft will drop you here in Collins Circle
1400 Washington Avenue
Albany NY 12222
Parking
From the main entrances on Washington Ave. take University Drive East to the Indigenous Staff parking lot for free event parking, and walk to Campus Center West (there will be signs)
Event Location
Once inside the Campus Center, follow signs to the elevator or stairs to find the Ballroom on the 2nd floor.
Hampton Inn
5 min drive
I-90
To I-90 East
Washington Avenue
Main Entrance
University Drive West
University Drive East
To Alumni Quad-2.3 miles, Downtown Campus-2.8 miles
Collins Circle
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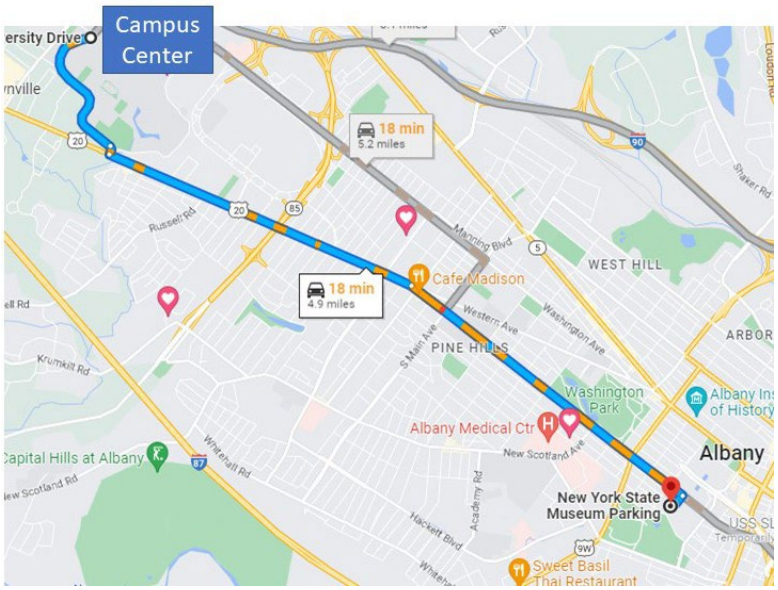
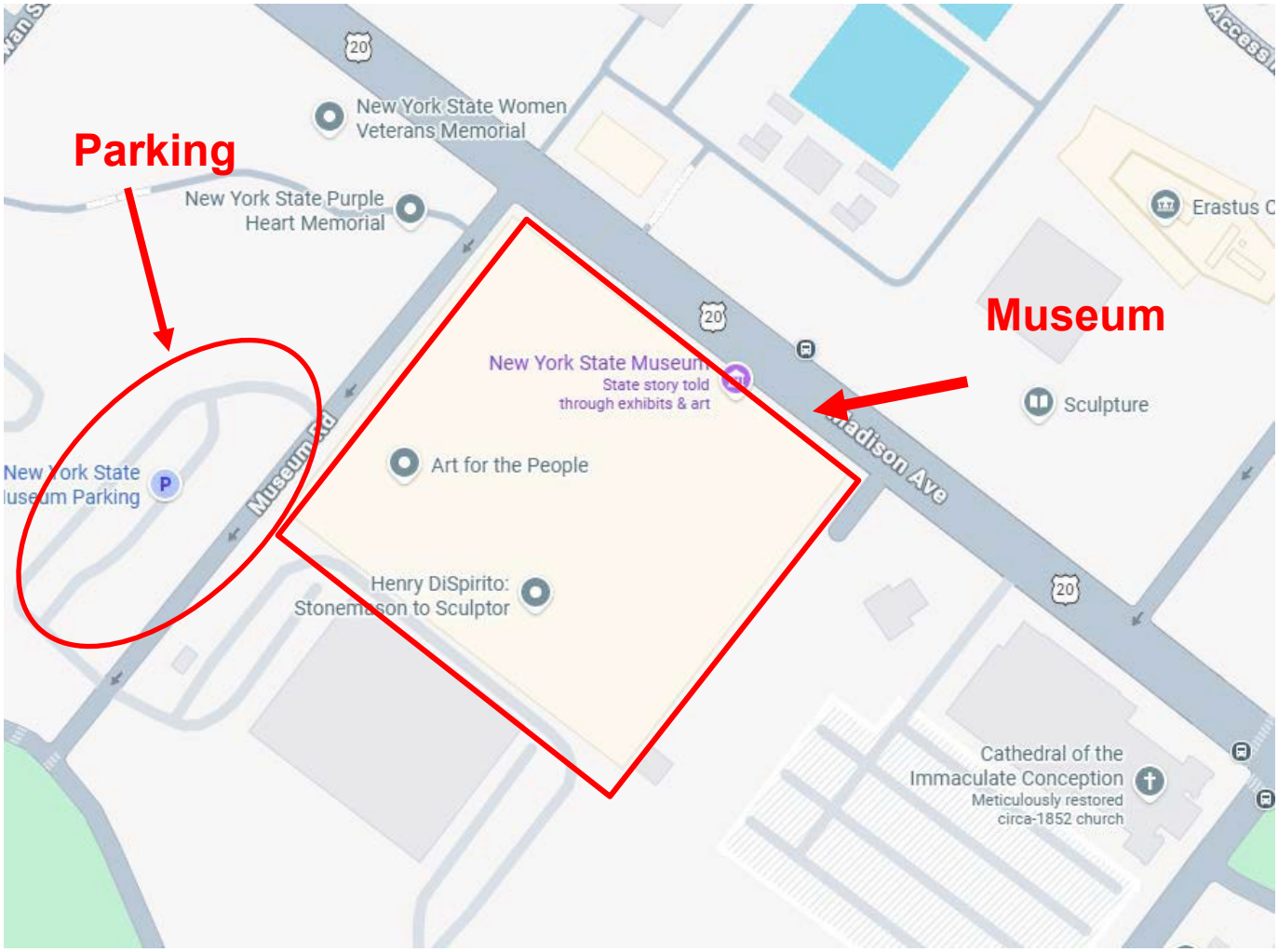
Carillon Dr E
Center Dr W
Indigenous Dr
Tri-Centennial Dr

Monday, March 16th Dinner Venue: New York State Museum
222 Madison Ave, Albany, NY 12230



Google Maps Link to Parking: https://maps.app.goo.gl/PLWzczmSdnbdt8BZ6
Google Code: J6XP+8M Albany, New York



Google Maps Link to Museum: https://maps.app.goo.gl/sK7CeopSThZxrwbv8
Google Code: J6XQ+CG Albany, New York

2026 Upstate NY RNA Symposium Dinner
NY State Museum

Drinks & Small Tuscan Bites

4:00-6:30 PM

Imported & Domestic Cheeses, Fresh Seasonal Fruits, Fresh Vegetable Crudité, Stuffed Olives, Fresh Mozzarella & Tomato Salad with Basil, Roasted Vegetable Salad, Assorted Artisan Breads

Main Dinner – Buffet Style

6:30 – 8:00 PM

Arugula Salad

Arugula, Poached Pears, Bing Cherries, Goat Cheese, Citrus Vinaigrette (dressing is GF, DF)

Dinner Rolls & Butter

Entrées

Baked Salmon – Citrus & Chive Sauce (GF)

Chicken Corleone – Sautéed Chicken with Sun-Dried Tomatoes, Spinach & Roasted Garlic Sauce (DF)

Vegetable Lasagna – Fresh vegetables, pasta, cheese & homemade sauce (V)

Carving Station

Beef Brisket – Brown Sugar–Chili BBQ (GF) & Creamy Horseradish Sauce (contains dairy)

Sides

Rosemary Red Potatoes (GF, DF, VG)

Green Beans Almondine (GF, DF, VG)

Coffee & Tea Service

Freshly brewed Coffee, Decaf & Hot Tea

Dessert Platters

Cookies, Brownies, Lemon Bars

GF = Gluten free
DF = Dairy free
V = Vegetarian
VG = Vegan

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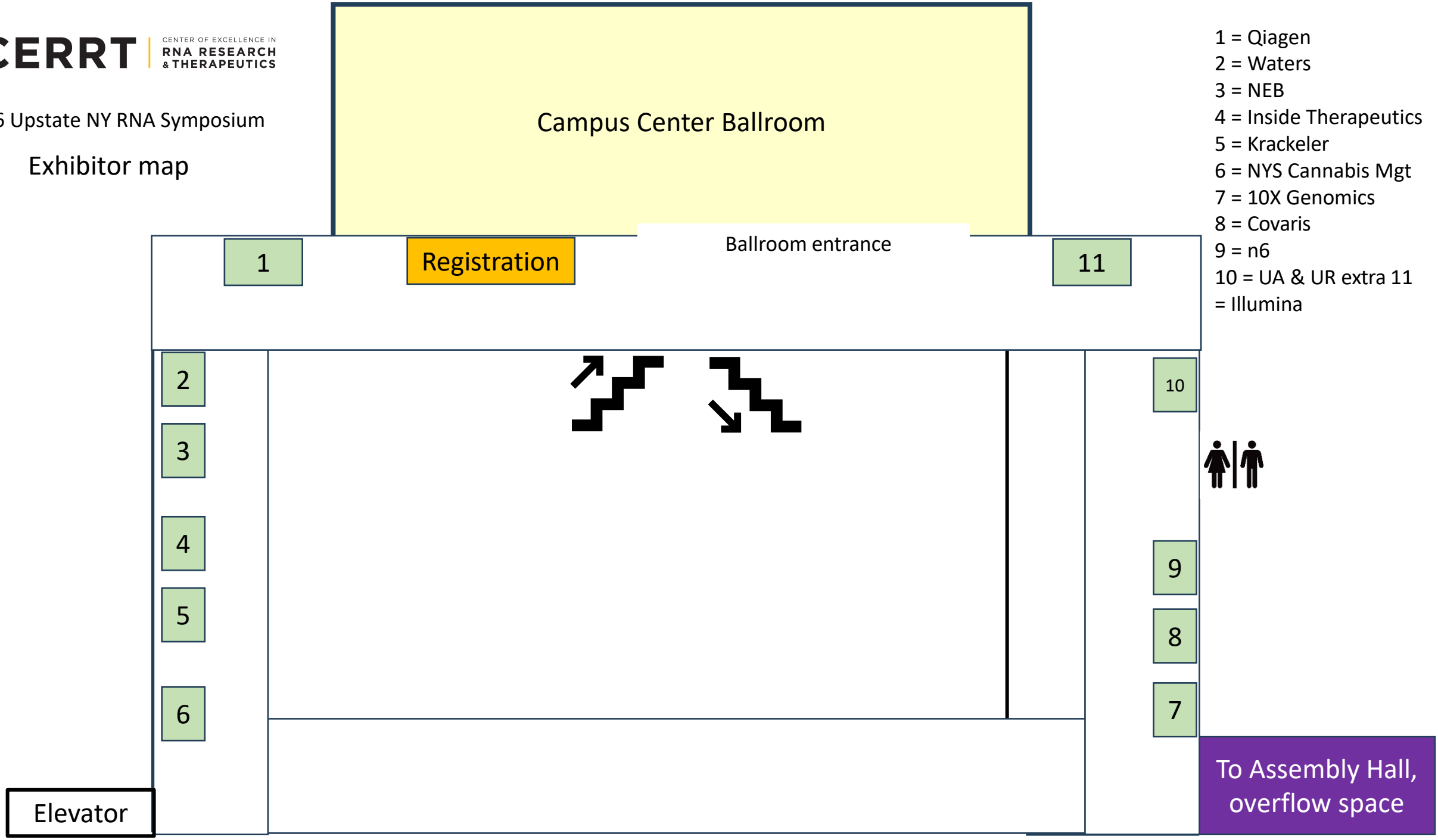
2026 Upstate NY RNA Symposium

Exhibitor map



1 = Qiagen
2 = Waters
3 = NEB
4 = Inside Therapeutics
5 = Krackeler
6 = NYS Cannabis Mgt
7 = 10X Genomics
8 = Covaris
9 = n6
10 = UA & UR extra 11
= Illumina

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2026 Upstate NY RNA Symposium Poster Information

Blue = Selected Talk Abstract, Green = Selected Lightning Talk

First Name	Last Name	Poster Number	Title	Poster Session
Naa Nuerki	**Adade**	**1**	Inosine-containing mRNA Triggers the Integrated Stress Response	**1**
Jyoti	**Adala**	**2**	Defining the landscape of R-Loop- and G-Quadruplex-Forming Sequences in the Highly Repetitive Ribosomal DNA Locus	**2**
Nurat	**Affinnih**	**3**	Investigation of Ilhéus virus infection in mosquito and mammalian cell lines	**1**
Mohammed	**Ali**	**4**	Investigating differential intron sensitivity to PUF60, a gene that is deleted or amplified in developmental syndromes and cancers	**2**
Moses	**Arthur**	**6**	Enhancing Hybridization Chain Reaction Assay Sensitivity Through Nanoporous Filtration	**2**
Dylan	**Baker**	**7**	Epigenetic Mechanisms Linking Acetate to Transcriptional and Social Behavior Changes in a Genetic Model of Autism Spectrum Disorder	**1**
Aiswarya	**Balakrishnan**	**8**	Functional role of novel CPSF73-UBE3D-DNAAF2 complex in mRNA 3'-end processing	**2**
Nilesh	**Banavali**	**9**	Predicting antibiotic binding to ribosomes	**1**
Nathaniel	**Beam**	**10**	Defining Epitranscriptomic Shifts in Myotonic Dystrophy Type 1	**2**
Alissa	**Beam**	**11**	The Effect of Integrator Subunit 11 Mutations on Integrator Function and Neuronal Development	**1**
Samira	**Bell**	**12**	PACER LncRNA Structure, Function, and Regulation of Arachidonic Acid Signaling	**2**
Logan	**Beringer**	**13**	A High-Throughput Luminescent Platform for Functional Profiling and Drug Discovery Targeting Viral 2'–5' Phosphodiesterases	**1**
Ashif	**Bhuiyan**	**14**	Optimization of CAGrb01 Analogs for Targeting Expanded CAG Repeat RNA in Spinocerebellar Ataxias	**2**
Amarnath	**Bollu**	**15**	Synthesis of Amide Modified Nucleoside Dimers for Site-specific Incorporation into siRNAs	**1**
Kerry	**Brown**	**16**	Use your SCULL! Short ORFs deliver ribosomes to downstream genes in mycobacteria - **Selected Talk**	**2**
Anna	**Carhart**	**17**	Comparing flexibility of simulated vs experimental single-stranded RNA using molecular dynamics and time-resolved fluorescence	**1**
Riley	**Carter**	**18**	Raman Spectroscopy for the Detection and Identification of Oral Fluid Stains on Common Substrates	**2**
Pramita	**Chakma**	**19**	Development of novel small molecules as potential therapeutics for Myotonic Dystrophy	**1**
Mouli	**Chakraborty**	**5**	Engineering Small-Molecule RNA Degraders to Target Disease-Relevant Structured RNAs	**1**
Aditi	**Chaubey**	**20**	3'UTR Alternative Polyadenylation Drives Metabolic Reprogramming and Functional Heterogeneity in Cancer-Associated Fibroblasts	**2**
Lily	**Cisco**	**21**	Treatment of a severe DM1 mouse model with verapamil, amlodipine, and ranolazine.	**1**
Evan	**Corwin**	**22**	Generation of a dual luciferase reporter construct to investigate translational frameshifting in SARS-CoV-2	**2**
Elianna T.	**Cruz Gonzalez**	**23**	Commonalities and differences between ATF4 and ATF3 upon induction of different branches of the integrated stress response (ISR)	**1**
Dwiti Krushna	**Das**	**25**	Grating-Coupled Fluorescence Plasmonics (GC-FP) Biosensor: Expanding from Protein to Nucleic Acid Detection	**1**
Pearl	**De-Veer**	**26**	Radiation-Induced Senescence Remodels Salivary Gland Epithelial Cell States and Is Modulated by Fisetin Treatment	**2**
Justin	**Dingman**	**27**	Next-Generation Mass-Spectrometry Sequencing of Full Length mRNA	**1**
Jack	**Dowling**	**28**	The adenovirus splicing regulator L4-33K coordinates viral splicing with genome replication - **Selected Talk**	**2**
Dylan	**Ehrbar**	**29**	Aging is characterized by significant shifts in tRNA modifications and codon usage bias and altered translation **- Selected Talk**	**1**
Jacen	**Emerson**	**30**	Alternative Splicing as a Mechanism of Oligodendrocyte Impairments in Spinocerebellar Ataxia Type 3 - **Selected Talk**	**2**
Javier	**Estrada**	**31**	Quantitative Lysosomal Profiling of Cellular Senescence Using LysoTracker Fluorescence Imaging and Automated Single-Cell Analysis	**1**

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2026 Upstate NY RNA Symposium Poster Information

Blue = Selected Talk Abstract, Green = Selected Lightning Talk

First Name	Last Name	Poster Number	Title	Poster Session
Alexander	**Evans**	**32**	Optical Melting and Thermodynamic Analysis of G-T Mismatch Pairs for Catalytic Hairpin Assembly	**2**
Sara	**Farshineh Saei**	**33**	5-Triazolyl Uracil Improves Nucleobase Stacking, Pyrimidine Recognition, Biological Activity, and Specificity of RNA-Binding Triplex-Forming Peptide Nucleic Acids	**1**
Olivia	**Fraser**	**34**	Characterization of the Mitochondrial FASTKD2 RNA Binding Protein	**2**
Jeffrey	**Gabell**	**35**	From Codons to Conductance: tRNA supplementation and informed codon optimization for therapeutically enhancing CFTR channel function	**1**
Jack	**Geary**	**36**	Methods for interrogating translation of lncRNAs	**2**
Hen	**Gelshtein**	**37**	Roles of Alternative Protonation in the Structure and Function of Biological RNAs	**1**
Saumya	**Gupta**	**38**	Beyond Zinc Fingers: The Role of the MBNL1 Linker in Splicing Control	**2**
Sakura	**Hamazaki**	**39**	From toxic RNAs to physiology, an investigation of HSALR mouse model of DM1	**1**
Mark	**Handley**	**40**	A stress-response to endogenously-produced inosine-containing mRNA	**2**
Emmett	**Hanson**	**41**	Pathogenic genome detection on paper using CRISPR and inherently fluorescent DNA nanoclusters	**1**
Jayamini	**Harasgama**	**42**	RNA Chemical Probing to Analyze the Structural Dynamics of the SARS-CoV-2 3'UTR	**2**
Ryan	**Hardy**	**43**	The Integrator Complex is Required for HSV-1 Infection	**1**
Leon	**Harvey**	**44**	Investigating the Role of RNA Helicases in Splicing Changes Caused by Cancer-Associated SF3B1 Mutations	**2**
MaryClaire	**Haseley**	**45**	HEXIM1 integrates transcriptional stress and cell cycle arrest following dual disruption of Integrator and NELF	**1**
Kate	**Hayes**	**46**	Global Transcriptional Regulation of Tissue-Specific Development	**2**
Yichen	**He**	**47**	Short RNA duplex formation in artificial cytoplasms	**1**
Olivia	**Hiltke**	**48**	Insights into the Structure and Function of RNA Using In Vivo-Like Folding Stability	**2**
Porama Zafreen	**Hoque**	**49**	Synergistic Correction of Splicing Defects in Myotonic Dystrophy Type 1 via Combination Therapy	**1**
Sean	**Jones**	**50**	Characterization of the SigD regulon and its role in susceptibility to hydrophobic antibiotics in Mycobacterium abscessus	**2**
Young Mee	**Jung**	**51**	Deep-UV Resonance Raman and Two-Dimensional Correlation Spectroscopic Analysis of mRNA Encapsulation in Lipid Nanoparticle Therapeutics	**1**
Nobuhiko	**Kamoshita**	**52**	Stimulatory and Non-Stimulatory Downstream RNA Structures for Viral Stop Codon Readthrough	**2**
David	**Kariuki**	**53**	Synthesis of Amide-Linked RNA Phosphoramidite Dimers for Backbone Engineering of CRISPR Guide RNAs	**1**
Anees Mohammed	**Keedakkatt Puthenpeedikakk**	**54**	REFINEMENT OF THE AMBER FORCE FIELD FOR RNA: IMPROVING THE DESCRIPTION OF NON-BONDED INTERACTIONS	**2**
Safi Khiati	**Khiati**	**55**	In vitro transcription of tRNASec and its effects on human cells	**1**
Nora	**Kiledjian**	**56**	Androgen deprivation therapy activates the ISR to rewire mRNA translation	**2**
Sravya	**Kovvali**	**57**	Detection of serum proteins with aptamer-based DNA nanoswitches	**1**
Nada	**Kabbara**	**58**	Controlled reassociation of polycrossover DNA molecules into duplexes	**2**
Nicholas	**Lane**	**59**	SNRPF Levels Modulate the Spliceopathy in Myotonic Dystrophy	**1**
Mahla	**Lashkari**	**60**	Using Glucometer for Genome Detection Through in vitro Translation Using RNA Switch and CRISPR technologies	**2**
Jung Yeon	**Lee**	**61**	Unbiased Sequencing of RNA Modifications for Determining True RNA Sequence Using Mass Spectrometry Readout	**1**

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Blue = Selected Talk Abstract, Green = Selected Lightning Talk

First Name	Last Name	Poster Number	Title	Poster Session
Qishan	**Lin**	**62**	Development of UPLC-MS/MS Method for Global RNA Chemical Modification Analysis in Saccharomyces Cerevisiae	**2**
Xuhang	**Liu**	**63**	Machine learning-based small-RNA liquid biopsy enables accurate classification of benign, malignant, and metastatic breast diseases -**Selected Talk**	**1**
Jiss	**Louis**	**64**	Investigating cis-elements within the DMPK transcript to modulate toxic CUG RNA levels in myotonic dystrophy type 1	**2**
Soneya	**Majumdar**	**65**	A Novel Actinobacteria-Specific Ribosome Hibernation Factor in Mycobacterium tuberculosis - **Selected Talk**	**1**
Abby	**Manning**	**66**	Characterization and function of a novel Integrator-associated complex - **Selected Talk**	**2**
Amirhossein	**Manzourolajdad**	**67**	3D-based RNA Switch Design	**1**
Amy	**Mascorro**	**68**	Dual Targeting ASO-based RiboTAC degrades CUG RNA and Rescues Mis-Splicing in HeLa-based DM1 Cell Model	**2**
Massa	**Masri**	**69**	Spinocerebellar Ataxia 2 Whole Blood Transcriptomic Analysis Reveals Novel Immune Biomarkers and Widespread Mis-Splicing	**1**
Chetna	**Mathur**	**70**	Distinct Codon Usage and tRNA Modifications Define Regulatory Control of Oncogenes in Cancer	**2**
Ian	**McClain**	**71**	Photochemistry of Oligonucleotide Chemistry	**1**
Katherine	**McCown**	**72**	Functional Assessment of Enzyme MiaA in the Conversion of Adenosine to Isopentenyladenosine in tRNA	**2**
Morgan	**McGrath**	**73**	Integrated stress response directs adaptive mRNA translation in prostate cancer following irradiation	**1**
Tristan	**Melfi**	**74**	DNA nanostructures for drug delivery across lipid membranes	**2**
Steven	**Mesite**	**75**	TAMARA: a Simple and Efficient Microfluidic Nanoparticle Formulation System Tailored for Preclinical Stuidies	**1**
Megan	**Miaro**	**76**	Prediction of non-canonical base pairs using structural constraints from sequence comparison	**2**
Subodh	**Mishra**	**77**	Therapeutic targeting of DM1 disease mechanism using a quercetin–vorinostat combination	**1**
Shirin	**Mohammadian**	**78**	The Folding Roadmap: Tracing RNA's Intermediate Landscapes via GROMACS	**2**
Katrine	**Mongin**	**79**	Structural Determination of Insulin Receptor mRNA with SHAPE-MaP Chemical Probing	**1**
Fin	**Morris**	**80**	mRNA based Senescence Detection system	**2**
Vinod	**Morya**	**81**	A 3D printed mini-gel electrophoresis system for rapid and inexpensive DNA nanoswitch biosensing	**1**
Andrew	**Munoz Gamba**	**82**	Phenotypic and genotypic signatures of adaptation of Zika virus after serial passaging in mosquito and mammalian hosts - **Selected Talk**	**2**
Rina	**Nagao**	**83**	Dynamic competition between nuclear PIA-RNA stabilization and decay shapes mitochondrial activity	**1**
Brianna S.	**Nelthrope**	**84**	Dysregulation of Alternative Splicing is a Transcriptomic Phenotype of SCA3 Mouse Models	**2**
Esther	**Neves**	**85**	The Ribosom's Enigmatic Players: Unveiling the Roles of Ribosomal RNA and RNA-Binding Protein Interactions	**1**
Taiwo (Annabelle)	**Ogunyale**	**87**	Schwann Cells Drive Salivary Gland Fibrosis via ECM Remodeling	**1**
Humphrey	**Omeoga**	**88**	The tRNA Writer MnmA Translationally Regulates Key Transcription Factors Critical to Environmental and Physiological Changes	**2**
Ebenezer	**Omojowolo**	**89**	Characterization of DIAPH1 Membrane Localization: Structural Predictions and Experimental Validation	**1**
Onyinyechi	**Onuoha**	**90**	Unraveling the Distinct Roles of the Alpha-like Subunits in RNA Polymerase I and III Complex Biogenesis	**2**
Alexander	**Parks**	**91**	Development of a Small Molecule Integrator Inhibitor to Probe its Role During Erythropoiesis	**1**
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P-001: **Naa Nuerki Adade**

Inosine-containing mRNA Triggers the Integrated Stress Response

Naa Nuerki Adade [(1)(2),] Jacob H Schroader [(1)(2),] Emmanuel Adade [(1)(2),] Alex Valm [(1)(2),] Cara Pager [(1)(2),] J. Andrew Berglund [(1)(2),] Mark Handley [(1),] Gabriele Fuchs [(1)(2),] Kaalak Reddy [(1)(2)]

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Inosine is an important RNA modification that is highly regulated. However, inosine may also arise aberrantly in RNA through cellular accumulation of the non-canonical nucleotide inosine triphosphate (ITP). Inosine monophosphate (IMP) is a precursor for generating the canonical purine nucleotides ATP and GTP, but may itself become phosphorylated into ITP during this process. Cellular ITP levels are normally restricted by the inosine triphosphate pyrophosphatase (ITPase) enzyme, which hydrolyzes ITP to generate IMP. Loss of ITPase activity due to biallelic variants in the *ITPA* gene in humans causes a fatal multisystem disorder characterized by inosine misincorporation into RNA, although the molecular consequences of this misincorporation remain unclear.

In this study, we show that cellular transfection of inosine-containing mRNAs induces stress granule formation. This response is accompanied by elevated phosphorylated eukaryotic initiation factor-2α (p-eIF2α) protein levels, a hallmark of the integrated stress response (ISR). Furthermore, we observed an increase in phosphorylation of the double-stranded RNA sensor protein kinase R (PKR), one of the main activators of p-eIF2α. These results suggest that PKR-dependent p-eIF2α activation regulates the cellular response to inosine-containing mRNA. Altogether, our study reveals that inosine misincorporation into mRNA is an unappreciated form of cellular stress with implications for pathogenesis of ITPase deficiency.

P-002: **Jyoti Adala**

Defining the landscape of R-Loop- and G-Quadruplex-Forming Sequences in the Highly Repetitive Ribosomal DNA Locus

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Bruce A Knutson, Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY

Ribosomal DNA (rDNA) is the gene locus that encodes for ribosomal RNA (rRNA), which is essential for producing ribosomes and maintaining cellular protein synthesis. The rDNA locus is a tightly regulated and repetitive genetic element organized in a head-to-tail cluster of approximately 200–300 repeats. These repeats form nucleolar organizing regions (NORs) in acrocentric chromosomes. The rDNA locus is transcribed by RNA polymerase I to generate 18S, 5.8S, and 28S rRNA, which are processed and partially assembled in the nucleolus to form ribosomal subunits (ribosomes). rDNA transcription dysregulation or aberrant rRNA modification can lead to disease states, including craniofacial developmental disorders and cancer. Given the high transcriptional activity and GC-rich nature of this region, we hypothesized that rDNA locus favors the formation of non-canonical DNA structures, such as R-loops and G-quadruplexes (G4s). R-loops consist of an RNA-DNA hybrid with a displaced single-stranded non-template DNA strand. G4s form through Hoogsteen hydrogen bonding of guanine quartets and are stabilized by monovalent cations. Stable R-loops and G4s play diverse regulatory and pathological roles by influencing transcription, replication, and genome stability. However, experimental detection of these structures in rDNA is challenging due to the repetitive nature of rDNA, which complicates mapping and has been ignored in most genome-wide studies. While studies in yeasts and plants support the presence of R-loops and G4s in rDNA, comprehensive investigation of the human rDNA locus remains limited. This gap can be addressed using computational tools designed to predict non-canonical structures based on sequence features in nucleic acid sequences. Here, we show that R-loop forming sequences (RLFS) and potential canonical G-quadruplex forming DNA sequences (pG4CS) are non-randomly distributed across the rDNA locus and are evolutionarily conserved, highlighting their potential role in maintaining rDNA stability. In conclusion, the rDNA locus is prone to form stable non-canonical DNA structures. The non-arbitrary distribution and evolutionary conservation of non-canonical DNA structures across the rDNA locus underscore their potential roles in regulating transcription, rRNA processing, and maintaining genomic stability.

P-003: **Nurat Affinnih**

Investigation of Ilhéus virus infection in mosquito and mammalian cell lines

Nurat Affinnih, Dr. Shawn Gianola, Dr. Cara Pager

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Ilhéus virus (ILHV) is a mosquito-borne flavivirus that is maintained in an enzootic transmission cycle between birds and mosquitoes. The virus has been found in mosquitoes, birds, and humans in Central and South America and the Caribbeans. The most common symptoms of ILHV are fever, headache, muscle pain, and encephalitis. Given the potential for the virus to cause significant disease, there is a need to understand ILHV infection dynamics and pathogenesis. To do this, we infected Aedes albopictus C6/36 mosquito, monkey kidney Vero, and human lung adenocarcinoma A549, and human hepatocellular carcinoma Huh7 cells at multiplicity of infection of 0.01-1 and harvested cell culture media at 0-96 hours post infection (hpi). We then performed plaque assays to examine the infection kinetics associated with ILHV. We found that there was peak infectivity of ILHV in C6/36, Vero, A549, and Huh7 cells at 96, 48, 24, and 48 hpi, respectively. Finally, we used RT-qPCR to assess viral RNA levels in C6/36 and Vero cells. We found higher viral RNA levels in Vero cells compared to C6/36 cells. Further experiments will explore the infection kinetics in other mosquito, bird, and mammalian cell lines as well as investigate the innate immune response. Ultimately, this project will elucidate ILHV infection in a range of cell types and generate knowledge about the innate immune response to ILHV infection.

P-004: **Mohammed Ali**

Investigating differential intron sensitivity to PUF60, a gene that is deleted or amplified in developmental syndromes and cancers

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PUF60 is a splicing factor related to the polypyrimidine-tract binding protein U2AF2. PUF60 is deleted in developmental disorders such as Verheij syndrome, and amplified in approximately 8% of cancers. Thus both increases and decreases in PUF60 expression can have profound physiological effects. However, little is known about how changes in PUF60 expression impact global splicing patterns. Specifically, we aim to determine whether PUF60 amplification and deletion influence the same splicing events in opposite directions, or affect distinct exons. To investigate this, I employed CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) in mouse embryonic stem cells (mESCs) to transcriptionally upregulate or downregulate Puf60. Successful establishment of the CRISPRa/i system uncovered extensive transcriptional, post-transcriptional, and post-translational regulation of Puf60 protein expression. To this point, we observed that Puf60 protein levels in normal mESCs drop dramatically when the cells reach a critical density, leading to cell death. This suggests that Puf60 may be targeted by ubiquitin ligases, with cells maintaining stringent control over Puf60 protein levels in response to stress. Analysis of phosphoprotein data revealed phosphorylation sites at the N-terminus of PUF60 that match ERK consensus motifs. Consistent with this hypothesis, treatment with a MEK inhibitor led to a significant downregulation of the protein at an early time point. Using our validated cell lines, we investigated the effects of Puf60 modulation on splicing outcomes. We find that altering Puf60 expression impacts specific splicing events, including its own splicing autoregulation, providing valuable insights into the functional consequences of PUF60 dysregulation.

P-006: **Moses Arthur**

Enhancing Hybridization Chain Reaction Assay Sensitivity Through Nanoporous Filtration

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The Hybridization Chain Reaction (HCR) is an enzyme-free isothermal nucleic acid amplification technique that uses DNA hairpins to amplify specific DNA or RNA sequences. While HCR has been demonstrated to have the ability to detect mRNAs in cells with single-copy sensitivity, its application in diagnostics has been limited by background signal and insufficient sensitivity at low target concentrations caused by free hairpins. To address this, there is a need to selectively separate amplified HCR products from unreacted hairpins to enhance the sensitivity and reduce the limit of detection (LOD) of the assay. Hairpins are composed of 36 nucleotides and are approximately 8 nm long in folded form. In contrast, HCR products consist of long nicked dsDNA helices formed by the hybridization of many hairpins, yielding structures ≥ 80–100 nm in length. Due to this difference in length, we hypothesized nanoporous filtration to be an ideal strategy for selectively retaining HCR products while removing folded hairpins, thereby improving the signal-to-background ratio and lowering the assay's limit of detection. Nanoporous silicon nitride membranes with an average pore size of 60 nm were chosen as the filtration barrier. A two-stage approach combining HCR amplification with subsequent nanoporous filtration was implemented. This approach was tested using model DNA and RNA triggers and evaluated using fluorescence microscopy and spectrofluorometry. For the DNA and RNA triggers, the limit of detection was found to be 10 zM and 3 aM, respectively. This method provides a simple strategy to enhance HCR-based diagnostic and research applications that require highly sensitive nucleic acid detection.

P-007: **Dylan Baker**

Epigenetic Mechanisms Linking Acetate to Transcriptional and Social Behavior Changes in a Genetic Model of Autism Spectrum Disorder

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Autism Spectrum Disorder is a neurodevelopmental condition characterized by social deficits and repetitive behaviors. Both genetic and environmental factors contribute to ASD, including mutations to Shank3 gene and an altered gut microbiome. Shank3 knockout (KO) mice exhibit microbiome alterations, reduced levels of short-chain fatty acid (SCFA) acetate, and social deficits. Antibiotic treatment was found to exacerbate social impairments and levels of acetate, whereas acetate supplementation rescues social behavior and induces transcriptional changes in the medial prefrontal cortex (mPFC). The molecular mechanisms underlying acetate-driven transcriptional remodeling in the mPFC remain unknown.

Male and Female Shank3 KO mice and Wt controls were divided into the following treatment groups starting at post-natal day 21: Control water, Antibiotic, acetate, or Antibiotic+ acetate. When animals reached PND60 they were behaviorally tested and mPFC samples collected for westernblot analysis.

Analysis revealed a decrease in pan acetylation in Shank3 deficient mice at baseline. Treatment with antibiotics caused a decrease in pan acetylation which reaches significance in KO mice compared to Wt controls. Treatment with acetate and antibiotic + acetate was found to significantly increase pan acetylation in both Wt and Shank3 KO mice compared to control counterparts.

These results indicate that acetate alters brain gene expression and behavior via influencing histone acetylation in the mPFC. Next, we will assess changes to specific histone marks implicated in ASD including H3K27AC. To ascertain how acetate changes histone acetylation future experiments will knockdown expression of the enzyme required for histone acetylation (acetyl-CoA synthase 2) in mPFC followed by behavioral, transcriptional and epigenetic analysis.

P-008: **Aiswarya Balakrishnan**

Functional role of novel CPSF73-UBE3D-DNAAF2 complex in mRNA 3'-end processing

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Proper 3’-end processing of the pre-mRNA is crucial for RNA polymerase II termination, mRNA stability, transport, and translation. Even the slightest disruptions to this process can lead to a multitude of diseased states. The Cleavage and Polyadenylation (CPA) machinery carries out this process with Cleavage and Polyadenylation Specificity Factor 73 (CPSF73) serving as the endonuclease subunit that cleaves the pre-mRNA. Although the nuclear role of CPSF73 is well established, the mechanism by which it is assembled in the cytoplasm with other CPA members and trafficked into the nucleus remains largely unknown.

We have identified a novel hetero-trimeric complex comprising CPSF73, ubiquitin protein ligase E3D (UBE3D), and dynein axonemal assembly factor 2 (DNAAF2) in the cytoplasm. In collaboration with the Tong laboratory, we have solved the cryo-EM structure of this trimeric complex. To define the functional role in mRNA 3'-end processing, we employ a combination of genetic knockouts and degron-based rapid depletion systems coupled with PolyA-ClickSeq (PAC-Seq) 3'-end sequencing techniques in mammalian cells. Complete loss of UBE3D by CRISPR knockouts resulted in co-depletion of CPSF73. PAC-Seq analysis on UBE3D knockouts revealed widespread 3'UTR lengthening in several genes due to the shift in cleavage towards the distal poly(A) sites. This suggests that disrupting the cytoplasmic binding partners of CPSF73 can impact its nuclear function. To further validate the functional role of each member of this trimeric complex, we are establishing degron lines for rapid and temporal depletion.

P-009: **Nilesh Banavali, PhD**

Predicting antibiotic binding to ribosomes

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Individual codons in mRNA are “translated” by ribosomes and their accessary factors to individual amino acids, which they then connect sequentially to form functional proteins. This protein synthesis mechanism is essential for all living organisms, which is why ribosomes are primary targets for antibiotics in pathogen bacteria. Many structures showing such antibiotic binding to bacterial ribosomes have been solved since the first high-resolution structures for bacterial ribosomes were determined at the turn of the century. Nevertheless, bacterial ribosomes have species-specific differences, and unanticipated features continue to be revealed in recent structures. We determined the hibernating 70S ribosome structure of Borrelia burgdorferi (Bbu), the causative agent of Lyme disease. The Bbu ribosome uniquely has two ribosomal proteins (uL30 and bL37) combined into one uL30 protein through an alpha-helical linker located near the peptidyl transferase center – the catalytic site of the ribosome and a target of multiple antibiotics. It is expensive and cumbersome to individually determine a structure for each antibiotic bound to each specific pathogen bacterial ribosome. It is therefore necessary to find effective and inexpensive computational methods to accurately predict binding of any antibiotic to any ribosome. In this work, we employ structural analogy, restrained energy minimization, and single-point binding free energy computations to predict binding of 35 different clinically relevant antibiotics to the small subunit of the Bbu ribosome. These single-point binding free energies are sensitive to small conformational changes in the antibiotic as well as its environment, which are both shown to be prevalent in previous structural data. The implications of these results in structure-guided design and the prospect of narrow-spectrum antibiotics for specific bacterial pathogens are discussed.

P-010: **Nathaniel Beam**

Defining Epitranscriptomic Shifts in Myotonic Dystrophy Type 1

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RNA modifications in the form of the enzyme-catalyzed epitranscriptome regulate processes such as RNA splicing, localization, translational fidelity, and the cell cycle.1–3 The epitranscriptome is reprogrammed in response to stress caused by increased reactive oxygen species (ROS) and DNA damage to regulate gene and protein expression.4-5 Expression of RNA from DNA repeat expansions can stress cells and disrupt cellular function, with expanded CUG repeats from the 3' UTR of the Dystrophia Myotonica Protein Kinase (DMPK), resulting in aberrant splicing, RNA foci formation, and increased levels of senescence in Myotonic Dystrophy Type 1 (DM1).6–9 The consequences of toxic RNA, such as elevated senescence and mitochondrial dysfunction, along with the physical symptoms of muscle wasting and early balding, lead some to consider it a progeroid condition.10 Preliminary data have confirmed increased levels of senescence in DM1 fibroblasts at basal conditions and that these cells are more sensitive to genotoxic stress. Longitudinal RNA-seq data reveal upregulation of DNA damage and senescence-associated genes, including p21, that worsen over time. Inversely, there is broad down-regulation of mitochondrial genes and RNA modifiers. Changes in these genes correlate strongly with clinical markers of DM1, such as repeat length and splicing index. We hypothesize that alterations in RNA modification deposition impair the cell's ability to respond to stress inherent in the disorder, leading to increased levels of DNA damage and senescence. Our study aims to define the spectrum of modifications dysregulated in DM1 in response to cellular stress and describe the contribution of RNA modification systems to senescent programs in DM1 models. We will perform absolute quantification RNA sequencing (AQRNA-seq) and an mRNA enhanced version to identify sequence-specific modifications of tRNA and mRNAs, including the repeat-expanded DMPK. Providing the sequence-specific location of RNA modifications will allow us to determine specific pathways and shifts in RNA pools that occur between disease states and stress induction. We will then test whether altering the expression of these RNA-modifying enzymes can alleviate or exaggerate DM etiology and/or senescence onset. These findings could help explain the heterogeneity found between patients and tissue types, along with opening new therapeutic strategies for DM1. Connecting the epitranscriptome to DNA damage and senescent programming may shed light on the induction of senescence and the aging process.

P-011: **Alissa Beam**

The Effect of Integrator Subunit 11 Mutations on Integrator Function and Neuronal Development

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Brain development is under precise spatiotemporal genetic control, and dysfunction in gene regulation can result in severe neurodevelopmental disorders (NDDs). A key point of control for gene expression occurs at the transcriptional level, specifically at the RNA polymerase II (RNAPII) promoter-proximal pausing stage. There are two fates of paused RNAPII: release into productive elongation or termination via Integrator (INT). INT is a metazoan-specific complex that induces termination through its endonuclease and phosphatase activities. INTS11 is the active endonuclease and recently biallelic variants have been identified as potentially causative of severe neurodevelopmental defects. Despite the link to NDDs and the overall importance of INTS11, the majority of the INTS11 mutations have yet to be thoroughly characterized. I am using a multi-pronged approach to understand the impact of INTS11 mutations on INT function. To study the effect of INTS11 missense mutations on general INT function, I will rescue INTS11 loss from HEK293T INTS11 Auxin-inducible degron lines. I will measure the capability of each variant to rescue INTS11 loss through RNA-seq. Variants will be further characterized by their ability to disrupt interactions with known INTS11-interactors using coimmunoprecipitation and quantitative mass-spectrometry. These two approaches will nominate INTS11 mutations for introduction into human embryonic stem cells (hESCs) which will then be differentiated into brain organoids and analyzed via a multiple-omics approaches to observe the mutations effects on neuronal development. Altogether, this work will provide novel insight into the importance of INTS11 in brain development.

P-012: **Samira Bell**

PACER LncRNA Structure, Function, and Regulation of Arachidonic Acid Signaling

Samira Bell, Samuel Desind, Carol Lutz

Rutgers Graduate School of Biomedical Sciences

Our laboratory investigates the roles of long noncoding RNAs (lncRNAs) and micro RNAs (miRNAs) in lung cancer, focusing on their regulation of inflammation and arachidonic acid metabolism. The enzyme cyclooxygenase-2 (COX-2), a rate-limiting producer of prostaglandins and a key mediator of inflammatory response, is regulated by several lncRNAs and miRNAs. The lncRNA p50-associated COX-2 extragenic RNA (PACER) is known to regulate COX-2 transcription and is upregulated in lung cancer. Our data suggest that PACER is critically involved in COX-2 transcription and dysregulation in lung cancer cells. We show that PACER and COX-2 mRNA expression are regulated by similar feedback mechanisms. Wound healing assays indicate that shRNA knockdown of PACER significantly impacts LUAD cell (A549) migration. Using the bioinformatic tool LncLOOM, we identified conserved miRNA binding sites within PACER. We are investigating whether these miRNAs add complexity to the arachidonic acid signaling pathway and COX-2 regulation through interactions with PACER. To assess the translational relevance of this regulatory axis, we are also studying the murine PACER ortholog, Ptgs2-Os, to determine whether it exhibits similar effects on COX-2 expression in mouse models. In parallel, we are using selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) to resolve the secondary and tertiary structures of PACER and Ptgs2-Os at single-nucleotide resolution, with a focus on evolutionarily conserved miRNA binding motifs. Together, these findings aim to uncover conserved structural and regulatory mechanisms that govern COX-2 expression in cancer and inflammation.

P-013: **Logan Beringer**

A High-Throughput Luminescent Platform for Functional Profiling and Drug Discovery Targeting Viral 2′–5′ Phosphodiesterases

Logan Beringer - Master Student (Department of Chemistry, RNA Institute, University at Albany)

Yu-Sheng Chen - PhD Candidate (Department of Chemistry, RNA Institute, University at Albany)

Jia Sheng - Principle Investigator (Department of Chemistry, RNA Institute, University at Albany)

Viruses have evolved diverse strategies to evade host innate immune defenses and promote their replication. Viral Protein 3 (VP3) functions as a viral phosphodiesterase that mimics human PDE12 by degrading 2′–5′ oligoadenylates (2′–5′A), thereby preventing activation of the RNase L pathway and blocking RNase L—mediated cleavage of viral dsRNA during infection. Homologous viral phosphodiesterases, including those encoded by other rotaviruses, share this conserved mechanism of 2′–5′A cleavage. Despite the virological and biological importance of VP3, sensitive and quantitative methods for measuring its enzymatic activity remain limited. Here, we report the development of a highly sensitive, high-throughput luminescence-based enzymatic assay for VP3 using a one-pot strategy that couples VP3 activity with T4 polynucleotide kinase (PNK) and the AMP-Glo™ (Promega) assay. In this system, VP3 cleaves 2′–5′A to generate 2′,3′-cyclic adenosine monophosphate (A>p) and adenosine, which is concurrently converted to adenosine monophosphate (AMP) by T4 PNK in the presence of ATP, producing ADP during phosphate transfer. The AMP-Glo™ system depletes residual ATP, then converts AMP to ADP, and subsequently converts ADP to ATP for luciferase-based detection. This coupled enzymatic cascade amplifies product formation into a quantifiable luminescent signal, with ADP generated by T4 PNK providing additional amplification beyond AMP formation, thereby enabling highly sensitive, real-time kinetic measurement of VP3 activity in a high-throughput format. This integrated one-pot platform establishes the first highly sensitive luminescence-based kinetic assay for VP3 and provides a robust, quantitative, and high-throughput method for enzymatic characterization, with broad applicability to homologous viral phosphodiesterases.

P-014: **Ashif Bhuiyan, PhD**

Optimization of CAGrb01 Analogs for Targeting Expanded CAG Repeat RNA in Spinocerebellar Ataxias

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Spinocerebellar ataxias (SCAs) are a group of inherited neurodegenerative disorders characterized by progressive loss of coordination. Several subtypes, including SCA1, SCA2, SCA3, SCA6, SCA7, and SCA12, are caused by expanded CAG repeats that produce CAG repeat RNAs and, in most cases, polyglutamine (polyQ) proteins. Despite their severity, no disease-modifying therapies are available for these CAG repeat RNAs. Our study previously identified a small molecule, CAGrb01, that significantly lowers CAG repeat RNA levels in patient-derived cell models of multiple SCAs and in a mouse model of SCA1 and demonstrated blood–brain barrier penetration. However, its clinical potential is limited by poor aqueous solubility. To address this limitation, I performed in silico docking of over 6,000 structurally diverse CAGrb01 analogs against CAG RNA and selected 30 candidates based on docking scores and predicted solubility (logS). The analogs were evaluated, and CAGrb01-A1 was identified as a new lead candidate. It demonstrated solubility up to 50 μM and reduced toxicity relative to the parent compound in HEK293T and A11 cells. Nano-dual luciferase and multiplex RT-qPCR assays confirmed suppression of expanded CAG RNA and polyQ protein expression. Further structure–activity analysis informed the design of 8 improved derivatives. Our data suggests that small molecules that bind expanded CAG RNA hold promise as potential therapeutics for the large family of CAG expansion diseases.

P-015: **Amarnath Bollu, PhD**

Synthesis of Amide Modified Nucleoside Dimers for Site-specific Incorporation into siRNAs

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The small interfering RNAs (siRNA) are mimics of miRNAs but designed to be more selective and specific in targeting the mRNA for RNA interference (RNAi).1a,b The major challenge for siRNAs is the exo/endo nucleases mediated degradations. The chemical modifications such as 2′-fluoro, 2′-O-methyl nucleosides, thiophosphate backbone are being implemented, however still exo/endo nuclease mediated degradations were observed.1b,2 From our previous studies, replacing the phosphate backbone of siRNA guide strand with amide modification (Figure 1A) retained high RNAi activity for the PIK3CB on-target mRNA in HeLa cells (IC50 < 100 pmol) with high on-target selectivity.3a,b To further study the amide modified siRNAs involving different human cells and in vivo conditions it is necessary to further modify the amide nucleosides and their siRNAs. The aim of the present study is to synthesize amide modified nucleoside dimers having 2′-fluoro and/or 2′-O-methyl nucleosides, and incorporate them at site-specific positions of the guide strands of designed siRNAs, and validate their RNAi activity in the human cells. Synthesis of the nucleoside amide dimers (Figure 2B) and phosphoramidates will be discussed.

Selected Speaker – Session 6: **Kerry Brown, PhD**

Use your SCULL! Short ORFs deliver ribosomes to downstream genes in mycobacteria

Kerry Brown (1), Keith Derbyshire (1)(2), Todd Gray (1)(2), Joseph Wade (1)(2)(3)

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In mycobacteria, ~ 25% of mRNA transcripts are leaderless, meaning that they completely lack a 5' UTR. Analysis of ribosome profiling data from Mycobacterium smegmatis and Mycobacterium tuberculosis indicate that many leaderless mRNAs encode a short ORF upstream of an annotated gene. The stop codons of many such short ORFs overlap the start codon of the downstream gene, suggesting that the two ORFs are translationally coupled such that translation of the upstream ORF promotes translation of the downstream ORF. Hence, we propose that these short, coupled, upstream leaderless ORFS ("SCULL" ORFs) function to promote translation initiation of the downstream gene. Mutational analysis of reporter constructs using SCULL ORFs suggest that the same ribosome that translates the SCULL ORF also translates the coupled downstream ORF. We hypothesize that in mycobacteria, leaderless, coupled sORFs serve as an alternative ribosome loading mechanism that is distinct from the canonical 30S-dependent translation initiation mechanism. Analysis of other species' genomes suggest that this phenomenon is prevalent amongst diverse prokaryotes.

P-017: **Anna Carhart**

Comparing flexibility of simulated vs experimental single-stranded RNA using molecular dynamics and time-resolved fluorescence

Anna Carhart, RNA Institute UAlbany

Dr. Alan Chen, Chemistry Dept, UAlbany

Briana Yanson, RNA Institute UAlbany

We are using computational methods in conversation with collaborator-run experimental studies to simulate a modified 2-aminopurine molecule. The aminopurine's modifications cause it to fluoresce under specific conditions which permit us to track fluorescence "on and off" states to better understand its molecular structure and overall behavior. Using the molecular dynamics simulation tool GROMACS, we are able to observe both stacking states associated with fluorescence - quenched and unquenched - as well as the rotational correlation of the modified 2-aminopurine.

P-018: **Riley Carter**

Raman Spectroscopy for the Detection and Identification of Oral Fluid Stains on Common Substrates

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Body fluid traces found at a crime scene serve as a key source of DNA evidence. Raman spectroscopy has attracted significant interest in forensic applications because of its ease of use, quick analysis, and non-destructive nature. Particularly, it has proven to be remarkable in identifying body fluids. However, biological stains are often found on interfering substrates that limit the practical use of this technology. To overcome this limitation, we have developed an approach for the analysis of oral fluid stains on different substrates. Oral fluid was deposited onto plastic soda bottles, rubber, and clear plastic baggies to simulate the oral fluid stains that might be found as evidence. Raman spectra were acquired from the stains using 785 nm laser excitation, with measurements collected across different regions of each stain. Our results indicate that this approach can be effectively applied to detect traces of oral fluid on various substrates, particularly homogeneous surfaces, using simple chemometric methods. When fully developed, this approach could be applied in forensic investigations to detect traces of oral fluid on homogeneous substrates.

P-019: **Pramita Chakma**

Development of novel small molecules as potential therapeutics for Myotonic Dystrophy

Pramita Chakma[1,2], Jesus A. Frias[1,3], Sawyer M. Hicks[1,3], Hormoz Mazdiyasni[1], Nada Elniel[1,2], Subodh K. Mishra[1], Clara Yeboah[1,3], Sweta Vangaveti[1], John D. Cleary[1], Kaalak Reddy[1,3], and J. Andrew Berglund[1,3,] Ting Wang[1*]*

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Myotonic dystrophy is an autosomal multisystemic disorder which currently lacks approved treatments that target the expanded CUG/CCUG repeats (CUG exp /CCUG exp) RNA. DM type 1 (DM1) and type 2 (DM2) are caused by the expression of expanded CUG and CCUG repeats RNA, which sequester the muscleblind-like (MBNL) family of RNA binding proteins leading to DM associated splicing defects. The Berglund lab has previously identified diamidine and furamidine which rescued DM1 dysregulated alternative splicing events, however their potential was limited by toxicity and off-target effects. To address these limitations, we have designed and synthesized a series of modified polycyclic compounds (MPCs) through systematic modifications of the core scaffold and the end cap structures. An optimized synthetic route was developed for rapid access to MPC analogs, aiming to enhance their splicing rescue, improve solubility, and increase blood brain barrier (BBB) penetration. This approach identified two lead compounds, MPC03 and MPC04, that rescued DM1 dysregulated splicing events at low nanomolar concentrations with no distinct toxicity and limited off target effects in multiple DM1 cell models. In addition, we also aim to study mechanistic insights into interaction between MPCs and CUG/CCUG repeats RNA. Taken together, these findings will support MPCs as potential therapeutics candidates for myotonic dystrophy.

P-005: **Mouli Chakraborty**

Engineering Small-Molecule RNA Degraders to Target Disease-Relevant Structured RNAs

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Structured RNAs are essential regulators of gene expression and are increasingly recognized as contributors to the pathogenesis of cancer and other RNA-mediated diseases. Despite their significance, therapeutic targeting of RNA remains challenging because many structured RNAs are not amenable to modulation by traditional small-molecule approaches. This study presents a chemical-biology strategy to convert RNA-binding small molecules into RNA degraders that selectively eliminate disease-associated transcripts. The approach centers on the design of nuclease-independent RNA degraders by conjugating established RNA-binding scaffolds with chemical warheads that facilitate phosphodiester bond cleavage. A focused library of Dovitinib–PEG conjugates featuring diverse warhead chemistries, including imidazole-based derivatives, was synthesized. Cellular assays revealed that several conjugates significantly reduced miR-21 levels, demonstrating that small molecules can be engineered to induce targeted RNA degradation independently of endogenous nucleases. To evaluate the generalizability of this platform, the strategy was applied to MALAT1, a cancer-associated long non-coding RNA with defined structural motifs. Modification of a known MALAT1-binding small molecule into degrader constructs resulted in decreased MALAT1 levels, as measured by RT-qPCR and RNA-FISH, and exhibited enhanced potency relative to the parent compound. Collectively, these results establish that RNA-binding small molecules can be rationally engineered into functional RNA degraders, thereby expanding the chemical toolkit for targeting structured RNAs implicated in human disease.

P-020: **Aditi Chaubey**

3′UTR Alternative Polyadenylation Drives Metabolic Reprogramming and Functional Heterogeneity in Cancer-Associated Fibroblasts

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense, fibrotic stroma that can comprise up to 90% of the tumor mass. This stromal compartment actively shapes tumor progression and therapeutic response. Cancer-Associated Fibroblasts (CAFs) are the predominant cellular component of the tumor microenvironment and play central roles in remodeling the tumor landscape. CAFs are heterogeneous, consisting of inflammatory, myofibroblastic, and antigen-presenting subtypes with context-dependent pro- and anti-tumorigenic functions. While these populations have been phenotypically defined, the molecular mechanisms driving their transcriptional heterogeneity remain unclear.

Our previous work identified 3′UTR-Alternative Polyadenylation (APA) as a key contributor to gene dysregulation in PDAC. APA regulates mRNA stability, localization, and translation, thereby influencing cellular processes such as proliferation, migration, and metastasis. We have shown that increased 3′UTR shortening is enriched in pro-tumorigenic inflammatory CAFs. However, the functional role of APA in CAF biology has not been fully characterized.

To address this, we used human-derived CAF cell lines and pharmacologically modulated APA in vitro, followed by PAC-Seq to assess transcriptome-wide 3′UTR dynamics. Our results demonstrate that APA-dependent changes disrupt key metabolic regulators in CAFs, reducing their ability to support tumor cell growth. Inhibition of APA promotes a migratory and senescent phenotype and alters the expression of genes essential for CAF function and metabolism. We further identify a novel mechanism by which the metabolic gene SLC7A11 is regulated through APA. Collectively, our findings establish APA as a critical post-transcriptional regulator of CAF function and stromal heterogeneity in PDAC.

P-021: **Lily Cisco, PhD**

Treatment of a severe DM1 mouse model with verapamil, amlodipine, and ranolazine.

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Our lab showed the benefit of verapamil, amlodipine, or ranolazine in a bi-channelopathy mouse model for myotonic dystrophy type 1 (DM1). All three treatments improved reduced survival, reduced muscle strength, prolonged time of righting and myotonia, and severe transient weakness. We were then interested in testing if verapamil, amlodipine, or ranolazine treatment could improve survival and health in DM1 mouse models that exhibit the full panoply of altered splicing and myopathic features.

We chose to treat the HSALR/Mbnl1-/- mouse model with verapamil, amlodipine, or ranolazine to see if there was similar benefit to what was seen in the bi-channelopathy mouse. The HSALR/Mbnl1-/- mouse combines CTG-repeat expansion with loss of MBNL1, which are both seen in DM1. We measured muscle force generation, respiratory function, body weight, time of righting reflex, and overall survival.

Treatment with verapamil or amlodipine allowed us to investigate if blocking Ca2+ entry via CaV1.1 alone is able to increase lifespan and overall health in HSALR/Mbnl1-/- mice. Targeting myotonia with ranolazine allowed us to determine if reducing a dominant skeletal muscle feature improves survival and muscle function. We found that treating with verapamil, amlodipine, or ranolazine improved survival, increase muscle strength, improved respiratory function, increased body weight, and reduced time or righting in the HSALR/Mbnl1-/- model.

These results suggest that targeting CaV1.1 conduction with verapamil or amlodipine has therapeutic benefit, even in a complex model for DM1. Further, long term treatment with ranolazine improved survival and health in the HSALR/Mbnl1-/- mice. The next step will be to test if there is a synergistic impact of combining a calcium channel blocker with an anti-myotonic. Since these drugs are FDA approved, rapid translation to the clinic would be expected.

P-022: **Evan Corwin**

Generation of a dual luciferase reporter construct to investigate translational frameshifting in SARS-CoV-2

Evan Corwin (Department of Biological Sciences and The RNA Institute, University at Albany),

Gabriele Fuchs, Ph.D. (Department of Biological Sciences and The RNA Institute, University at

SARS-CoV-2 has an ssRNA genome that is translated upon infection. The first open reading frame, ORF1a, contains a stop codon, producing a truncated polyprotein. Programmed ribosomal frameshifting can occur at a frameshifting stimulatory element (FSE) between ORF1a and ORF1b which pushes ribosomes into the -1 frame, allowing translation of the full-length ORF1ab. ORF1ab encodes necessary proteins for viral proliferation like RNA dependent RNA polymerase. ORF1a and ORF1ab are translated in a specific ratio. Previous work suggests that SARS-CoV-2 may also undergo non-canonical translation initiation. One form of non-canonical translation initiation common to viruses is the use of internal ribosome entry sites (IRESs). However, the relationship between translation initiation, ribosome spacing, and frameshifting efficiency is unknown. To measure how the translation initiation rate affects ribosome frameshifting, we are developing a dual luciferase reporter construct. We inserted the SARS-CoV-2 FSE into a firefly luciferase-nanoluciferase construct and confirmed the dual luciferase assay could be run in HEK-293T cells following transfection. The firefly luciferase is in the 0 frame while nanoluciferase is in the -1 frame, thus the ratio of firefly to nanoluciferase readout serves as our measure of frameshifting efficiency. We then inserted a stem loop to inhibit canonical translation initiation. Next, we will insert IRESs from PV (poliovirus), EMCV (encephalomyocarditis virus), HCV (hepatitis C virus), and CrPV (cricket paralysis virus) from our existing Renilla-IRES-firefly luciferase constructs, as well as the SARS-CoV-2 5'UTR, into our frameshifting reporter. These viral IRESs are known to have different translation initiation efficiencies. Finally, we will transfect HEK-293T cells with our final constructs and perform the dual luciferase assay to determine how the rate of translation initiation impacts ribosome frameshifting in our reporter. Currently, our results suggest our desired reporter construct can be generated and this dual luciferase assay can be performed for our purposes.

P-023: **Elianna T. Cruz Gonzalez**

Commonalities and differences between ATF4 and ATF3 upon induction of different branches of the integrated stress response (ISR)

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The integrated stress response (ISR) is a canonical intracellular mechanism vital for restoring and maintaining homeostasis in response to four main stressors, endoplasmic reticulum (ER) stress, amino acid starvation, heme deficiency, and viral infection. These stressors activate the kinases PERK, GCN2, HRI, and PKR, respectively, which phosphorylate eIF2α at serine 51, suppressing global cap-dependent translation while promoting selective translation of stress-response genes. Among these are the well-known modulator of the ISR, activating transcription factor 4 (ATF4) and its downstream target, activating transcription factor 3 (ATF3). ATF3 and ATF4 are regulators of the ISR but appear to have opposite roles during Zika virus (ZIKV) infection. However, the genes and pathways individual to and shared between the transcription factors remains unknown. Using transcriptomics and genetic knockout models, we present a broad view of genes and pathways affected by the regulatory activity of ATF3 and ATF4. We found that canonical ER stress and unfolded protein response (UPR) pathways are largely dependent on ATF4 activity, whereas ATF3 regulates genes involved in mRNA splicing upon induction of ER stress. Under amino acid starvation both ATF3 and ATF4 models showed higher gene expression, compared to ER stress and ZIKV infection, suggesting their high involvement in stress-specific responses. Ribosomal RNA processing and ribosome biogenesis were primarily ATF3-dependent, while autophagy pathways appeared independent of ATF3. Anti-viral processes and production of type 1 interferons upon infection with ZIKV have been previously identified as ATF3-dependent pathways, our analysis expands to show these pathways are independent of ATF4, while regulation of other immune genes depends on ATF4 activity. Our findings present a detailed overview of pathways shared and dependent to ATF3 and ATF4 during the ISR, providing a basis for future studies into ATF4 and ATF3 activity upon activation of different branches of the ISR.

P-024: **Daphne Rincon**

Cross-Disease Therapeutic Approaches for CAG Microsatellite Expansion Diseases

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Abstract: CAG microsatellite expansion diseases including spinal and bulbar muscular atrophy (SBMA), Huntington's Disease (HD), and spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7, and 12 all share the production of CAG expansion RNAs and, in most cases, the expression of toxic poly- glutamine containing proteins. These features result from expansions in disease-specific genes. Despite these shared features, there is an absence of effective cross-disease therapeutics and clear understanding of the molecular mechanisms driving the disease pathogenesis. We have identified novel small molecules with efficacy across various models of CAG SCAs. However, the clinical translation of these molecules remains limited. Our approach focuses on exploring the potential of FDA-approved small molecules. We have developed and validated a screening system based on a CAG-expressing cell line and have completed an initial compound screen. This system allows us to assess the effects of FDA-approved compounds on cell viability, CAG repeat RNA levels, and polyglutamine protein accumulation. Our lead candidates could potentially advance the development of therapeutic strategies with broader applicability across multiple CAG expansion disorders.

P-025: **Dwiti Krushna Das**

Grating-Coupled Fluorescence Plasmonics (GC-FP) Biosensor: Expanding from Protein to Nucleic Acid Detection

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Disease diagnosis and biomarker screening require developing highly sensitive and adaptable biosensing platforms. This research focuses on a biosensing platform known as grating-coupled fluorescence plasmonics (GC-FP), which utilizes periodic nanoscale gold gratings to amplify fluorescence emission through surface plasmon-coupled emission (SPCE). When polarized light excites surface plasmons at the metal-dielectric interface, fluorophores near the surface couple their emission into these plasmon modes, resulting in ~100-fold signal enhancement compared to conventional flat-surface fluorescence. This enhancement improves the detection of low-abundance targets in complex biological samples while maintaining spatial specificity through multiplexed arrays.

GC-FP chips are fabricated at the Albany NanoTech 300 mm wafer cleanroom facility using photolithography, resulting in 500 nm pitch gratings coated with ~70 nm gold and ~3 nm titanium. Biological reagents such as antigens, antibodies, aptamers, or oligonucleotides are deposited using a precision piezo-driven microarrayer (Scienion sciFLEXARRAYER S3) in spatially defined arrays. Samples and reagents are flowed across the chip via automated microfluidics, and fluorescently labeled targets are detected using a Centinela imaging platform (Ciencia, Inc.). We have previously demonstrated GC-FP-based serological detection of Lyme disease and COVID-19 antibodies with excellent sensitivity and specificity. Recent developments have translated the platform into point-of-care formats, including dipstick and lateral flow configurations.

Building on this foundation, current work focuses on extending GC-FP to nucleic acid-based detection. Short oligonucleotide probes printed on the grating surface capture complementary DNA or RNA targets through room-temperature hybridization. Preliminary results demonstrate detection of DNA-DNA hybridization events down to 100 pM of the target strands, with specificity confirmed through spatially resolved multiplexing in which only complementary probe regions exhibit fluorescence.

In parallel, we are exploring aptamer-based detection of small molecules such as dopamine using a modular duplex-bubble switch design. A fluorophore-labeled DNA aptamer hybridized to a quencher-bearing displacement strand remains quenched until target binding induces conformational change, increasing fluorophore-quencher separation and generating a SPCE-enhanced fluorescent signal.

We are also investigating switchback DNA strand displacement on the GC-FP platform, tracking fluorescence changes as invading strands displace switchback structures to form conventional duplexes.

P-026: **Pearl De-Veer**

Radiation-Induced Senescence Remodels Salivary Gland Epithelial Cell States and Is Modulated by Fisetin Treatment

Pearl De-Veer[1], Sergo Gabunia[1], Ifunanya Godslove[1], Victoria Gellatly[1], Kennedi Weston[1], Dierdre A. Nelson[1], Catherine Ovitt[2], Melinda Larsen[1]

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Radiation therapy for head and neck cancer frequently results in persistent salivary gland dysfunction associated with impaired epithelial maintenance and repair. Increasing evidence implicates cellular senescence as a driver of radiation-induced tissue injury; however, the specific epithelial populations affected, and their contribution to gland hypofunction remain incompletely defined. Using a murine model in which 15 Gy irradiation is delivered to the neck region to mimic the cumulative effects of IR in cancer patients, we examined structural and molecular alterations within salivary epithelial cells. Radiation exposure was associated with depletion of $Smgc^+$ intercalated duct cells accompanied by increased γH2AX accumulation and reduced lamin B1 expression, consistent with a senescence-associated phenotype. Irradiated mice were treated with the senolytic flavonoid, fisetin. Functional assessment demonstrated increased saliva production in fisetin-treated irradiated mice compared with vehicle-treated controls. Single-cell transcriptomic analysis revealed fisetin-associated shifts in epithelial cell population distributions, including increased representation of SMG acinar cell populations compared with vehicle-treated irradiated controls, suggesting remodeling of the epithelial landscape following treatment. Collectively, these findings support a model in which radiation induces senescence-associated disruption of epithelial populations contributing to gland hypofunction and suggest that targeting senescence pathways may represent a therapeutic strategy to restore salivary gland function following IR.

P-027: **Justin Dingman, PhD**

Next-Generation Mass-Spectrometry Sequencing of Full Length mRNA

Dr. Justin Dingman (SUNY Albany), Dr. Shenglong Zhang (PI, SUNY Albany), Dr. Jung Yeon Lee (SUNY Albany), Dr. Sergei Reverdatto (SUNY Albany), Dr. Jiangfen Jian (DirectSeq Bioscience), Dr. Tony Frudakis (DirectSeq Bioscience), Dr. Ruixin Yang (DirectSeq Bioscience), Tianchen He, (SUNY Albany)

Messenger RNA (mRNA) therapeutics have rapidly advanced into clinical development, elevating primary sequence integrity to a critical quality attribute requiring comprehensive and quantitative validation. Current analytical approaches — including partial-digestion LC–MS/MS and nanopore direct RNA sequencing — either rely on reference-guided fragment alignment or lack the accuracy and exhaustiveness required for regulatory-grade verification of long, chemically modified transcripts. Here we present an integrated next-generation mass spectrometry platform enabling reference-free, de novo sequencing of full-length mRNA at single-nucleotide resolution using LC–MS[1]. The workflow combines scaffold-generating enzymatic digestion, complementary mass-ladder fragmentation chemistry, and a computational reconstruction framework that assembles overlapping de novo reads without prior sequence input. Applied to the 1,937-nt NIST Research Grade Test Material mRNA (RGTM 10202) and a 1,737-nt N1-methylpseudouridine–substituted mRNA standard, the platform achieved ~100% highest-confidence base calling with complete sequence reconstruction and reproducible performance across independent experiments. In addition to confirming intended product sequences, the method quantitatively resolved coexisting sequence impurities, including cap and terminal heterogeneity, with stoichiometric precision. By enabling chemically interpretable, exhaustive sequence validation independent of predefined reference alignment, this platform establishes mass spectrometry as a scalable foundation for regulatory-grade characterization of therapeutic mRNAs and emerging RNA modalities.

Selected Speaker – Session 6: **Jack Dowling**

The adenovirus splicing regulator L4-33K coordinates viral splicing with genome replication

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Adenovirus (AdV) is a dsDNA virus that relies on host RNA processing factors to promote viral splicing. Adenovirus encodes its own splicing regulator, L4-33K, to shift viral splicing towards weak 3′ splice sites during the late stages of infection. Mechanisms of L4-33K function are unknown, including whether it contributes to other late viral processes such as genome replication. Re-analysis of existing IPOND-mass spectrometry data during AdV infection reveals that L4-33K is enriched at sites of replicated DNA. Additionally, I have found that L4-33K is strongly enriched in the chromatin during infection. To identify which host proteins L4-33K co-opts to alter viral splicing, I performed FLAG co-immunoprecipitation followed by mass spectrometry. Analysis of the FLAG-33K interactome reveals that L4-33K interacts with cellular proteins involved in two separate molecular processes: RNA splicing and DNA replication. Using siRNAs to target RNA splicing factors interacting with FLAG-33K revealed that RBM17 is essential for splicing of viral transcripts carrying weak 3′ splice sites. This is consistent with previous reports identifying RBM17 as a regulator of cellular transcripts carrying weak 3′ splice sites. FLAG-33K also strongly interacts with NAP1L1 and NAP1L4, core histone chaperones that mediate the reorganization of nucleosomal histones as well as linker histone H1. L4-33K expression is sufficient to recruit NAP1L proteins into the chromatin and siRNA depletion of these proteins impacts AdV progeny production. We have also identified that excessive L4-33K production during AdV infection changes the morphology of viral replication compartments, suggesting that L4-33K has a role in viral genome replication. Finally, predicted structures of L4-33K using AlfaFold3 reveals that L4-33K is structurally similar to cellular core histone proteins. We postulate that L4-33K is serving as a viral histone mimic which binds to nascently replicated DNA to impact not only viral genome fate but also modulate alternative splicing through RBM17.

Selected Speaker – Opening Session: **Dylan Ehrbar**

Aging is characterized by significant shifts in tRNA modifications and codon usage bias and altered translation

Dylan Ehrbar (1)(2), Kennedi Weston (1)(2), Evan Davis (3), Qishan Lin (2)(4), Peter Dedon (5)(6), Melinda Larsen (1)(2), J. Andres Melendez (2)(3), Thomas J Begley (1)(2)

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Aging is a major risk factor for chronic disease and is closely linked to cellular senescence, a stable state of cell-cycle arrest driven by telomere attrition and stress. While transcriptional reprogramming is a hallmark of senescence, the contribution of translational regulation, particularly tRNA modifications, remains poorly defined. tRNA modifications can impose codon-specific effects on translation, with tRNA and codon usage representing regulatory layers that may shift with age. We analyzed transcriptional and translational codon usage bias across 54 human tissues from approximately 1,000 donors, comparing genes up- and down-regulated with age. Across most tissues, we observed strong, highly significant age-associated shifts to one of two dominant codon usage patterns in up-regulated genes, preference for G/C-ending (GC3) or A/U-ending (AU3) codons. Consistent with these tissue-level findings, IMR90 human lung fibroblasts exhibited a shift from AU3 codon usage during replication to GC3 usage upon senescence, closely resembling primary ex vivo fibroblasts and selected aged tissues. Senescent IMR90 cells also showed significant reductions in 13 tRNA modifications relative to replicative cells, a trend similarly observed in aged salivary glands, including loss of N3-methylcytidine (m3C). tRNA modification and codon usage in IMR90's was accompanied by altered polysome occupancy for 2827 RNAs and a pronounced age-associated decline in translational efficiency of the pro-proliferative transcription factor MYB, whose codon usage is linked to m3C-modified tRNAs. Our ongoing studies are testing whether restoring m3C-dependent translation can rescue youthful translational programs and decrease aspects of the senescent phenotype.

Selected Speaker – Session 3: **Jacen Emerson**

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

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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

P-031: **Javier Estrada**

Quantitative Lysosomal Profiling of Cellular Senescence Using LysoTracker Fluorescence Imaging and Automated Single-Cell Analysis

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Cellular senescence is commonly evaluated using SA-β-Gal staining, p16/p21-associated markers, and growth arrest, yet many assays remain difficult to quantify reproducibly and are not easily scaled across conditions. A consistent feature of senescence is remodeling of the lysosomal compartment, motivating lysosome-targeted fluorescent probes as an imaging readout. LysoTracker labels acidic vesicular organelles and is widely used, but it is often applied qualitatively rather than as a standardized quantitative measure.

We are establishing a fluorescence microscopy workflow to quantify senescence-associated lysosomal remodeling using LysoTracker and automated single-cell feature extraction. The assay combines LysoTracker with DAPI to enable nuclear-based segmentation and per-cell measurement, and includes SA-β-Gal as an orthogonal benchmark when needed. Human fibroblasts are used as a primary model system, with planned evaluation across passage-driven replicative senescence and comparison to stress-induced paradigms such as DNA damage and oxidative stress. Imaging is performed with fixed acquisition settings (objective, exposure, gain) to support cross-plate and cross-day comparability. Images are processed with an automated pipeline that segments nuclei from DAPI, defines per-cell regions, and extracts LysoTracker-derived features including mean and integrated intensity, lysosomal area fraction, puncta size statistics, and texture descriptors that quantify spatial organization. Quality control filters are incorporated to minimize artifacts from debris, saturation, and segmentation failures. Outputs are summarized as per-cell feature distributions and per-well aggregate scores to support systematic comparisons across conditions and timepoints.

This workflow is designed to provide a quantitative, microscopy-friendly layer for senescence assessment that captures cell-to-cell heterogeneity and supports development of a graded senescence score rather than binary classification. The framework is readily scalable to multi-condition experiments and compatible with AI-assisted analysis for higher-throughput imaging studies of senescence and stress responses.

P-032: **Alexander Evans**

Optical Melting and Thermodynamic Analysis of G-T Mismatch Pairs for Catalytic Hairpin Assembly

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Nucleic acid amplification tests (NAATs) are critical tools for the detection of viral infections. The gold standard NAAT is PCR, with a limit of detection (LOD) of 20 copies of target RNA/mL of sample. One of the major challenges with PCR is the need for these tests to be performed in centralized laboratories with experienced technicians and reagents that are expensive and sensitive to contamination and temperature changes. As an alternative, catalytic hairpin assembly (CHA) is an enzyme-free nucleic acid amplification technique using 2 metastable DNA hairpins to recognize and amplify the presence of target nucleic acids (DNA or RNA). Designing efficient CHA reactions requires optimization of intermolecular free energy of binding. One of the methods used to optimize hairpin free energy is to incorporate G-T mismatch pairs in the stem regions, destabilizing the hairpins, and allowing the CHA reaction to occur efficiently in the presence of the target. Unfortunately, nearest neighbor thermodynamic parameters of G-T mismatches have not yet been determined, making free energy modeling of hairpins and computational modeling of these reactions a challenge. To address this gap and determine the free energy of these nearest neighbor pairs with G-T mismatches, optical melting experiments for all of the possible nearest neighbor pairs were performed. Analysis of optical melting data showed that G-T mismatch pairs are stabilizing base pairs, with free energies ranging from -0.12 to -0.95 kcal/mol. However, G-T mismatches are still less stable than canonical G-C base pairs (-1.28 to -2.24 kcal/mol), making them suitable for destabilizing DNA hairpin structure. This new thermodynamic G–T mismatch data will improve upon the current set of DNA nearest neighbor thermodynamic parameters, and will also improve computational modeling of CHA.

P-033: **Sara Farshineh Saei**

5-Triazolyl Uracil Improves Nucleobase Stacking, Pyrimidine Recognition, Biological Activity, and Specificity of RNA-Binding Triplex-Forming Peptide Nucleic Acids

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Peptide nucleic acid (PNA) closely mimics the structure of DNA and RNA, with a key difference being the replacement of the negatively charged backbone with a neutral one. This unique characteristic enhances PNA hybridization with complementary DNA or RNA, enabling distinct binding modes such as triplex formation. However, the application of triplex-forming PNAs is largely limited to specific purine-rich sequences. A major challenge in this field remains the Hoogsteen recognition of pyrimidines. Although nucleobases designed for pyrimidine recognition possess perfectly aligned hydrogen bond donors and acceptors, molecular modeling suggests a loss of base stacking. In this study, 5-triazolyl uracil was introduced adjacent to the pyrimidine recognition region to restore stacking interactions. Experimental findings confirmed an increased binding affinity for pyrimidine recognition within a model system. Moreover, the modeling data aligned with these results, showing enhanced stacking in sequences containing 5-triazolyl uridine. The increased binding affinity improved the biological activity of triplex-forming PNAs targeting microRNA precursors in a cell-based dual fluorescent protein assay. RNA-seq analysis showed that a PNA recognizing a mixed sequence of three nucleobases (G, A, and U) had higher specificity than a similar PNA featuring a uniform purine recognition tract. Overall, this study demonstrates the potential of 5-triazolyl uracil in addressing the challenge of pyrimidine recognition in triplex-forming PNAs.

P-034: **Olivia Fraser, PhD**

Characterization of the Mitochondrial FASTKD2 RNA Binding Protein

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Human FASTK proteins comprise the largest mitochondrial RNA binding family. FASTK family members exhibit distinct roles in mitochondrial gene regulation, including nascent RNA processing, mRNA stabilization, and ribosomal RNA maturation. Emerging evidence highlights the central role of FASTK RNA binding proteins in preserving mitochondrial function; and yet, the molecular basis for FASTK RNA binding and selectivity remains unknown. FASTK members are proposed to exert RNA sequence dependent binding activity, potentially explaining their distinct roles in gene control. However, due to a paucity of experimental structures, the molecular determinants which enable recognition of distinct RNA targets remain unclear. Family member FASTKD2 is an essential mitochondrial ribosome (mitoribosome) assembly factor known to engage the 16S ribosomal RNA. Dysregulation of FASTKD2 impairs mitoribosome biogenesis, energy production, and is associated with mitochondrial encephalopathy, underscoring the pressing need to define its mechanism of action. To define the molecular basis for FASTKD2 RNA binding, we reconstituted human FASTKD2 for structure-function studies, a milestone due to both the absence of literature precedent and challenge of producing FASTK family proteins. We demonstrate that recombinant FASTKD2 is monomeric in solution and exerts a strong binding preference for unstructured RNA. A systematic RNA binding study reveals the binding affinity of FASTKD2 for model RNA substrate and defines a minimal RNA footprint. We determined the molecular mass of the RNA-bound FASTKD2 complex to show it associates with RNA at a 1:1 stoichiometry. We generated a model prediction for RNA-bound FASTKD2, supported by orthogonal structural data, to nominate putative RNA binding surfaces. FASTKD2 variants generated by targeted mutagenesis of residues predicted to form an extended charged surface will be characterized to define their roles in RNA binding and selectivity. Together, this work sheds light on the elusive RNA binding requirements for FASTKD2 and the broader FASTK protein family

P-035: **Jeffrey Gabell**

From Codons to Conductance: tRNA supplementation and informed codon optimization for therapeutically enhancing CFTR channel function

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Cystic fibrosis (CF) is a monogenic autosomal recessive disorder caused by loss-of-function mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Several classifications of the disease exist with various susceptibility to therapeutic intervention, to which there is a need for patients whose conditions aren't treatable through available pharmacologic means. CFTR gene complementation remains a promising treatment for all CF-causing mutations. To date, these strategies have utilized an intron-less, codon optimized CFTR cDNA to achieve therapeutically meaningful levels of CFTR expression. Codon optimization (CO) is a common approach to maximize transgene expression in vectors for more preferred or rapid decoding during translation. When combined with gene therapies, CO assists in achieving therapeutically meaningful levels of expression needed to remediate disease phenotypes and overcome barriers such as delivery. However, there are a myriad of factors that influence rate of translation and contribute to the optimality of a transcript that are often not all considered in available CO algorithms, and show varying influence on CFTR specific channel function. To this end, we have developed screening reporters that target specific codon usage within CFTR while isolating the effect of individual tRNA isodecoders delivered heterologously. The goal is to explore the efficacy of natural and non-natural tRNA supplementation to target elongation bottlenecks and further inform the creation of what we are calling 'functionally optimized' CFTR transcripts by identifying specific codons susceptible to improved rates of decoding. From these screens, we have determined a list of tRNAs and codons in the CFTR transcript that are promising targets. Indeed, we have observed in creating selective, minimal, screen-informed synonymous mutations in the CFTR transcript, we are able to improve channel function when transiently expressed in functional assays. Importantly, what we learn here can be adapted to other transcripts for generation of therapeutics.

P-036: **Jack Geary**

Methods for interrogating translation of lncRNAs

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Long non-coding RNAs (lncRNAs) are a diverse class of transcripts traditionally defined by their lack of protein-coding capacity. However, accumulating evidence suggests that a subset of lncRNAs can engage the translational machinery to produce short AUG-initiated peptides, often called micropeptides. Whilst canonical translation is a tightly controlled process that results in the expression of a protein, typically greater than 100 amino acids, from an RNA transcript initiated at an AUG start codon, multiple lncRNAs can utilize AUG codons to produce much shorter proteins that retain functionality. Ribosome profiling studies have revealed ribosome-protected fragments mapping to annotated lncRNAs, suggesting active translation. Notable examples include LINC00961, which encodes the micropeptide SPAR that regulates mTORC1 signaling, and HOXB-AS3, which produces a peptide that modulates metabolic reprogramming in colon cancer cells. These findings suggest that some lncRNAs function as bifunctional transcripts, acting both as regulatory RNAs and as templates for peptide synthesis. Here, we utilize polysome profiling and candidate translation factor knock-down studies to investigate the translational potential of a candidate lncRNA that has not previously been reported to produce a micropeptide. Understanding translation from lncRNAs expands the coding potential of the transcriptome and redefines boundaries between coding and noncoding RNA biology.

P-037: **Hen Gelshtein**

Roles of Alternative Protonation in the Structure and Function of Biological RNAs

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Base pairing between nucleobases is crucial for RNA molecules to adopt the correct structures and carry out essential functions. Beyond canonical Watson Crick Franklin base pairs, RNA frequently can have noncanonical interactions such as G•U and A+•C mismatches. These A+•C pairs often show shifted pKa values [1,2], which can lead to protonation changes and the formation of charged nucleobases. Further, these mismatches can also adopt tautomeric forms [3] and present shifted geometries that can resemble canonical base pairs and may cause errors during processes like template driven synthesis or lead to off-target effects in gene regulation. In this work, we investigate a G•U wobble pair within an RNA identified by structural informatics [4] as a candidate for alternative protonation states. We used 1H and 31P NMR to characterize the pH dependence and pKa of the G•U base pair and further performed optical melting to characterize pH- and temperature-dependent behavior of G•U base pairs. We have observed a shifted pKa of G•U base pairs that is dependent on salt concentration and observed effects of salt concentration on thermal stability of G•U-containing RNA.

P-038: **Saumya Gupta**

Beyond Zinc Fingers: The Role of the MBNL1 Linker in Splicing Control

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Objective: The muscleblind-like 1 (MBNL1) RNA-binding protein is an important regulator of alternative splicing (AS), a process that generates multiple isoforms of a single pre-mRNA transcript. MBNL1 sequestration in myotonic dystrophy (DM) leads to aberrant splicing and disease phenotypes. MBNL1 is composed of four CCCH zinc fingers (ZF) tandemly arranged into 2 domains, ZF1-2 and ZF3-4 separated by a disordered 76 amino acid linker. The ZF domains bind to YGCY (Y= C or U) RNA motifs. ZF2 and ZF4 contain primary RNA binding sites whereas ZF1 and ZF3 serve as stabilizing domains1. Previous studies have predicted the linker plays a role in splicing regulation2,3, however exact mechanism of its function and relationship with the ZFs are not well understood. We hypothesize that the linker region separating ZF1-2 and ZF3-4 plays an important role in regulating AS, with different functions for inclusion and exclusion events.

Methodology: To address this hypothesis, we designed synthetic MBNL1 constructs with linker modifications, overexpressed them at various concentrations in HEK293 cells that have low endogenous MBNL1 expression and calculated percent spliced in values.

Result: (i) Dose dependent MBNL1 overexpression in this system shifts splicing significantly for multiple events, (ii) a modified MBNL1 containing a deletion of 19 amino acids in the linker abated splicing of inclusion events.

Conclusion: Comparing wildtype MBNL1 to linker mutant MBNL1 revealed that the linker is a critical component of MBNL1 protein in regulating splicing, functions independently of the ZF3-4 domain and regulated splicing of inclusion and exclusion events differently.

Practical Implication: The overall goal of the project is to identify the regions and amino acids within the linker that modulate MBNL1 mediated splicing activity, and explore if these modifications can complement the current therapeutics strategies to alleviate MBNL sequestration in DM.

P-039: **Sakura Hamazaki**

From toxic RNAs to physiology, an investigation of HSALR mouse model of DM1

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Myotonic Dystrophy type 1 (DM1) is a multisystem disease that affects every organ system, with muscle weakness and wasting accounting for the majority of deaths through respiratory failure. DM1 is caused by a CTG repeat expansion in the 3' untranslated region of the DMPK gene. When transcribed, these repeats form toxic RNA hairpin structures that sequester RNA binding proteins, including muscleblind-like 1 and 2 (Mbnl1, Mbnl2) splicing factors. Sequestration of these splicing factors have been linked to the dysregulation of alternative splicing of thousands of transcripts, which overall display a reversion from adult to fetal splice-patterning. A mouse model harboring approximately 250 repeats in a human skeletal actin transgene (HSALR), which exhibit extensive splicing abnormalities in the hindlimb muscle with overt myotonia, has become the standard preclinical model for assessing novel therapeutics and probing disease mechanisms. However, with lingering questions regarding the differential effects of toxic repeats in specific muscle groups and efforts to accurately study mortality in DM1, important considerations need to be made when selecting appropriate models. Specifically, we show that this mouse model lacks derangements in respiratory physiology expected in DM1. Interestingly, we also show that the diaphragm, a respiratory muscle largely effected in individuals affected by DM1, does not express the HSALR transgene and consequent toxic RNA repeats. Without expression of the repeats in the diaphragm, this model cannot mirror the progressive respiratory phenotype, and thus severely limiting our ability to test novel therapeutics that target the skeletal muscle symptoms. Furthermore, this study highlights the need to assess the genetic and physiologic consequences of toxic RNA repeats directly in the diaphragm and not purely rely on extrapolations from hind limb muscles.

P-040: **Mark Handley, PhD**

A stress-response to endogenously-produced inosine-containing mRNA

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Biallelic loss-of-function variants in ITPA cause a severe neurodegenerative syndrome characterized by microcephaly, seizures, hypotonia, developmental failure, and frequently by dilated cardiomyopathy (MIM 616647). ITPA encodes inosine triphosphate pyrophosphatase (ITPase), an enzyme that removes the non-canonical purine nucleotides (deoxy)inosine triphosphate (dITP/ITP) from cellular pools. In the absence of ITPase activity, ITP is misincorporated into RNA, but the subsequent consequences when inosine-containing RNAs are produced are unknown. We find that exogenous inosine-containing mRNA can elicit pronounced transcriptional changes reminiscent of the innate immune and integrated stress responses. We now show that endogenously produced inosine-containing mRNA can elicit a similar response. Control and ITPase-null H9c2 cardiomyoblast cell lines were treated with inosine nucleoside. With mass spectrometry, RNA from treated ITPase-null cells was shown to exhibit dose-dependent increases in inosine misincorporation. Thus, the nucleoside is taken up by the cells and undergoes successive rounds of phosphorylation. Further, the ITP generated is a substrate for ITPase in the control cells but for RNA polymerase in the ITPase-null cells. Oxford Nanopore direct mRNA sequencing confirmed increased base-substitution rates in RNA from the treated ITPase-null cells and established inosine misincorporation into endogenous mRNA. Western blotting demonstrated increased eIF2α phosphorylation. Together, these data show that endogenously-produced inosine-containing mRNAs can trigger cellular stress.

P-041: **Emmett Hanson**

Pathogenic genome detection on paper using CRISPR and inherently fluorescent DNA nanoclusters

Emmett Hanson - Senior Chemistry Undergraduate in Yigit Lab, UAlbany Chemistry Department and RNA Institute

Mehmet Yigit - Professor, UAlbany Chemistry Department and RNA Institute

Foodborne pathogens have proven to be a significant threat to global health. Here, we present the development of a cost-effective multiple pathogenic genome detection system using CRISPR-Cas12a and fluorescent DNA-templated silver nanoclusters (FNPs) on a paper substrate. Specifically designed guide RNAs for Campylobacter jejuni, Escherichia coli (STEC), and Listeria monocytogenes (C, E and L) recognize target genomic fragments leading to FNP degradation and significant fluorescent reduction visualized through fluorescent imaging.

To improve diagnostic clarity, a two-step CRISPR-Cas12a reaction was performed to reverse signal output allowing for an ON-retention reporting mode. Using both reporting modes, all combinations of individual and multiple target genomic regions were detected. Finally, full Listeria genome detection was demonstrated on a paper substrate showing consistent detection of as few as 40 copies of full genomic DNA. This unique nucleic acid detection platform allows for a reprogrammable target selection and simultaneous detection of multiple pathogens while also reducing cost and preparation time making it a promising advance in point-of-care pathogen detection for resource limited settings.

P-042: **Jayamini Harasgama, PhD**

RNA Chemical Probing to Analyze the Structural Dynamics of the SARS-CoV-2 3'UTR

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The highly conserved secondary structural motifs of SARS- CoV-2 3’ untranslated region (UTR) are critical regulators of viral replication and transcription. The possible molecular switch between the bulged stem loop (BSL) and the pseudoknot (PK) is believed to be the driving force for this regulatory function. Despite extensive research, the formation of the pseudoknot (PK) structure within the 3’UTR remains elusive; its highly transient nature within the host cell suggests a rapid conformational flux that complicates detection. Our approach utilizes peptide nucleic acids (PNA) in conjunction with an advanced RNA chemical probing to interrogate these subtle structural dynamics of the 3’ UTR.

We utilized dimethyl sulfate (DMS) MaP to elucidate the purported structural shift between the BSL and PK of the 3’UTR. PNAs were synthesized to target and bind the in-vitro transcribed 3’UTR conserved region under physiological conditions. RNA/PNA complexes were then reacted with DMS. Modified RNAs were reverse transcribed and utilized in high-throughput sequencing, analyzed by Seismic RNA algorithm to elucidate the structural dynamics stabilized by the PNA binding.

Among the different classes of PNAs used in the study, including triplex-forming, antisense, and PNA clamps, one antisense PNA favored the stabilization of the pseudoknot, while the rest of the PNAs stabilized the BSL conformation. A full-length replicon system of SARS-CoV-2 developed in our laboratory will be used in cell-based assays together with in-cell chemical probing to further validate these findings. By modulating the structural dynamics of the viral genome, our work seeks to establish these motifs as novel therapeutic targets for SARS-CoV-2 and emerging coronaviruses.

P-043: **Ryan Hardy**

The Integrator Complex is Required for HSV-1 Infection

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Herpes simplex virus type 1 (HSV-1) is a broadly prevalent pathogen, with 67% of the world's population having been infected. HSV-1 is a DNA virus wholly reliant on host RNA Polymerase II (RNAPII) to transcribe its genes and the viral genome is known to encode proteins important to ensure this happens. The Integrator (INT) complex is a seventeen-subunit complex (INTS1-15, PP2A-A, and PP2A-C) that acts on promoter-proximal paused RNAPII to induce transcription termination and the cleavage and degradation of nascent RNA. There have not been any studies conclusively demonstrating connections between INT subunits and HSV-1 infection thus representing a knowledge gap in the field.

We have developed an auxin-inducible degron (AID) system to rapidly deplete Integrator subunits. Using the system, we demonstrate that the rapid depletion of INT subunits via auxin-induced degradation leads to heavily attenuated infection of HSV-1 in 239T cells. However, other viruses, such as Human Coronavirus OC43, are observed to be unaffected by INT depletion, suggesting HSV-1 uses specific interactions with the INT complex to promote viral propagation. To further characterize this observation, we purified native INT complexes during early and late infection points and characterized associated proteins using quantitative mass spectrometry. We found that specific viral proteins associated with INT upon infection, including immediate early proteins ICP0, ICP4, and ICP22. Further, late timepoints revealed that viral proteins are likely 'remodeling' INT-containing complexes to reduce INT association with transcription machinery. We hypothesize that, through specific protein interactions via immediate early viral proteins, HSV-1 utilizes the host INT complex to attenuate host gene transcription and promote the production of viral transcripts. Future work consists of determining the specific mechanism through which INT promotes HSV-1 infection as well as determining specific INT subunit interactions with viral proteins.

P-044: **Leon Harvey**

Investigating the Role of RNA Helicases in Splicing Changes Caused by Cancer-Associated SF3B1 Mutations

Leon Harvey, Mohammed Ali, Xueyang He

University of Rochester

The spliceosome is a dynamic ribonucleoprotein (RNP) complex responsible for accurately processing pre-mRNA to mRNA. It accomplishes this with five small nuclear RNPs (snRNPs) and other proteins, including NTP-dependent RNA helicases. SF3B1, the largest subunit of the SF3B complex, is tasked with 3' splice-site (3'ss) positioning and is frequently mutated in a multitude of cancers and pre-malignancies. Mutant SF3B1 alters the splicing of many transcripts via alternative 3'ss selection. SF3B1 interacts with RNA helicases Prp5/DDX46 and Sub2/DDX39B, both playing a role in 3' ss. Recently, a third helicase — Prp43/DHX15 — was also implicated in this step of splicing. Additionally, loss of SUGP1, a coactivator of Prp43/DHX15, phenocopies SF3B1-Mut alternative 3' ss. We hypothesize that SF3B1 mutants alter their interactions with helicases during the splicing cycle. Different subsets of introns exhibit unique dependencies on those helicases and other splicing factors, accounting for various splicing patterns. By targeting these helicases with degron tag systems, we will characterize changes in splicing upon protein depletion and then compare the splicing changes resulting from SF3B1 mutations to gain mechanistic insight into these factors' interactions. Preliminary data from our lab have demonstrated this using the TCGA cohort database. Based on data from the 16 breast tumor patients, we could separate the patients expressing SF3B1K700E HEAT and SF3B1WT based on this hotspot mutation, using only the 3' ss usage differences. Uncovering the sets of introns sensitive to each helicase will shed light on the mechanism of action that leads cells expressing SF3B1K700E to develop into cancer cells.

P-045: **MaryClaire Haseley**

HEXIM1 integrates transcriptional stress and cell cycle arrest following dual disruption of Integrator and NELF

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Transcription requires tight, dynamic regulation for proper function. Shortly after initiation, RNA Polymerase II (RNAPII) pauses when NELF and DSIF bind, forming the paused elongation complex (PEC). To release RNAPII into productive elongation, pTEFb must phosphorylate the PEC. When pTEFb is not present, another transcriptional regulator, the Integrator complex (INT), binds the PEC and facilitates premature termination. HEXIM1, a regulator of pTEFb, has been shown to sequester pTEFb to block transcriptional elongation from occurring. This suggests that dynamic pTEFb regulation is essential to control proper transcription elongation. Not currently understood is the regulation of HEXIM1 and how it interfaces with transcription machinery during this paused state.

We developed a cell line where INT or NELF can be separately or in combination rapidly depleted. Using NGS approaches, we find that depletion of both INT and NELF synergistically alters transcription leading to the production of nearly unregulatable RNA polymerase complexes. Upon loss of INT/NELF, cells start to slow proliferation and begin to die. HEXIM1 is massively induced during double depletion, leading to a >20-fold increase in its protein levels. When HEXIM1 is knocked out in INT/NELF depleted cells there is an alleviation of the slowed proliferation. NGS approaches revealed that HEXIM1 is needed to fully activate critical genes when INT/NELF are absent. Using IP/MS we determined that HEXIM1 interacts with elongation machinery, suggesting that HEXIM1 could deliver pTEFb to stimulate transcription. These data support a working model in which transcriptional dysregulation—particularly when the INT/NELF checkpoint is compromised—leads to an induction of HEXIM1 as a feedback mechanism.

P-046: **Kate Hayes**

Global Transcriptional Regulation of Tissue-Specific Development

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The Mediator complex is a molecular bridge connecting transcription factors bound to distal enhancers and RNA Polymerase II at promoter elements to enable enhancer-dependent regulation of Pol II-mediated transcription, thus defining the complex as a global transcription regulator. The current understanding of the Mediator complex has largely focused on its role as a ubiquitous accessory to the pre-initiation complex. However, we posit that the Mediator complex regulates cell type specific development in a cell autonomous manner, conflicting with its ubiquitous regulatory role.

We observe that zebrafish harboring a point mutation in med24, a subunit of the Mediator, exhibit an altered mouth morphology that impairs feeding and consequently reduces survivability. Analysis of the tissues surrounding the mouth reveals coordinated defects in cartilage, bone, neuronal and muscle differentiation in these mutants. We hypothesize that Med24 plays a key role in regulating muscle development, and that disruption of this process secondarily affects neighboring tissues through altered signaling interactions.

To determine how Med24 influences multiple tissue types and to elucidate the molecular interactions among these tissues during development, we performed spatial transcriptomic analysis using the 10x Genomics Xenium platform. We custom designed our panel of 100 genes probe set comprising of known transcription and signaling factors regulating cartilage, bone, muscle and neuronal development. The resulting dataset captured transcriptomic profiles at single-cell resolution while preserving spatial localization within the tissue. Preliminary analysis of the muscle marker Myod1 shows a decreased level of RNA expression in relation to specific cartilage structures in the lower jaw, indicating a spatially-dependent reduction of transcription factors regulating muscle development. Perturbations in the transcriptional landscape will identify how multiple craniofacial tissues coordinate for proper differentiation during craniofacial development as well as how the loss of Med24 alters these interactions.

P-047: **Yichen He**

Short RNA duplex formation in artificial cytoplasms

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The dynamics of RNA folding in cells has not been extensively studied. Previous research from our lab led to the development of a minimal Escherichia coli artificial cytoplasm called Eco80, which has 80% of the metabolites from E. coli [1]. The binding thermodynamics of some RNA sequences have been assessed in Eco80 and show generally weaker stability [2], but their binding kinetics have not been studied. By using stopped-flow rapid mixing on several short RNA duplexes with fluorophore and quencher labels, we were able to collect kinetic data under different solution conditions. Using temperature-dependent experiments, we collected kinetic rate constants and activation energy of duplex formation for several sequences, which were close to literature references. We also finished some basic Eco80 tests. By performing quenching and pulse-chase experiment using unlabeled RNA bottom strand, we were able to collect kinetic constant data of the duplex formation and diassociation. Comparing the ΔΔG data of the RNA duplex formation obtained from stopped-flow experiments in 1 M NaCl buffer and in Eco80 to our previous thermodynamic data, we found that they are in good agreement. Furthermore, we found that the lowerstability of RNA duplex in Eco80 may because of both lower association rate and higher dissociation rate. Our future experiments will focus on finding out which components in Eco80 lead to these kinetic differences.

P-048: **Olivia Hiltke**

Insights into the Structure and Function of RNA Using In Vivo-Like Folding Stability

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RNAs regulate gene expression and cellular processes, often relying on specific conformations for function. RNA folding is hierarchical and sequence-dependent, with nearest neighbor thermodynamic models commonly used to predict secondary structure. These models are based on optical melting experiments under 1M NaCl conditions, which poorly represent the cellular environment. To address this, we developed a new model in Advanced DMEM (Adv. DMEM) buffer, which mimics mammalian extracellular ionic composition. This in vivo-like model provides RNA folding parameters for helical base stacks and loop motifs. Optical melting experiments revealed that helical stacks, particularly tandem GU pairs, are less stabilizing in Adv. DMEM. Loop parameters were generally destabilizing but highly sequence- and loop-type dependent, with internal loops displaying idiosyncratic behavior. Structure prediction benchmarking revealed minimal differences in structure predictions overall, except for tRNAs, which showed improved prediction reliability and enhanced cloverleaf stability. Notably, tRNAs lack internal loops, suggesting further studies in Adv. DMEM could refine secondary structure predictions. This in vivo-like parameter set is included in the RNAstructure software package. By grounding these parameters in a physiologically relevant environment, we improve the biological relevance of RNA secondary structure predictions and establish a foundation for studying RNA folding under in vivo conditions.

P-049: **Porama Zafreen Hoque**

Synergistic Correction of Splicing Defects in Myotonic Dystrophy Type 1 via Combination Therapy

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Objective: Myotonic Dystrophy Type 1 (DM1) is a genetic disorder caused by a CTG repeat expansion in the DMPK gene, leading to the sequestration of muscle-blind-like (MBNL) proteins and RNA splicing disruption. Correcting mis-splicing by reducing or blocking toxic RNA to restore MBNL function is a promising therapeutic approach. Antisense oligonucleotides (ASOs) and small molecules (SMs) exhibited encouraging pre-clinical results in DM1 cells and mouse models, with ASOs recently demonstrating partial splicing rescue in clinical trials. Our research aims to determine the therapeutic potential of ASO-SM and SM-SM combinations in correcting the spliceopathy in DM1.

Methodology: Our group has previously demonstrated the efficacy of a novel series of small molecules, termed modified polycyclic compounds (MPCs), in correcting mis-splicing in DM1 cell and mouse models. In this study, we have investigated the effects of combination therapies using ASOs with the small molecule histone deacetylase inhibitor, vorinostat, as well as combinations of MPCs and vorinostat, in patient-derived cell lines via RT-PCR. Currently, we are performing RNA sequencing to evaluate the efficacy and off-target effects of combination treatments compared to single-agent therapies and extending our study to additional small molecules. The most effective combinations will be further tested in DM1 mouse models to validate their therapeutic potential.

Results: Our data indicate these combinations may produce additive or synergistic effects at lower doses, significantly enhancing splicing correction and reducing toxic RNA levels compared to single-agent therapies. ASO-SM combinations and SM-SM combinations led to over 85% splicing correction in DM1 myoblasts and over 90% correction in myotubes following 72-hour and 96-hour treatments, respectively.

Conclusions: Combinatorial therapies show significant promise in correcting spliceopathy while allowing dose reduction, enhancing therapeutic efficacy, and potentially minimizing off-target effects and toxicity.

Practical Implications: This approach could significantly advance existing DM1 treatments by offering a more effective and safer intervention for patients.

P-050: **Sean Jones**

Characterization of the SigD regulon and its role in susceptibility to hydrophobic antibiotics in Mycobacterium abscessus

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The mycobacterial cell wall plays a major role in bacterial physiology, including protection against hostile environments and chemicals such as antibiotics. The cell envelope consists of an inner leaflet of mycolic acids esterified to arabinogalactan, which is bound to peptidoglycan. The outer leaflet is composed of free, unbound glycolipids and in some species such as Mycobacterium abscessus, glycopeptidolipids (GPL). As environmental stressors are constantly changing, expression of genes involved in synthesizing and maintaining cell wall components needs to be regulated in response to the changing conditions. One of the ways mycobacteria can respond to such stimuli is through extracyctoplasmic function (ECF) sigma factors. These sigma factors are often held inactive by their respective anti-sigma factors, until they are activated by a certain extracellular signal. One of these ECF sigma factors in M. abscessus is SigD. In our current work, we characterize the regulon of SigD and show that it regulates different cell wall related and secreted proteins, becoming activated through cell wall disruption such as ethambutol treatment. This work reveals that under normal conditions, SigD helps to decrease sensitivity to hydrophobic antibiotics and lower cell permeability by maintaining the cell wall through increased expression of genes such as those in the antigen 85 complex and genes involved in glycolipid synthesis and transport.

P-051: **Young Mee Jung**

Deep-UV Resonance Raman and Two-Dimensional Correlation Spectroscopic Analysis of mRNA Encapsulation in Lipid Nanoparticle Therapeutics

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Messenger RNA (mRNA) therapeutics have rapidly emerged as a powerful modality for disease treatment, yet their clinical success critically depends on efficient stabilization and delivery. Because mRNA is inherently unstable, lipid nanoparticle (LNP) encapsulation is essential; however, direct and quantitative characterization of the mRNA encapsulation state remains a major analytical challenge. Here, we introduce a deep-UV resonance Raman (DUVRR) spectroscopic approach combined with two-dimensional correlation analysis to quantitatively distinguish free and encapsulated mRNA in LNP-based model vaccine systems. By employing 266 nm excitation, resonant with the intrinsic UV absorption of mRNA, we achieve strong molecular selectivity and sensitivity to mRNA structural environments. Raman spectra were collected from samples with systematically decreasing lipid concentrations while maintaining a constant mRNA level. Distinct vibrational signatures associated with mRNA–lipid interactions were observed. Principal component analysis revealed lipid-dependent spectral variations, particularly in the 1270–1800 cm^{-1} region. Notably, two-trace two-dimensional correlation spectroscopy (2T2D-COS) enabled extraction of the 1322 cm^{-1} band as a quantitative marker for mRNA–lipid interactions. This work represents the first demonstration of 2T2D-COS for quantitative analysis in complex biochemical systems. Our results highlight the unique capability of DUVRR spectroscopy to directly probe mRNA encapsulation at the molecular level and establish a powerful analytical framework for optimizing and validating LNP-based mRNA therapeutics.

P-052: **Nobuhiko Komoshita**

Stimulatory and Non-Stimulatory Downstream RNA Structures for Viral Stop Codon Readthrough

Some RNA viruses infecting higher eukaryotes employ stop codon readthrough to translate a downstream open reading frame required for viral replication. As one type of translational recoding, the nucleotide sequence surrounding the stop codon (stop codon context) plays a pivotal role and has been widely used for classification. In contrast, the requirement for a downstream RNA structure appears to depend on the stop codon context and may further be influenced by experimental conditions or additional factors.

To clarify the determinants and possible consensus features of stimulatory elements, published downstream structures from four animal virus families are reviewed, including four stimulatory structures, one biochemically confirmed non-stimulatory structure, and one structure that has not been fully characterized. As no ribosome-complexed structures are currently available for stop codon readthrough, ribosome-bound frameshifting structures are also considered for comparison and modeling.

The gammaretrovirus stimulatory element represents a typical hairpin-type pseudoknot spanning approximately 50 nucleotides with an eight-nucleotide spacer and is regarded as a prototype of stimulatory structures.

In contrast, a non-stimulatory extended stem–loop (~70 nucleotides) identified in an insect triatovirus — whose lack of stimulatory activity has been biochemically validated — initiates the structure within the termination codon, and its structure has been determined as a published crystal structure. These features were used to interpret its non-stimulatory property, as up to ~10% readthrough efficiency can still occur in the absence of a downstream structure.

Given that various cellular genes can adopt diverse RNA structures, the presence of both stimulatory and non-stimulatory structures may represent a generally plausible scenario, highlighting their potential for different applications.

Stimulatory elements in viral RNAs could serve as antiviral targets, whereas readthrough events involving non-stimulatory structures provide useful models for exploring readthrough therapy via near-cognate tRNAs.

P-053: **David Kariuki**

Synthesis of Amide-Linked RNA Phosphoramidite Dimers for Backbone Engineering of CRISPR Guide RNAs

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CRISPR/Cas9 genome editing has broad therapeutic potential; however, the instability and high polyanionic charge density of guide RNAs (gRNAs) limit their performance in biological environments. Neutral amide modified RNA backbone provides a direct strategy to modulate charge and stability while preserving native base pairing & comparable backbone geometry. Our laboratory previously demonstrated that amide linked modified RNAs are well tolerated in the protospacer adjacent motif (PAM)-distal region of CRISPR RNAs and retained Cas9 activity.

Building on those preliminary results from our lab, we continue synthesizing amide -linked RNA dimers (CG, CA CC, GC) that will be incorporated into the Cas9 gRNAs. Synthesis routes involve complex multi-step chemistry to install the carboxylic acid and amine groups on the upper (3' position) and the lower (5' position) ribose residues of the dimer, respectively. Synthetic routes for the carboxylic acid monomer involves acylation, selective hydroxyl protection followed by radical allylation & oxidative cleavage of alkene or Wittig & hydrogenation reactions for the G monomer whereas 5'amino monomer is synthesized via 5' azide intermediate followed by its reduction to the corresponding amine. These dimers are further converted into a phosphoramidite derivatives suitable for standard solid phase RNA synthesis.

This strategy provides a modular and reproducible platform for backbone-neutral RNA synthesis, enabling systematic incorporation of amide linkages in CRISPR guide RNAs with a goal of improving CRISPR/Cas 9 specificity.

P-054: **Anees Mohammed Keedakkatt Puthenpeedikakkal**

Refinement of the amber force field for RNA: Improving the description of non-bonded interactions

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RNA performs a wide range of functions, including coding, regulating, and expressing genes. Molecular dynamics (MD) simulation can be used to investigate the time evolution of RNA conformations and to complement or interpret experimental observations. Amber uses a fixed-charge force field model to estimate the potential energy of a molecule from its atomic coordinates. Studies suggest that the stacking free energies observed from the current RNA force field are too favorable compared to the experimental data. The non-bonded interactions are modeled by a Lennard-Jones potential and Coulomb's law; these parameters have not been updated since 1995. We are refitting the Amber parameters to potential energies determined by quantum mechanics. Structures from the Protein Data Bank are used to harvest base pairs, base stacks, base-phosphates, base-sugar, and backbone-backbone interactions. These structures were clustered on interatomic distances using density peak clustering, and the cluster centers obtained were chosen as representative structures. To account for the polarization from the solvent, charges were fitted to represent the time-averaged electrostatic field from the solvent, sampled through the molecular dynamics trajectory of these cluster centers with OPC water. Symmetry-adapted perturbation theory (SAPT) calculations were performed using these representative structures and solvation charges to determine the non-bonded interaction energy. The non-bonded parameters of the Amber fixed-charge potential form were fit by nonlinear regression to the observed interaction energies.

P-055: **Safi Eddine Khiati**

In vitro transcription of tRNASec and its effects on human cells

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Reactive oxygen species (ROS) are produced during metabolism and can cause DNA, protein and lipid damage. ROS levels can be mitigated by cellular antioxidant systems that include catalase superoxide dismutase (SOD) and selenocysteine-containing glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs). There are 25 selenocysteine containing proteins with 18 dedicated to ROS mitigation and maintaining redox balance, with their translation requiring specialized cis (internal stop codon, SECIS element) and trans factors (tRNASec and translation factors). We in vitro transcribed (IVT) tRNASec (unlabeled and fluorescently labeled), purified it and transfected it into human embryonic kidney (HEK293G) cells. We have successfully in vitro transcribed and purified tRNASec and tRNALeu(CUG) and we can use our system to produce other tRNA species. We have used fluorescently labeled tRNASec to introduce IVT-tRNASec into human cells. We are currently assessing the effects of IVT-tRNASec in transfected HEK293G cells under normal and ROS-stress conditions. Our goal is to develop novel tRNA-based reagents with the potential to act as an antioxidant to mitigate ROS and reprogram cellular translation.

P-056: **Nora Kiledjian**

Androgen deprivation therapy activates the ISR to rewire mRNA translation

Nora T. Kiledijian [(1)], Sayantani Das [(1),] Katharina E. Hayer [(2)], Morgan J. McGrath [(1),] Vincent Dong [(1),] Ioannis Paraskevaidis [(1),] Ioannis I. Verginadis [(1),] Matthew D. Weitzman (20), Crystal S. Conn [(1)]

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Activation of the Integrated Stress Response (ISR) has been identified as a marker for aggressive prostate cancer (PrCa) which correlates with worse patient prognosis. The ISR is an adaptive pathway triggered by intrinsic and extrinsic stressors present within rapidly dividing cancer cells and within the tumor micro-environment. It serves as a protective mechanism that limits extraneous protein synthesis while also promoting the translation of select mRNAs to allow cells to more efficiently respond to the stressors. Previous research has indicated that ISR activation can accelerate tumor growth and metastasis in PrCa. Here, we have uncovered that some commonly used treatments for PrCa — including androgen deprivation therapies (ADT) — can activate this pathway, potentially enabling a more aggressive cancer to emerge in patients treated with these therapies. Our experiments reveal that ADTs rapidly activate a signaling cascade within PrCa cells that leads to activation of the ISR. We are currently working to identify the mechanism by which this ISR activation by cancer therapies occurs in PrCa cells. Using sequencing from highly ribosome bound mRNA, we have identified large scale translational changes resulting from ADT induced ISR activation, with selective translation occurring amidst the global translation reduction caused by ISR activation. The mechanism through which specific mRNAs are selected for translation amidst a global translational shutdown during ISR activation has not yet been fully elucidated in PrCa. Our ongoing efforts are to examine whether specific RNA binding proteins, RNA modifications, secondary structure, or other RNA features may be marking specific transcripts for increased translation in response to cancer therapy. By identifying how selective translation occurs in this context, we can provide novel insights into improving current targeting strategies for the treatment of advanced PrCa.

P-057: **Sravya Kovvali, PhD**

Detection of serum proteins with aptamer-based DNA nanoswitches

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The sensitive detection of biologically relevant proteins is critical for both clinical diagnostics and fundamental research, yet many existing assays remain expensive, time-consuming, or technically complex. Developing simple, modular platforms that enable reliable detection of diverse protein targets could reduce barriers for applications in healthcare and research. To address this need, we incorporated aptamers into the detector strands of our established DNA nanoswitch platform. The DNA nanoswitch is a nanostructure constructed from M13 DNA, with detector DNA strands positioned in a way such that binding of a target to the detector strands induces a controlled conformational change of the linear scaffold into a looped structure. Thrombin, a key clotting factor, and VEGF, a central regulator of angiogenesis, were selected as target biomolecules for aptamer-based detection. Binding of the protein target induces nanoswitch looping, producing a detectable signal via gel electrophoresis. Using this approach, VEGF- and thrombin-responsive nanoswitch activity was confirmed, establishing proof of principle. Conditions for optimal detection were established, and simultaneous detection of thrombin and VEGF was performed, demonstrating the specificity of the nanoswitches. This aptamer-based nanoswitch platform offers a low-cost, versatile method for detecting clinically relevant proteins. Expanding its compatibility will broaden diagnostic applications while providing valuable insight into the detection limits of aptamer-based systems.

P-058: **Ethan Krupa**

Development of a Zika virus single cell fluorescent reporter

Ethan Krupa, Cara T. Pager

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Zika virus (ZIKV) is a flavivirus associated with severe neurological and developmental defects in babies born to mothers infected in the first trimester. In this project, we propose to introduce a Mango aptamer (that binds a fluorescent molecule) into the 3' untranslated region (UTR) of the viral RNA. In another investigation we developed a mutant virus in which we expanded an A-rich region in the ZIKV 3' UTR to contain 12 tandem adenosines. In this project, we will use site-directed mutagenesis to insert the Mango aptamer in the middle of the 12 adenosine stretch, then use this construct to investigate if the Mango aptamer affects translation of the construct in cultured cells, as well as determine if the RNA can be fluorescently tracked. If the inserted Mango aptamer does not affect translation and efficiently associates with a fluorophore, we will next insert this 3' UTR into a subgenomic reporter replicon to assay ZIKV translation and replication, as well as the infectious clone construct that will enable the production of infectious virus particles containing the Mango aptamer sequence. If successful, the development of this powerful ZIKV reagent will permit the tracking of viral RNA at the single-cell level.

P-059: **Nicholas Lane**

SNRPF Levels Modulate the Spliceopathy in Myotonic Dystrophy

Nicholas Lane[1,2], Jiss M. Louis[1,2], Claudia D. Lennon[1], Melissa A. Hale[3], Nicholas E. Johnson[3], J. Andrew Berglund[1,2] and Kaalak Reddy [1,2]

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Myotonic Dystrophy (DM) is a multisystemic disease caused by the expansion of a CTG repeat in the 3'-UTR of the DMPK gene (DM1) or a CCTG repeat within CNBP intron 1 (DM2). These repeat expansions lead to the production of toxic CUG/CCUG RNA, which sequesters the MBNL family of splicing regulators into ribonuclear foci, causing widespread mis-splicing that is linked to symptoms in DM. While larger expansions are generally associated with more severe symptoms, this correlation does not explain the immense disease heterogeneity present with DM.

Our lab previously uncovered the spliceosome as a novel class of modifiers of the spliceopathy in DM1. Recent analysis of RNA sequencing from congenital DM (CDM) patient muscle samples revealed strong correlations with the core spliceosome protein SNRPF and mis-splicing within the CDM cohort. Cellular experiments in our HeLa DM1 model, showed that modestly knocking down SNRPF levels improved mis-splicing, through both a reduction in CUG RNA levels and MBNL1 upregulation. Furthermore, SNRPF knockdown in DM2 patient fibroblasts revealed rescue of mis-splicing and upregulation of MBNL1. Our work suggests that the expression levels of certain core spliceosome components such as SNRPF modulate the spliceopathy in DM patients through MBNL upregulation and toxic RNA reduction. These findings have implications for uncovering the biological determinants of the extreme disease heterogeneity in DM.

P-060: **Mahla Lashkari**

Using Glucometer for Genome Detection Through in vitro Translation Using RNA Switch and CRISPR technologies

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There is a critical need for accurate, accessible, and user-friendly point-of-care pathogen detection methods outperforming traditional methods. Cell-free biosensors employ the selectivity of cellular machinery without living cells' constraints, offering significant advantages in biotechnological applications. Programmable RNA toehold switches represent a powerful platform for translational regulation. These switches activate protein synthesis only when a specific input DNA binds and unfolds the hairpin through structural rearrangement. This mechanism enables precise control of gene expression and facilitates the development of highly specific and versatile molecular biosensors. In this study, we prepared and controlled activation of an RNA toehold switch that, upon triggering, drives β-galactosidase synthesis. The expressed enzyme catalyzes the conversion of lactose into glucose, enabling quantitative detection using a glucometer. Using this platform, we selectively detected pathogenic genomic sequences from five distinct organisms through glucometer readouts. This multimodal system provides result validation through two complementary fluorogenic and chromogenic outputs.

P-061: **Jung Yeon Lee, PhD**

Unbiased Sequencing of RNA Modifications for Determining True RNA Sequence Using Mass Spectrometry Readout

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More than 180 known RNA modifications regulate RNA folding, stability, translation, and function, and their dysregulation is linked to over 100 diseases, including cancer, neurodegeneration, diabetes, and immune disorders. However, current sequencing platforms are largely blind to RNA modifications, preventing determination of the true RNA sequence (RNome) — which includes both canonical nucleotides and their modifications. To address this limitation, we have developed a next-generation mass spectrometry–based sequencing platform that uses molecular mass as a direct readout for de novo RNA sequencing. This approach enables unbiased, modification-aware sequencing at single-nucleotide resolution without reliance on cDNA conversion. Using this direct MS sequencing technology, we have determined true sequences — including site-specific modifications — in therapeutic RNAs such as CRISPR sgRNA and mRNA, as well as purified and bulk tRNAs at isodecoder resolution. This technology represents a paradigm shift toward exhaustive, true RNA sequencing and holds strong potential for epitranscriptomics and RNA therapeutic quality control.

P-062: **Qishan Lin, PhD**

Development of UPLC-MS/MS Method for Global RNA Chemical Modification Analysis in Saccharomyces Cerevisiae

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Post-transcriptional nucleoside modifications have long been recognized as key modulators of non-coding RNA structure and function. There is an emerging appreciation that the chemical modification of protein-coding messenger RNAs (mRNAs) also plays critical roles in the cell. There are over 150 known RNA modifications found in biology, However, only a handful have been identified in mRNAs. These chemical changes to nucleotides can alter gene expression and has recently been described as part of a so-called “epitranscriptome”. However, little is known about their biological roles. We sought to identify and quantify modifications present in the mRNAs of yeast cells using a high throughput ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method that measures the levels of 43 types of RNA nucleosides in parallel. our results expand the mRNA post-transcriptional modification (PTM) landscape in S. cerevisiae by examining its epitranscriptome profile under non-stressed, oxidative stress, heat-shock stress and glucose starvation conditions. Our results increase the diversity of mRNA modifications to identify 11 nucleoside modifications that may provide a potential mechanism for cells to rapidly respond to environmental stressors.

Selected Speaker – Session 8: **Xuhang Liu, PhD**

Machine learning-based small-RNA liquid biopsy enables accurate classification of benign, malignant, and metastatic breast diseases

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Mammographic screening is the primary modality for breast cancer detection but suffers from high false-positive rates that drive unnecessary and costly biopsies, causing psychological stress, and delaying the average time to diagnosis for patients with actual malignancies. We hypothesized that circulating small RNAs may provide a minimally invasive molecular complement to imaging for both risk stratification and perhaps systemic cancer staging. We prospectively collected serum from 320 women at MSKCC who were undergoing breast cancer screening or therapy and generated circulating small RNA profiles. Using these data, we trained machine-learning models to classify women as having benign breast disease, localized breast cancer, or metastatic breast cancer. Models based on serum small RNA signatures accurately distinguished benign, malignant, and metastatic diseases. Small nuclear RNA fragments (snRFs), an underexplored class of small RNAs, emerged as major contributors to model performance. A liquid biopsy based on ML-integrated small RNA and snRF signatures could be deployed alongside mammography to reduce unnecessary biopsies, accelerate diagnosis in women with malignancy, and noninvasively identify patients at high risk for underlying metastatic disease. These findings identify snRF/miRNA-informed liquid biopsy as a promising strategy for improving diagnostic precision and systemic staging in breast cancer.

P-064: **Jiss Louis, PhD**

Investigating cis-elements within the DMPK transcript to modulate toxic CUG RNA levels in myotonic dystrophy type 1

Jiss M. Louis[1], Jack Erickson-King[1], J. Andrew Berglund[1,2] and Kaalak Reddy[1,2]

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Myotonic dystrophy type 1 (DM1) is a multisystemic disease and the most common form of adult-onset muscular dystrophy. DM1 is caused by a CTG repeat expansion in DMPK. Transcription of the expanded repeats produces toxic CUG expansion RNA, leading to the sequestration of MBNL RNA-binding proteins into ribonuclear foci, which triggers extensive mis-splicing that has been linked to several DM1 symptoms. There are currently no approved disease-targeting treatments for DM1. We previously conducted a genome-scale screen and identified core spliceosome components that modulate DMPK levels and reduce ribonuclear foci in DM1 patient cells. Concomitant with DMPK downregulation, the inclusion of exons 14 and 15 of DMPK RNA decreased when selected core spliceosome components were knocked down. We hypothesize that cis-elements in DMPK can be targeted for therapeutic modulation. To test our hypothesis, I have conducted an antisense oligonucleotide (ASO) tiling screen in a genomic fragment of DMPK surrounding the repeat expansion. Relative CUG expansion RNA levels were measured in our HeLa DM1 screening cell line, and overall DMPK levels were monitored in DM1 patient myoblasts using RT-qPCR. Our ASO tiling screen in the HeLa DM1 cell model showed a selective reduction in CUG RNA levels with ASOs targeting exon 13, the exon-intron junctions, and the exon 15 sequence adjacent to the CUG repeats. In DM1 myoblasts, there was a ~50% reduction in overall DMPK mRNA levels with ASOs targeting exon 13 and intron 14. Thus, we have identified novel cis-elements that strongly modulate DMPK levels, potentially through altered splicing. My future work focuses on elucidating the specific mechanism by which DMPK splicing modulation leads to reduced levels and on evaluating the rescue of MBNL-mediated mis-splicing and ribonuclear foci formation. These findings have implications for the therapeutic development of DMPK splicing modulation using ASOs for the treatment of DM1.

Selected Speaker – Session 8: **Soneya Majumdar, PhD**

A Novel Actinobacteria-Specific Ribosome Hibernation Factor in Mycobacterium tuberculosis

Soneya Majumdar [(1)] #, Varsha Patil [(2)] #, Yunlong Li [(2)], Swati R. Manjari [(1),] Howard B. Gamper [(3),] Ya-Ming Hou [(3),] Nilesh K. Banavali [(1)(4)(5)], Anil K. Ojha [2)(4)(5),] Rajenda K. Agrawal [(1)(4)(5)]

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), persists within its host for long periods. Treatment of TB requires prolonged administration of antibiotics, some of which target Mtb ribosomes, the protein-synthesizing machine of the cell. We determined high-resolution cryo-EM structures of Mtb ribosomes obtained from biofilms under zinc-depleted conditions that are expected to induce ribosome hibernation. In these structures, in addition to finding the Mtb homolog of the hibernation promoting factor Mpy that binds to the decoding center in the small ribosomal subunit, we discovered an actinobacteria-specific protein, previously of unknown function, bound in the nascent polypeptide-exit tunnel (NPET) within the large ribosomal subunit. We refer to this NPET-bound protein as an actinobacteria-specific ribosome tunnel occlusion factor (RTOF). RTOF is found with vacant or E-tRNA-bound 70S ribosomes, but not in actively translating Mtb ribosomes, suggesting that its binding inactivates the ribosome. Loss of translation activity of the ribosome by RTOF was biochemically confirmed. RTOF makes strong interactions through the entire stretch of NPET extending up to the peptidyl-transferase center (PTC), such that it would block the passage of nascent polypeptide chain. The highly conserved FRRKSG motif of RTOF also interferes with the binding of antibiotic linezolid, which is used as a second line of antibiotics in treatment of TB, thereby maintaining a drug-free pool of hibernating Mtb ribosomes and imparting drug resistance against oxazolidinone class of antibiotics. Furthermore, FRRKSG motif also interferes with the accommodation of the formyl-methionyl moiety of initiator fMet-tRNA in the conformation required for translation initiation. Addition of mRNA and initiator fMet-tRNA displaces RTOF by triggering a significant alteration to the conformation of FRRKSG motif, thereby restoring translation activity of the ribosome. Together, these findings offer a RTOF-dependent mechanism of drug-resistance, as well as a reactivation mechanism of RTOF-bound ribosomes under optimal cellular conditions.

Selected Speaker – Opening Session: **Abby Manning**

Characterization and function of a novel Integrator-associated complex

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The Integrator complex (INT), a substantial 17-subunit, modular assembly, plays a pivotal role in the transcription of all RNA Polymerase II (RNAPII) genes. Its functions are well-established in two key areas: 3′ end processing at small nuclear RNA (snRNA) genes and the orchestration of premature termination at the promoter-proximal pause of protein-coding genes. A key unanswered question is whether unique subcomplexes of INT subunits occupy the promoters of different RNAPII biotypes. While the Little Elongation Complex (LEC) is known to associate with RNAPII at snRNA genes to facilitate transcription and recruit Integrator — enabling INTS11 to mediate 3′-end cleavage — the precise structural and mechanistic basis of this recruitment remains unclear. Through co-immunoprecipitation experiments, we have found that the LEC subunit ICE2 forms a previously uncharacterized complex with the INT Tail module, which we term the 'ICE-Tail' complex. AlphaFold modeling and comparison with existing cryo-EM structures reveal that the INT Tail module adopts a distinct conformation when bound to ICE2, forming the ICE-Tail complex. This complex appears structurally distinct from and mutually exclusive with the canonical full INT and LEC complexes, suggesting that ICE-Tail may function as a transient intermediate required for snRNA biogenesis. My preliminary data show that ICE2 interacts robustly with the INT Tail module. To define the constituency of the ICE-Tail complex, I will use an established auxin-inducible degron (AID) system, which also enables probing of transcriptional changes upon targeted depletion of ICE-Tail members. Our Structural characterization of ICE-Tail involves cryo-EM and mutational co-immunoprecipitation analysis. This work will fill critical knowledge gaps by identifying functionally essential interactions and generating novel structural models of this intermediate assembly. It also provides a key first insight into how INT may assemble and function differently across distinct RNA biotypes, suggesting that transcriptional machinery may customize its composition to regulate coding versus noncoding loci.

P-067: **Amirhossein Manzourolajdad**

3D-based RNA Switch Design

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RNA switching devices offer great therapeutic potential. Here, we present a computational pipeline to design RNA switches with desired ligand-binding preferences along with experimental validation. Our cross-disciplinary approach is as follows: (1) Atomic-resolution information about the 3D structure of RNA molecules determined from different experiments such as crystallography, NMR, and cryo-EM are gathered and used to train our geometric deep learning model, which learns nucleotide orientation and positioning in 3D. (2) Given an RNA molecule of interest as a template, our generative model produces hypothetical RNA sequences that conform to the desired 3D structure. (3) We then screen the generated sequences for high ligand-binding affinities using the AlphaFold software. (3) Top candidate sequences are synthesized and tested in lab for in-vitro functional validation. Initial ligand-binding melting curve and ITC data, validate our initial case-study of designing a synthetic FMN riboswitch. Results demonstrate the success of our approach in designing customized RNA molecules with reliable functionality.

P-068 **Amy Mascorro**

Dual Targeting ASO-based RiboTAC degrades CUG RNA and Rescues Mis-Splicing in HeLa-based DM1 Cell Model

Amy Mascorro [(1)(2),] Angela White [(1)(2),] Yu Sheng Chen [(1)(2)], Lucas Hooker [(1)(3),] Audrey Coons [(1)(3),] Shemari Blackwood [(1)(3)], Sanjida Ahmed [(1)(3)], Dr. Andrew Berglund [(1)(3),] Dr. Jia Sheng* [(1)(2)]*

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Myotonic dystrophy type 1 (DM1) is a repeat expansion disease caused by a CTG repeat expansion in the 3′UTR of the DMPK gene, producing toxic r(CUG)exp transcripts that sequester MBNL proteins and drive widespread mis splicing. Therapeutic strategies capable of selectively degrading r(CUG)exp remain urgently needed to reverse this RNA gain of function pathology. Building on Ribonuclease targeting chimera (RiboTAC) approaches, we developed a Dual Targeting RiboTAC (DuRTAC) engineered to simultaneously recognize r(CUG)exp and the DMPK 3′UTR while recruiting RNase L to cleave the pathogenic RNA. In vitro and cell based assays demonstrate that DuRTAC promotes sequence specific, RNase L–dependent degradation of r(CUG)exp, accompanied by release of MBNL proteins, reduction of nuclear RNA foci, and rescue of DM1 relevant splicing defects in a DM1 HeLa model. Early data indicate that dual target recognition enhances degradation efficiency relative to single target constructs. Ongoing work focuses on optimizing oligonucleotide chemistry, defining innate immune activation thresholds, and evaluating activity in DM1 patient derived cells. These results establish DuRTAC as a promising, modular platform for targeted destruction of repeat expanded RNAs in DM1.

P-069: **Massa Masri**

Spinocerebellar Ataxia 2 Whole Blood Transcriptomic Analysis Reveals Novel Immune Biomarkers and Widespread Mis-Splicing

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Microsatellite repeat expansion disorders are caused by one to six nucleotide regions of DNA that are repeated up to 1000 times. Non-pathogenic repeats make up about 3% of the human genome, but some repeat regions become problematic due to their instability and tendency to expand when the cell divides. Spinocerebellar Ataxias (SCAs) are neurodegenerative- and dominantly-inherited disorders affecting the cerebellum and other parts of the nervous system. They are caused by various genetic defects including repeat expansions. SCA2, the second most common SCA, is a severe subtype that is caused by a CAG repeat in the ATXN2 gene. SCA2 causes widespread symptoms such as gait ataxia, cerebellar dysarthria, postural instability, and dysmetria. The disease involves RNA repeat- and polyQ expansions, yet the mechanisms underlying its pathogenesis are not well understood. Particularly, the immune system's role in the disease's progression remains mainly unexplored. In this study, we investigated the immune system's response to the RNA and polyQ rep eat expansions in SCA2, and the mis-splicing that could contribute to its pathology. We hypothesized that SCA2 would trigger immune response pathways due to the accumulation of repeat RNA and polyQ protein, and lead to widespread mis-splicing. Using a dataset from bulk RNA sequencing of whole blood from SCA2 patients, we investigated the expression of immune response genes and mis-splicing events. From this study, we concluded that SCA2 shows a pattern of immune gene expression and mis-splicing. Future studies are important to determine whether immune gene changes are a driver of disease or a response to neurodegeneration, if mis-splicing plays a factor in those changes, and to understand the causes leading to mis-splicing.

P-070: **Chetna Mathur**

Distinct Codon Usage and tRNA Modifications Define Regulatory Control of Oncogenes in Cancer

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Oncogenes and tumor suppressor genes (TSGs) play opposing roles in cancer by promoting or restricting cellular growth, respectively. Beyond transcriptional control, translational regulation has emerged as a critical determinant of oncogenic programs, with codon usage bias and tRNA-based epitranscriptomic modifications acting as key regulatory layers. Codon-biased translation has been linked to proliferation, stress adaptation, and drug resistance across multiple cancers. Using advanced codon analytics, we systematically characterized and compared codon usage patterns in oncogenes and TSGs from humans and mice at both group and gene-specific levels. We demonstrate that human oncogenes and TSGs exhibit distinct and opposing codon usage signatures, with oncogenes strongly enriched for G/C-ending (GC3) codons and TSGs favoring A/U-ending (AU3) codons. Comparative analyses across 447 gene ontology groups reveal that human oncogenes possess one of the most distinctive codon usage profiles in the genome, underscoring the ability of codon bias to stratify functionally related pathways. Mouse oncogenes and TSGs display opposing yet less extreme codon bias, relative to humans, highlighting potential species-specific differences in oncogenic translation programs. Given the extreme codon usage of oncogenes, we hypothesized a dependency for optimal translation on specific tRNA-modifying enzymes. Mining The Cancer Genome Atlas, we identified five cancers with coordinated amplification of oncogenes and tRNA epitranscriptomic writers, including ALKBH8. Functional studies in ALKBH8-amplified bladder cancer cells revealed that ALKBH8 knockdown globally alters transcription, reduces the levels of the oncogenic MDM2 and CCND1 proteins, despite a global increase in translation. Together, these findings highlight codon usage bias in oncogenes and details how a defect in tRNA modification can alter gene expression, with both potentially exploitable for cancer therapeutics.

P-071: **Ian McClain**

Photochemistry of Oligonucleotide Chemistry

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Research into synthetic oligonucleotides continues to derive new methods of biomedical applications, and purification of the synthetic product continues to be one of the most difficult portions of solid-phase synthesis. Reverse-phase HPLC and anion-exchange HPLC methodology is effective for purification of long-chain oligonucleotides but is an expensive and time-intensive process which requires optimization to each new synthetic strand. In this study we introduce a new visible-light sensitive anchor comprised of a hetero-bifunctional coumarin molecule which cleaves with blue light (λ = 456 nm) in one quarter the time of our previous anchor. We applied this novel anchor with our previous click-purification strategy to purify 103-nucleotide sgRNA for CRISPR experiments which displayed comparable nuclease activity to sgRNA purified by conventional PAGE purification. In addition, the bifunctionality of this anchor afforded an avenue of research which continues growing, temporal control of CRISPR gene editing activity. We incorporated our bifunctional coumarin molecule into the guide RNA of CRISPR-cas9 system to afford time-based controls of RNase activity in both in vitro and in vivo applications.

P-072: **Katherine McCown**

Functional Assessment of Enzyme MiaA in the Conversion of Adenosine to Isopentenyladenosine in tRNA

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Transfer RNAs (tRNAs), which play an essential role in protein synthesis across all domains of life, contain the highest abundance of nucleoside modifications amongst the most common types of RNA: mRNA, tRNA, and rRNA. Here we study the N6-isopentenyladenosine (i6A) modification, a structurally bulky modification that occurs exclusively in certain tRNAs with anticodons ending in adenosine (A). In this study, we expressed MiaA, synthesized tRNA substrates, and used HPLC analysis to evaluate enzymatic activity on both native and modified tRNAs. Our future goal is to discover small molecule inhibitors of the MiaA enzyme.

P-073: **Morgan McGrath**

Integrated stress response directs adaptive mRNA translation in prostate cancer following irradiation

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Therapeutic resistance is a major contributor to prostate cancer mortality. The ability to combat resistance to standard-of-care therapies, therefore, has vast implications for patient outcomes. Transcriptional reprogramming in response to clinical therapies has been well studied, but adaptive translational responses in prostate cancer remain largely uncharacterized. Furthermore, while cancer cells are well-known to rewire translation to balance intrinsic oncogenic stressors, the application of therapeutic stressors imparts an additional layer of complexity onto the process of stress-inducible regulation of protein synthesis. With this study, we illustrate the impact of radiotherapy on both the global and specific translational behavior of prostate cancer cells, featuring radiation-directed Integrated Stress Response (ISR) activation as a therapeutically targetable constituent of the adaptive radioresponse. Exposure to ionizing radiation led to a reduction in global protein synthesis in prostate cancer cells. Amidst the radiation-sensitive decrease in global mRNA translation, select prostate cancer progression-associated transcripts were translationally increased. ISRIB, a small-molecule inhibitor of the ISR, countered the broad and selective effects of translational rewiring, restoring global protein synthesis in irradiated cells and xenograft tumors. Lastly, impairment of translational rewiring via inhibition of the ISR improved responses to radiotherapy in vivo, slowing tumor growth whilst mitigating adverse weight loss in irradiated mice. These findings establish translational control as a critical function of irradiated prostate cancer cells and present ISR inhibition as a novel avenue for both radiosensitization and alleviation of off-target radiation toxicity.

P-074: **Tristan Melfi**

DNA nanostructures for drug delivery across lipid membranes

Tristan Melfi, Liam Mackey, Sweta Vangaveti, Alan Chen

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With advancements in bioinformatics, structural biology, and other computational methods. The fact that DNA may take up forms different than the traditional b-form helix has become a more familiar fact. The reliability of base pairing interactions and stabilization through base stacking makes DNA a stable and versatile molecule. One particular interest is that of engineering sequences to fold into complex structures for a specific use. Using DNA nanostructures to accept small molecule therapeutics they can be introduced into a cell by diffusion across the membrane. In silico studies allow us to probe the efficacy of a structure at atomistic detail by performing pulling simulations to directly observe how a construct would bypass a lipid bilayer. Allowing experimental researchers to intelligently introduce modifications to maximize performance.

P-075: **Steven Mesite**

TAMARA: a Simple and Efficient Microfluidic Nanoparticle Formulation System Tailored for Preclinical Studies

Steven Mesite, Robin Oliverse (1)

[1] *Inside Therapeutics*

Lipid nanoparticles (LNPs) are fundamental to the success of RNA therapeutics due to their capacity to encapsulate, protect, and efficiently deliver RNA into target cells. LNP development requires understanding formulation composition (lipid and RNA types and purity) and process variables such total flow rate (TFR), flow rate ratio (FRR), and mixing geometry. These can all influence the critical quality attributes (CQAs) such as particle size, polydispersity index (PDI), encapsulation efficiency (EE%), and ultimately biological performance.

The screening required during preclinical development, can rapidly become cost-prohibitive due to the high cost of RNA materials, so very low batch volumes ~400 ul are desired. It is also important that none of the valuable product goes to waste.

Here, we present case studies developed with TAMARA, a microfluidic-based RNA-LNP formulation platform designed to seamlessly span the entire preclinical development workflow — from low-volume screening to larger-scale in vivo batch production — within a single system. The platform enables scale continuity without modification of formulation or processing parameters that may compromise LNP characteristics, while eliminating material losses.

RNA-LNPs were formulated using the TAMARA system with clinically validated ionizable lipids (SM-102- or ALC-0315-based formulations) across a broad scale range (200 µL to 30 mL). Process parameters (TFR, FRR, and chip geometries) were systematically varied to evaluate parameter sensitivity and scalability.

Biological performance was assessed through in vitro transfection efficiency and cell viability assays. Head-to-head comparisons were conducted against state-of-the-art toroidal mixers. Selected formulations were further evaluated in vivo to confirm functional potency.

P-076: **Megan Miaro**

Prediction of non-canonical base pairs using structural constraints from sequence comparison

Megan Miaro, David H. Mathews

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Non-coding RNA (ncRNA) have many biological roles including carrying genetic information, catalyzing reactions, and regulating gene expression. Around 98% of the human genome is made up of non-protein-coding sequences with ncRNA making up the majority of transcriptional output. The majority of these ncRNA have yet to be characterized.

As structure relates to function, ncRNA 3D structure predictions can provide key insights on the function of an RNA. These predictions currently suffer from a lack of accurate modeling, particularly in loop regions that contain non-canonical base pairs. Current methods in RNA secondary structure prediction focus on the prediction of canonical base pairs using thermodynamic parameters. There are no thermodynamic parameters available for the prediction of non-canonical base-pairs. Additionally, predicting conserved structural elements from homology is the most accurate approach to predict RNA secondary structures as homologous RNAs can share high structural similarity, even with low sequence identity.

We developed a program to predict RNA secondary structures including non-canonical base-pairs using a sequence comparison approach. TurboFold, from the RNAstructure package, predicts conserved secondary structures and a sequence alignment given a set of homologous sequences. Using TurboFold, we implemented a preexisting set of parameters named Nucleotide Cyclic Motifs (NCMs) that allow for the prediction of non-canonical base-pairs. The favorability of these motifs were learned from a database of RNA 3D structures. In benchmarks, we demonstrated that the NCMs perform worse than thermodynamic parameters at predicting conserved canonical base pairs. To address this, we use the accurate predictions of canonical base-pairs with thermodynamic parameters to constrain the search of non-canonical base pairs with NCMs. This significantly increases the prediction accuracy for canonical base-pairs, and it correctly predicts on average 70% of conserved non-canonical base pairs.

P-077: **Subodh Mishra, PhD**

Therapeutic targeting of DM1 disease mechanism using a quercetin–vorinostat combination

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant multisystemic neuromuscular disorder caused by CTG repeat expansion in 3′UTR of DMPK gene. Transcription of the expanded allele produces toxic gain-of-function CUG repeat–expanded RNA that aberrantly sequesters MBNL proteins into nuclear ribonuclear foci, leading to widespread mis-splicing and downstream dysfunction. These pathogenic pathways are mechanistically interconnected, such that modulation of a single pathway is unlikely to achieve broad molecular correction. Therapeutic strategies that engage multiple pathogenic pathways represent a critical need for effective molecular correction in DM1.

We recently identified quercetin as a CTG repeat-selective small molecule that reduces toxic CUG RNA, rescues DM1 mis-splicing in patient-derived cells, and in its bioavailable form, EMIQ, improves toxic RNA burden, splicing defects, and myotonia in DM1 mice. To evaluate a focused combination strategy, here we paired quercetin with the HDAC inhibitor vorinostat and quantified effects on MBNL-dependent splicing correction, selective toxic RNA reduction, and tolerability relative to each molecule alone.

We tested the quercetin–vorinostat combination in a DM1 MYOD1-engineered myogenic line and in DM1 patient-derived myotubes (DM05) and assessed in vivo efficacy in DM1 HSALR mice. In the DM1 MYOD1-engineered myogenic line, the mis-splicing of MBNL1 exon 5 showed 56% rescue with vorinostat (5 μM) alone and 45% rescue with quercetin (32 μM) alone, whereas the combination treatment showed 101% mis-splicing rescue. A similar additive pattern was observed for MBNL2 exon 5 (quercetin: 33%; vorinostat: 66%; combination: 100%). Consistent with data from the first DM1 cell line, in DM05 patient-derived myotubes, the mis-splicing of MBNL1 exon 5 showed 47% rescue with vorinostat (5 μM) alone and 57 % rescue with quercetin (32 μM) alone, whereas the combination treatment showed 102% mis-splicing rescue. A similar additive pattern was observed for MBNL2 exon 5 (quercetin: 50%; vorinostat: 56%; combination: 103%). In unaffected myogenic cells and control myotubes, combination treatment did not induce a statistically significant change in splicing, indicating that the observed splicing correction is selective to the DM1 disease context. Further, in DM1 HSALR mice, EMIQ and vorinostat combination treatment resulted in additive myotonia improvement relative to either single molecule treatment, supporting functional benefit of the combination approach. These findings support the quercetin and vorinostat combination as a disease-selective therapeutic strategy that resulted in additive rescue of representative MBNL-dependent splicing events, alongside functional improvement in myotonia in DM1 mice. We are currently performing RNA-seq in DM1 muscle cell models and HSALR tissues to define global transcriptomic changes and assess off-target effects.

P-078: **Shirin Mohammadian**

The Folding Roadmap: Tracing RNA's Intermediate Landscapes via GROMACS

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RNA molecules begin folding co-transcriptionally, as they emerge from RNA polymerase. These early structures can directly modulate transcriptional outcomes, pausing or promoting elongation. Riboswitches exemplify this regulation: ligand binding induces structural shifts, from an undocked to a docked conformation, that influence polymerase behavior. In the preQ$_1$ riboswitch, a pseudoknot intermediate stabilizes RNAP pausing, while ligand interaction promotes termination via conformational change. Because RNA folding is directional (5′→3′), it progresses through transient intermediates. Capturing these elusive states is key to understanding regulatory mechanisms. We applied GROMACS molecular dynamics with PLUMED biases to simulate and map RNA's intermediate conformations in silico.

P-079: **Katrine Mongin**

Structural Determination of Insulin Receptor mRNA with SHAPE-MaP Chemical Probing

Zohreh R. Zowzari, Katrine Morgin, Rob Merritt, Kaalak Reddy, Alan A. Chen, J. Andrew Berglund

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Alternative splicing of the insulin receptor (INSR) pre-mRNA at exon 11 is essential for normal insulin signaling, and its dysregulation is associated with several human disorders. The RNA-binding protein Muscleblind-like 1 (MBNL1) has previously been identified as a critical regulator promoting inclusion of exon 11; however, the structural basis of this regulation remains poorly understood. To investigate how MBNL1 influences INSR alternative splicing at the structural level, mRNA transcribed from an INSR minigene was analyzed using selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP). In this approach, RNA is treated with an electrophilic chemical probe that modifies the 2′-hydroxyl groups of conformationally flexible nucleotides. These modifications are detected as mutations during reverse transcription, enabling the generation of nucleotide-resolution reactivity profiles. SHAPE reactivity data were used to model the secondary and tertiary structures of INSR mRNA under cellular and in vitro conditions, both in the presence and absence of MBNL1. Structural predictions indicate that INSR mRNA adopts a more compact and double-stranded conformation when bound to MBNL1, consistent with the formation of an RNA–protein complex. These findings suggest that MBNL1-mediated structural rearrangements of INSR mRNA play a key role in regulating exon 11 inclusion. Improved understanding of INSR mRNA structure may facilitate the development of targeted therapeutic strategies, such as antisense oligonucleotides, to correct aberrant splicing.

P-080: **Fin Morris**

mRNA based Senescence Detection system

Fin Morris, J. Andre Melendez

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Cellular senescence is a state where cells stop dividing but remain metabolically active as result of exposure to a variety of stressors. The senescence pathway is an evolutionarily beneficial alternative to degenerating into a hyperproliferative cancer cell. These metabolically actively non-dividing cells secrete a myriad of proteins referred to as the Senescence Associated Secretory Phenotype (SASP) that create a permissive environment for disease development. We have taken advantage of a molecular mechanism regulating the SASP to develop a non-destructive RNA-based system for senescence detection. Real time detection of senescence is critical for testing interventional strategies that limit senescence and age associated disease development. Here we show how a mRNA construct incorporating two reporter molecules (TagBFP and eGFP) coupled by Interleukin-1alpha (IL-1a) can detect senescence in a mixed population. The protein is intended to be cleaved only in senescent cells, separating the reporter molecules into the nucleus and cytoplasm. Which will give a strong visual indicator of senescence in transfected cells. This research will provide a starting point for _in vivo_ research, where determining senescence is important for therapeutic purposes. This will also prove a valuable demonstration of programmable, pathway-specific RNA activity, which can be expanded to other cell types.

P-081: **Vinod Morya, PhD**

A 3D printed mini-gel electrophoresis system for rapid and inexpensive DNA nanoswitch biosensing

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Gel electrophoresis has been a cornerstone laboratory technique for decades, yet it is often viewed as cumbersome, costly, and has remained confined to laboratory settings. Recent advances in DNA nanotechnology have repurposed electrophoresis as a primary readout for some biosensing applications such as DNA nanoswitches, where a conformational change in a DNA structure indicates the presence of a target molecule. Conventional gel electrophoresis setups are less than ideal for such targeted applications, with moderate equipment cost, excessive reagent use, and time-consuming processes. In this work, we adopt a reductionist, application-driven approach to redesign gel electrophoresis specifically for DNA nanoswitch-based detection. We present a fully 3D-printable mini gel electrophoresis system that incorporates conductive plastic electrodes, demonstrating performance comparable to conventional systems using platinum electrodes. By carefully tuning the inter-electrode distance and optimizing running parameters, our system resolves the on/off states of DNA nanoswitches in as little as one minute. We further show that the device operates reliably at low voltages, including when powered by a USB power bank, and even enables instrument-free nanoswitch readout using an LED with a cell-phone camera. Our design substantially reduces the cost, voltage requirements, material usage, operational complexity, and experiment time. These improvements make gel-based biosensing more practical outside traditional laboratory environments, paving the way for broader adoption of gel electrophoresis in point-of-care and resource-limited settings.

Selected Speaker – Session 2: **Andrew Munoz Gamba**

Phenotypic and genotypic signatures of adaptation of Zika virus after serial passaging in mosquito and mammalian hosts

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Zika virus (ZIKV) is a flavivirus transmitted by Aedes aegypti mosquitoes that was introduced into the Americas in 2015. ZIKV infections cause severe neurological disease in humans, including congenital microcephaly and Guillain-Barré Syndrome. Unfortunately, no licensed antiviral treatments or vaccines have been approved. Encoded in the viral genome, the NS5 RNA-dependent RNA polymerase is inherently error-prone resulting in the accumulation of mutations as the virus alternates between mosquito and mammalian hosts. The significance of these new mutations to promote or restrict host adaptation remains poorly understood. In this research, we sought to investigate genetic determinants in the ZIKV genome during host adaptation. To this end, ZIKV was evolutionary passaged in mammalian (African monkey green-Vero) and mosquito (Aedes albopictus-C6/36) cells. The infections were undertaken in a single-host (only mammalian/mosquito cells) or with host-switching (alternating between both hosts). Specifically, ZIKV was passaged ten times at a multiplicity of infection of 0.01 plaque forming units/cell for 72 and 96 hours post-infection in Vero and C6/36 cells, respectively. Using plaque assays to phenotypically characterize virus infections, we observed one- to two-fold increases and decreases in ZIKV viral titers following host-switching. In contrast, in single-host infections, ZIKV titers increased two-fold by passage ten. An examination of the intra-host genetic diversity of input virus and that collected after passage five and ten, revealed a total of 4,675 variants. The NS5 gene had the highest accumulation of non-synonymous mutations independent of the host. Herein, 112 variants in the methyltransferase (MTase) domain were found. While the conserved amino acids associated with catalytic activity remained unchanged, many of the genetic changes were near and within the catalytic S-adenosyl-L-homocysteine site. These data suggest a previously unknown and novel role for the MTase domain in host adaptation and identify it as a potential target for therapeutic intervention.

P-083: **Rina Nagao**

Dynamic competition between nuclear PIA-RNA stabilization and decay shapes mitochondrial activity

Xavier Rambout [(1) (2)],**Rina Nagao[(1),(2)],**Victor Gu[(1),(2)] Justin Youngyunpipatkul[(3),(4)] Jadon Schlierf[(3),(4)] Christoph Pröschel[(2),(3),(4)] and Lynne E. Maquat [(1),(2)],**

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While RNA quality control tightly regulates gene expression, the scope of its regulatory functions in the nucleus remains unclear. Here, we describe chromatin-associated quality control of Poised-for-splicing Intron-containing polyAdenylated RNAs (PIA-RNAs), a class of partially processed pre-mRNAs. The nuclear cap-binding complex (CBC) bound to ARS2 (CBCA) serves as a central platform for nuclear RNA metabolism, coordinating the processing and quality control of nuclear RNAs. To date, the only known CBCA-binding factor that promotes mRNA biogenesis is NCBP3.

We find that the fate of PIA-RNAs is determined by competition between NCBP3 and Anillin, which we identify as a novel CBCA-binding adaptor of the nuclear RNA exosome, for binding to ARS2 within CBCA. Engagement of NCBP3 forms a reservoir of immature mRNAs for delayed splicing, whereas recruitment of Anillin promotes their degradation by the nuclear RNA exosome. Chromatin-associated RNA sequencing reveals that many PIA-RNAs, oppositely regulated by NCBP3 and Anillin, encode mitochondrial proteins. Consistently, Seahorse assays show that loss of NCBP3 leads to reduced mitochondrial activity, highlighting that this nuclear RNA quality control pathway contributes to mitochondrial homeostasis.

Together, these findings reveal an unexpected competition between nuclear pre-mRNA metabolic pathways, whose balanced activity is essential for mitochondrial function.

P-084: **Brianna S. Nelthrope, MS**

Dysregulation of Alternative Splicing is a Transcriptomic Phenotype of SCA3 Mouse Models

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Spinocerebellar ataxias (SCAs) comprise more than 40 genetically diverse neurodegenerative disorders, many of which are caused by CAG repeat expansions within coding regions that give rise to polyglutamine (polyQ) tracts. Although these repeat expansions drive progressive neurodegeneration, particularly in the cerebellum and brainstem, the molecular mechanisms linking the mutation to disease pathogenesis remain poorly understood. Recently, dysregulation of alternative splicing has emerged as a transcriptomic hallmark of CAG expansion SCAs. However, most studies have focused primarily on SCA1 models and have examined only the skipped exon (SE) event class.

To investigate disease-associated progression of alternative splicing and evaluate its potential as a transcriptomic biomarker for antisense oligonucleotide (ASO) therapeutic intervention, I performed RNA-sequencing analysis of YACMJD84.2Q-C57BL/6 transgenic mice (SCA3 Q84 mice) at early (8-week), mid (16-week), and late (65-week) disease stages following treatment with the ATXN3-targeting antisense oligonucleotide ASO-5. Widespread dysregulation of alternative splicing was detected across multiple splicing classes, including skipped exon (SE), mutually exclusive exon (MXE), alternative 5′ splice site (A5′SS), and alternative 3′ splice site (A3′SS) events, with SE events representing the most abundant class of missplicing. Gene ontology enrichment analysis using Metascape revealed that dysregulated SE events were enriched in pathways relevant to SCA pathogenesis, including neuronal projection organization, synaptic signaling, cytoskeletal regulation, and ion channel function. Importantly, ASO-5 treatment significantly rescued misspliced skipped exon events in both the cerebellum and brainstem of SCA3 mice.

Together identifying alternative splicing events in SCAs will deepen our knowledge and understanding of how dysregulation of splicing contributes to disease pathogenesis potentially leading to alternative splicing as a potential target engagement biomarker for therapeutic interventions.

P-085: **Esther Neves**

The Ribosome's Enigmatic Players: Unveiling the Roles of Ribosomal RNA and RNA-Binding Protein Interactions

Esther Neves, Rutgers New Jersey Medical School, Newark, NJ

Anthony Olarerin-George, Rutgers New Jersey Medical School, Newark, NJ

Ribosomal RNA (rRNA) comprises over 80% of all human cellular RNA and is primarily known for catalyzing protein peptide bonds and serving as the scaffold for the ribosome. However, there is still little known about the role of expansion segments, regions of rRNA which are available for cytoplasmic interactions. Considering how important and conserved the ribosome is for cellular survival, uncovering the function of the expansion segments could reveal novel functions of ribosomal and cellular biology. Previous studies have found that rRNA endogenously binds to many RNA-binding proteins (RPBs) that have unknown roles in ribosomal functions. It is likely that many of these RBPs are involved in mediating the dynamic processes of the ribosomes. Therefore, we postulate that expansion segments bind these RBPs to alter ribosomal activity under various cellular conditions. Previous studies have shown that long non-coding RNA and viral RNA are capable of sequestering RBPs that serve important regulatory functions. Recent data has also shown that the interaction between an oncogenic protein and rRNA resulted in changes to ribosomal biogenesis, cellular proliferation, and cellular stress. Based on these findings, we hypothesize that there are functional rRNA-RBP interactions that regulate translation and other cellular functions. The goal of the proposed studies is to determine how RBP-rRNA binding affects both ribosomal biology and any additional RBP pathways. Two specific aims are proposed to indicate whether 1) RBPs affect ribosomal biology and translation through changes to rRNA and 2) rRNA regulates the function of RBPs. Once these aims are completed, we expect to reveal novel functions of both rRNA and RBPs in regulating pathways vital to cellular homeostasis. Ultimately, this will elucidate the role of expansion segments in humans and expand the repertoire for the rRNA interactome.

P-086: **Rose Nowzari, PhD**

From RNA Structure to Therapeutic Design

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RNA structure plays a central role in alternative splicing regulation by RNA-binding proteins, yet the structural mechanisms underlying these processes remain poorly defined. My research focuses on understanding how Muscleblind-like 1 (MBNL1) recognizes and remodels RNA to regulate splicing, and how this knowledge can be exploited to develop structure-informed therapeutic strategies.

We first investigated the structural basis of MBNL1-mediated regulation of insulin receptor (INSR) exon 11 alternative splicing, a key event in insulin signaling and metabolic disease. Using selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP), we probed the structure of INSR mRNA derived from a minigene under cellular and in vitro conditions, in the presence and absence of MBNL1. SHAPE-guided modeling revealed that MBNL1 binding induces a more compact and double-stranded RNA conformation, consistent with formation of a stable RNA–protein complex that promotes exon 11 inclusion. These results provide direct structural insight into how MBNL1 regulates splicing through RNA remodeling.

Building on this mechanistic framework, we next examined therapeutic disruption of pathogenic MBNL1 sequestration in myotonic dystrophy, a multisystemic disorder caused by toxic repeat RNAs. We designed and computationally evaluated cyclic peptide inhibitors targeting MBNL1–RNA interactions using molecular dynamics simulations. The simulations revealed distinct and stable peptide–RNA binding modes and accurately captured the effects of subtle sequence and chirality changes on binding affinity, in strong agreement with available experimental data.

Together, these studies integrate RNA structural probing and molecular modeling to define how MBNL1 engages RNA in both physiological and disease contexts, providing a foundation for the rational design of RNA-targeted therapeutics to correct aberrant splicing.

P-087: **Taiwo (Annabelle) Ogunyale**

Schwann Cells Drive Salivary Gland Fibrosis via ECM Remodeling

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Schwann cells are specialized glia that support peripheral nerves and contribute to development, maintenance, and repair in multiple organs, including salivary glands. While nerve-associated stromal cells modulate extracellular matrix (ECM) remodeling in other fibrotic tissues, their role in salivary gland fibrosis remains poorly defined. Ductal ligation surgery was performed in adult female mice, and submandibular glands were collected at 0, 1, 3, and 7 days post-ligation for immunohistochemistry and single-cell RNA sequencing (scRNA-seq) to define Schwann cell states, ECM programs, and cell–cell signaling during injury, repair, and early fibrotic remodeling. ScRNA-seq identified distinct injury-associated Schwann clusters transitioning from a homeostatic to a repair-like state characterized by induction of canonical Schwann cell repair markers, including nerve growth factor (Ngfr) and c-Jun. These clusters demonstrated robust upregulation of matrisome genes, including Collagen I (Col1a1), Collagen 4 (Col4a1), and Periostin (Postn), directly establishing Schwann cell contribution to ECM deposition. Immunostaining validated Schwann cell proliferation, repair marker induction, and proximity to ECM proteins after ligation. Pathway and ligand–receptor analyses revealed TGFβ- and PDGF-signaling axes driving a novel profibrotic Schwann–fibroblast circuit in the injured gland. These data position Schwann cells as dynamic, active drivers of salivary gland fibrosis.

P-088: **Humphrey Omeoga**

The tRNA Writer MnmA Translationally Regulates Key Transcription Factors Critical to Environmental and Physiological Changes

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Wobble uridine (U34) modifications on tRNALys, tRNAGlu, and tRNAGln in Escherichia coli are written by the methylaminomethyl (mnm)-enzymes MnmA, MnmC, MnmE, MnmG and MnmH. Here we show that MnmA-writer deficient cells lack s2U and se2U modifications and exhibit slow growth and stress response phenotypes. The MnmA-writer deficiency drives global and stress-specific transcriptional and translational reprogramming, which we have linked to dysregulation of the RpoS, OxyR and FliA regulons. The RpoS, OxyR and FliA transcription factors all coordinate core adaptive pathways, including oxidative stress defenses, stationary phase programs, and motility/chemotaxis. Using advanced codon analytics based on gene-specific codon frequencies and Gaussian mixture modeling (GMM), we identified five distinct codon-usage clusters across the E. coli genome and have linked MnmA written modifications to the translational regulation of a cluster of genes enriched in both A and G ending codons for Lys, Glu and Gln, that includes RpoS, OxyR and FliA. Our studies demonstrate that MnmA written marks in tRNA play global roles in gene regulation and highlight the specific transcription factors that are translationally regulated. Furthermore, we demonstrate that codon bias and tRNA modification are linked to stress and motility responses in bacteria and provide a framework for studying translational regulation in complex gene expression systems.

P-089: **Ebenezer Omojowolo**

Characterization of DIAPH1 Membrane Localization: Structural Predictions and Experimental Validation

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Diaphanous 1 (DIAPH1) is a dimeric, cytosolic, multidomain formin that nucleates and elongates actin filaments during essential cellular processes including motility, phagocytosis, and cytokinesis. DIAPH1 is maintained in an autoinhibited conformation through intramolecular interactions between its Diaphanous Inhibitory Domain (DID) and Diaphanous Autoregulatory Domain (DAD). Activation occurs upon binding of the small GTPase RhoA to the GTPase Binding Domain (GBD), which destabilizes DID–DAD interactions and promotes actin polymerization, typically at the inner leaflet of the plasma membrane. Membrane localization is mediated in part by interactions of the N- and C-termini with lipid components; basic patches within the N-terminus associate with phosphatidylinositol 4,5-bisphosphate (PIP_2)-enriched membranes, while C-terminal interactions with regulatory proteins such as flightless-I modulate polymerase activity independently of DID. Despite extensive investigation, the in vivo mechanisms governing PIP_2-dependent membrane targeting of DIAPH1 remain incompletely understood.

Recent advances in structure prediction, notably AlphaFold and AlphaMissense — recognized for transformative contributions to protein structural biology and high performance in the Critical Assessment of protein Structure Prediction (CASP) competition — have provided new opportunities to characterize DIAPH1 structure and pathogenic variants. However, because AlphaFold models are trained predominantly on crystallographic datasets, their performance on dynamic or solution-phase conformations remains uncertain. Here, we integrate nuclear magnetic resonance (NMR) spectroscopy, cross-linking tandem mass spectrometry, and size-exclusion chromatography to evaluate structural and pathogenic predictions for DIAPH1 predicted by AlphaFold and AlphaMissense. Our results reveal significant discrepancies between computational predictions and experimentally derived intramolecular interactions, highlighting limitations in current predictive models for dynamic, multidomain proteins and providing new insights into the structural determinants of DIAPH1 regulation and membrane localization.

P-090: **Onyinyechi Onuoha**

Unraveling the Distinct Roles of the Alpha-like Subunits in RNA Polymerase I and III Complex Biogenesis

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RNA Polymerases (Pols) I and III are large multi-subunit complexes that synthesize rRNA, which is crucial for ribosome production and subsequent protein synthesis. Perturbations in Pol I and III transcription lead to dysregulated ribosome biogenesis, which impacts protein synthesis and results in a cascade of downstream cellular defects that affect cell growth and division. Pols I and III encode and share two distinct alpha-like subunits, POLR1C and POLR1D, that are evolutionarily conserved across all life forms. These subunits form a heterodimer, which is proposed to be crucial for assembly. Dysregulation of these alpha-like subunits is linked to diseases such as cancer, developmental disorders, and neurological defects. While the effects of mutations in the alpha-like subunits have been widely characterized, the functional roles of these subunits in Pols I and III complex biogenesis remain largely unexplored. Given the evolutionary conservation of the alpha-like subunits, we used AC19 and AC40, the yeast orthologs of POLR1D and POLR1C, to decipher the role of these subunits in Pols I and III complex biogenesis. We utilized the Auxin-inducible degron (AID) system to trigger the proteasomal degradation of AC19 or AC40 and subsequently analyzed the impact of the loss of either subunit on the integrity of Pols I and III complexes, as well as the protein abundance of other subunits within these complexes. Our findings reveal that the alpha-like subunits play distinct roles in the subunit expression dynamics and integrity of these polymerases. Although Pols I and III share a similar subunit architectural blueprint, our results suggest that Pols I and III have distinct, if not custom, assembly pathways, diverging not only from each other but also from other Pols. These findings provide new insights into Pol subunit interactions and possibly points of regulation that could inform therapeutic strategies for diseases linked to Pol dysregulation.

P-091: **Alexander Parks**

Development of a Small Molecule Integrator Inhibitor to Probe its Role During Erythropoiesis

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The chief regulatory step of gene expression critical for proper differentiation is at the stage of promoter proximal paused RNA Polymerase II (RNAPII) during transcription. During the proximal-pause, the cell must make decisions of RNAPII to proceed into productive elongation or to terminate the proximal-pause. During erythroid cell maturation into a red blood cell, erythroid cells repress unnecessary gene expression and overexpress globin genes to produce as much hemoglobin as possible before shutting down transcription entirely. While our labs have recently shown that regulation of promoter proximal paused RNAPII by Integrator has a key role in erythroid maturation, progress is hampered by the limited tool set for selectively inhibiting Integrator at specific stages of erythropoiesis.

To address this gap, we collaborated with the Scripps Research Institute to perform a small molecule screen aimed at identifying inhibitors of the Integrator complex. Using a cell based fluorescent reporter activated upon inhibition of INTS11, the catalytic subunit of Integrator, we identified a candidate compound that directly or indirectly suppresses INTS11 activity. This small molecule activates a GFP reporter of Integrator inhibition and induces expression of known Integrator target genes to levels comparable to those observed following genetic depletion of Integrator subunits. In HEK293T cells, compound treatment also upregulates immediate early genes and components of the integrated stress response, phenocopying Integrator loss. Collectively, these findings identify a candidate chemical inhibitor of Integrator and provide a new tool to dissect the role of Integrator mediated RNAPII pause regulation during erythropoiesis.

P-92: **Haleigh Pascual**

Killing Two Birds with One Stone: Dual Inhibition of RNA Polymerases I and III as a Novel Strategy for Targeting Ribosome Biogenesis in Cancer

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Dysregulated ribosome biogenesis is a hallmark of cancer. RNA Polymerase I (Pol I) and RNA Polymerase III (Pol III) are critical enzymes responsible for ribosome biogenesis and protein synthesis, both of which are often upregulated in cancer cells to support rapid cell growth. Given their central role in tumor cell proliferation, Pol I and Pol III represent attractive therapeutic targets. Pol I transcribes most of the ribosomal RNA (rRNA) that forms the structural and catalytic core of the ribosome. Likewise, Pol III transcribes the 5S rRNA and tRNAs necessary for translation of mRNA into mature proteins. Current inhibitors targeting Pol I and Pol III have shown promise individually; however, no strategy exists to simultaneously inhibit both Pols I/III. Inhibition of Pol I and Pol III have emerged as a promising therapeutic strategy, with preliminary data suggesting that dual inhibition of these complexes may have enhanced anti-cancer effects. The current bottleneck in the field is the limited understanding of a dual peptide inhibitor optimized to target both Pols I/III.

This project focuses on evaluating the therapeutic potential of simultaneously targeting Pol I and Pol III, with a particular emphasis on developing a peptide inhibitor that selectively disrupts the POLR1C/POLR1D heterodimer subcomplex which is shared between Pol I and Pol III and is crucial for the assembly of both complexes. To develop more specific and selective approaches to target Pols I/III dysregulation in cancer, we employ structure-based design to develop peptide inhibitors against the POLR1D/POLR1C subcomplex. Specifically, we utilize the Peptiderive application on the Rosetta Online Server that Includes Everyone (ROSIE). Here, we present our current findings that the combined inhibition of Pols I/III and the development of a dual peptide inhibitor that targets both Pols I/III will show maximized therapeutic efficacy as a novel anti-cancer therapeutic.

P-093: **Lia Prager**

Promoter elements governing RhlR-dependent quorum sensing in P. aeruginosa

Lia Prager, UAlbany - Biomedical Sciences

Jon Paczkowski, Wadsworth Center & UAlbany - Biomedical Sciences

Pseudomonas aeruginosa is an opportunistic pathogen that utilizes quorum sensing (QS) to regulate virulence and direct biofilm formation. QS is a cell-cell bacterial communication mechanism that uses signaling molecules called autoinducers (AI) to drive group behaviors such as virulence factor production and pathogenesis. QS-mediated pathogenesis in P. aeruginosa is dependent on the transcriptional activator RhlR, which is controlled by the RhlI-synthesized AI N-butyryl-L-homoserine lactone (C4HSL) and the metallo-β-hydrolase PqsE. The Pseudomonas quinolone signaling (Pqs) circuit of the QS network is regulated by the Rhl circuit to initiate biosynthesis of the PQS signaling molecule. Although PqsE exhibits functional redundancy with RhlR, previous studies showed that it enhances the DNA binding affinity of RhlR. Each effector, C4HSL and PqsE, exerts its influence at overlapping and distinct promoters to alter the transcriptional output of target genes in the RhlR regulon. Previous research showed that RhlR can bind specific promoters independently of C4HSL but is still highly dependent on PqsE for the transcriptional activation of those genes. Conversely, most promoters require PqsE for binding but still require C4HSL for activation. Our preliminary data show that there are three distinct modes of binding and activation by RhlR to progress QS-mediated functions: (1) when RhlR is unbound by AI but bound by PqsE, (2) when RhlR is bound by AI, but not PqsE, and (3) when RhlR is bound to both AI and PqsE. The goal of this proposal is to determine the promoter elements that dictate the temporal regulation of RhlR-mediated gene expression across the QS regulon, with a specific focus on the distinct contributions of PqsE and C4HSL dependencies.

P-094: **Caroline Pritchard**

Interactive RNA Exploration: Bridging Biology and Art through AI-Powered AlphaFold

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This science communication and outreach project proposes an innovative, interactive science museum installation designed to inspire curiosity with AI and molecular biology through exploration of the central dogma, sequence to structure. Visitors, particularly children, will assemble their own RNA sequences using colorful, tactile ribonucleotide blocks on a sensor-equipped table. Each unique sequence will be computationally transcribed into RNA (computer vision, image segmentation), translated into codons, then instantly visualized as a dynamic 3D protein structure via AlphaFold (an AI-based protein folding prediction tool, running via local continuous instance on a desktop-scale AI supercomputer). Proteins are displayed live on a screen to explore, modify, and compare with real examples, like insulin.

The exhibit aims to: (1) illuminate the remarkable power and versatility of RNA, (2) demonstrate how cutting-edge AI tools are transforming what we know by supercharging the speed of RNA research, (3) engage visitors through hands-on interactives revealing how the smallest building blocks of RNA have immense impact on life, and (4) inspire a deeper connection to scientists and innovators driving these advanced technologies. Each year the Museum of Innovation and Science (Schenectady, NY) welcomes tens of thousands of visitors, including students, families, educators, and lifelong learners. This exhibit will serve as a vital bridge connecting local audiences to the forefront of modern biological discovery, emphasizing the concept that small changes in code can effect big changes in protein structure and function while increasing accessibility of ‘RNA’ as a household term and ‘AI’ as a tangible tool.

Future applications include outreach opportunities (1) facilitating meaningful interaction between researchers and rare-disease communities at conferences, where users are instead modifying and modeling disease relevant proteins; (2) building a larger community art exhibit, where all proteins designed over a period of time are cached then displayed in a planetarium.

P-095: **Emily Rennells**

Bioorthogonal glycan labeling of tRNA

Emily Rennells, Maksim Royzen

Department of Chemistry and RNA Institute, University at Albany

Recently, a class of biological RNA, called glycoRNA has been discovered and little is known about the possibilities for biological roles. RNA will be selectively labeled with glycans using bioorthogonal chemistry. The glycan linker is designed to contain a tetrazine structure that will be available to perform bioorthogonal click chemistry. The glycan-tetrazine linker will undergo an inverse electron demand diels alder click reaction with a designed tRNA structure containing a transcyclooctene (TCO) modification. The reaction allows for the tRNA to permeate into the cell membrane, leaving the glycan on the cell surface. The design of this molecular probe will allow for glycoRNA's biochemical properties to be investigated by fluorescently labeling the complexes. Synthetic analogues of glycoRNA structures will be made to investigate their behavior in live cells. Forming a library of glycoRNA structures is essential for understanding the possibilities of this approach for novel drugs and designing future targeted therapeutics.

P-096: **Esperanza Rosas**

Development of screening assay for identification of novel IRES and IRES-like sequences

Gabriele Fuchs

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Translation is a highly coordinated four-step process requiring various proteins and RNA species to aid in protein production. The four steps include initiation, elongation, termination, and ribosome recycling. However, when canonical translation is impacted, there are other modes of translation that can occur. These other modes of translation are known as non-canonical and are typically facilitated by secondary RNA structures. The secondary structures include Internal Ribosomal Entry Sites (IRESs) and hairpins forming from repeat motifs. IRESs facilitate translation by directly recruiting the ribosome to the mRNA, bypassing the cap recognition and requirement of the ribosome to the cap. Although IRES-mediated translation is well understood in the viral context, in the mammalian context, IRES-mediated translation is not. Mammalian IRESs have been identified and implicated in cellular pathways related to stress, development, and the cell cycle. However, mammalian cellular IRESs do not have any conserved structure or sequence, making their discovery incidental. Here, we have developed a screening assay for the identification of novel IRES and IRES-like sequences. Using an established back-splicing bicistronic reporter construct, we demonstrate novel methods for the intentional discovery of candidate sequences that can facilitate cap-independent translation.

P-097: **Cady Roselli**

Investigating the Role of tRNA Sulfurtransferase-Mediated Modification in Regulating Growth and Cellular Properties in E. coli

Cady Roselli [(1) (2)], Humphrey Omeoga [(1), (2)], Thomas J Begley [(1) (2)]

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Reactive oxygen species (ROS) can be produced as cellular byproducts or come from exogenous sources. Unchecked ROS levels can damage nucleic acids, proteins and cellular organelles causing disease in humans. Importantly though, ROS can also act as signaling molecules, impacting proliferation, differentiation and even metastasis. Thus, cells have developed antioxidant defense mechanisms such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzymes to detoxify ROS and prevent damage. The Tus and Mnm enzyme families in bacteria are responsible for sulfur transfer and modification of wobble uridine on tRNA, respectively. Modifications to tRNA promote translational fidelity, tRNA stability and modulates codon-anticodon interaction. Here we showed that Tus deletion strains, which are linked to tRNA modification deficiencies, showed significantly reduced catalase activity and spent significantly longer time in lag phase, by about three hours. The tus mutants also had reduced growth and decreased colony forming (CFU) ability. Our findings from tusA mutants as well as downstream tusB mutant, mnmA and mnmH mutants suggests this modification also impacts bacterial cell motility, chemotaxis and biofilm formation which will be tested via swarm and biofilm formation assays. Understanding pathways of tRNA modifying enzyme systems and their roles in stress response and virulence factor formation can lead to clinical applications related to antibiotic development.

P-098: **Maksim Royzen**

Temporal control of CRISPR-Cas9 using bio-orthogonal chemistry.

Bhoomika Pandit, Sweta Vangaveti, Gabriele Fuchs

Department of Chemistry and RNA Institute, University at Albany

The CRISPR–Cas9 system has become a widely used tool for genome engineering. We will present a new method for small-molecule control of CRISPR-Cas9 using bio-orthogonal chemistry between tetrazine (Tz) and trans-cyclooctene (TCO). We carried out molecular modeling studies and identified a unique position on single guide RNA (sgRNA) that can be site-specifically tagged with Tz without disrupting its activity. We also synthesized a series of TCO-modified CRISPR suppressors. When exogenously added, they click to the Tz-tagged sgRNA, perturb the system and drastically reduce the nuclease activity. The most successful suppressor is a TCO- modified six amino acid long cell-penetrating peptide, which shows excellent cell permeability. The presentation will show that our method to control CRISPR-Cas9 nuclease activity is general by applying it to three different sgRNAs. We will also show that our method works in solution, as well as live HEK293 cells. We utilized flow cytometry to demonstrate temporal control of CRISPR-Cas9 targeting GFP. Lastly, we will show the therapeutic potential of our method by targeting vascular endothelial growth factor A (VEGFA).

P-099: **Suman Saha**

Expediting biologics production through the "sxRNA-Select" cell line development platform

Dr. Mike Dolan[2], Dr. Francis Doyle[2], Prof. Scott Tenenbaum[1,2,] Prof. Susan Sharfstein[1]

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Currently, the biopharmaceutical industry faces significant challenges in efficient development of high producing mammalian cell lines for complex biologic therapeutics, particularly for complex modalities such as bispecific and trispecific antibodies, which require precise ratiometric expression of multiple chains. Current antibiotic and metabolic selection methods are costly, time consuming, and can negatively impact cellular health and protein yield due to continuous expression of metabolic or selection markers. Consequently, cell line development timelines often exceed 6 months. To address these challenges, we developed 'sxRNA Select', a novel RNA-based positive selection platform. 'sxRNA Select' utilizes a structurally interacting RNA (sxRNA) system that enables positive selection based on the stoichiometric expression of an engineered microRNA (sxRNA key) directly linked to the gene of interest (GOI, e.g., a recombinant therapeutic antibody). The sxRNA key, transcribed from the GOI intron, interacts with a transiently introduced sxRNA lock mRNA, activating the translation of a surface marker or a fluorescent protein (e.g., CD4 or GFP) for a limited 48-to-72-hour window, facilitating rapid selection using magnetic beads or FACS. Furthermore, the level of the selection marker will directly correlate with GOI expression, allowing for the enrichment of high producing clones. Preliminary results demonstrate that in a CHO-S model, we will be able to generate and isolate high expressing clones in ~10 days using sxRNA-Select, which is significantly shorter time than conventional glutamine synthetase or antibiotic selection. Moreover, the selection process is compatible with standard transfection reagents, scalable electroporation, and diverse GOI payloads. By eliminating constitutive selection marker expression, sxRNA-Select reduces metabolic burden, which may help in reallocating translational machinery towards GOI production. This innovative plug-and-play platform could offer a rapid alternative and scalable strategy for cell line development and has a strong potential to accelerate and enable efficient production of next generation biologics.

P-100: **Spoorthy Sandras**

Integrative Structural Analysis of Disease-Associated αA-Crystallin Mutants Reveals C-Terminal Destabilization

Dr.Alexander Shekhtman, Dr.Jayanti Pande

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Alpha-A crystallin (αA-crystallin) is a small heat shock protein that plays a critical role in maintaining lens transparency through its chaperone-like activity and dynamic oligomeric assembly. Mutations in αA-crystallin, particularly G98R, are strongly associated with early-onset cataracts; however, the structural mechanisms underlying mutation-driven dysfunction remain incompletely understood.

To address this gap, we performed a residue-level structural investigation of wild-type αA-crystallin, the G98R mutant, and the R21Q/G98R double mutant. Using solution NMR spectroscopy, we systematically mapped mutation-induced perturbations across the protein, with particular focus on the C-terminal region (residues 149–173), a segment critical for oligomer stability and inter-subunit interactions. To contextualize experimental observations within the oligomeric framework, we reconstructed the 6T1R oligomer model and integrated missing structural elements. Molecular dynamics simulations were then conducted to evaluate mutation-dependent changes in structural stability, residue flexibility, and oligomer packing behavior.

Our findings reveal that the G98R substitution induces not only localized structural alterations but also long-range conformational perturbations that propagate toward the C-terminal region, reshaping oligomeric interactions. The double mutant exhibits compounded destabilization effects, suggesting cooperative structural disruption. Together, these results provide mechanistic insight into how cataract-associated mutations rewire αA-crystallin structural dynamics and compromise its chaperone function.

This integrative NMR–computational framework establishes a mechanistic model linking point mutations to oligomer destabilization, offering structural resolution to disease-associated dysfunction in small heat shock proteins.

P-101: **Md. Sharear Saon, PhD**

Identifying and characterizing partially and fully shifted G•U wobbles arising from alternative protonation of RNA

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RNA exhibits diverse functionalities, including those of an enzyme (ribozyme), a molecular sensor (riboswitch), and even a therapeutic agent. It also played a central role in the origin of life under the RNA world hypothesis. This functional versatility is remarkable given the relatively limited intrinsic molecular diversity of RNA. While covalent nucleotide modifications have been extensively studied, far less is known about non-covalent alterations of the nucleobases. These non-covalent changes can be classified as novel charged or tautomeric forms of the bases, which involve changes in protonation state and proton location, respectively. Like covalent modifications of RNA, non-covalent modifications may provide additional stability and functionality to the RNA. Here, we applied a cheminformatic approach to identify non-covalent alterations in RNA that support two rare orientations of the G•U wobble base pair. In the first, either the G or U can adopt a deprotonated or rare enol tautomeric form, resulting in a canonical G-C-like geometry, which we annotate as a partially shifted G•U wobble. In the second orientation, only the U adopts a deprotonated or enol tautomeric state, forming a fully shifted G•U wobble in which the O6 atom of G protrudes into the major groove. We provide widespread structural support of these proton-altered G•U wobbles within RNA 3D structures in the Protein Data Bank. We suggest that the partially shifted G•U wobble could be a source of mutagenesis during replication of RNA viruses. The cheminformatics pipeline developed here can be extended to other non-Watson-Crick-Franklin base pairs to uncover additional hidden protonation states and expand our understanding of RNA structural chemistry

Selected Speaker – Session 7: **Jack Schwartz**

Regulation of Insulin Receptor Translation by a Conserved uORF

Jack Schwartz, Lindsey Waitwright, Peng Yao

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Diabetes mellitus (DM) is the third most common chronic illness in the United States, affecting approximately 1 in 7 adults. Patients with DM are unable to properly control their blood sugar levels, increasing their risk of complications including cardiovascular disease, kidney disease, and stroke. Insulin signaling, the pathway disrupted in diabetic patients, regulates cellular glucose uptake, protein synthesis, cell proliferation, and fatty acid metabolism. In mouse models of type 2 diabetes mellitus (T2DM), overexpression of the insulin receptor (INSR) results in improved phenotypes for diabetic mice. Therefore, drugs to increase INSR expression offer an exciting, novel therapeutic strategy for T2DM, but a thorough understanding the regulation of INSR expression is required to guide the development of pharmacological and genetic strategies to modulate INSR expression. We have identified a conserved upstream open reading frame (uORF) in the 5'-UTR of the INSR mRNA. Using dual reporter assays, we demonstrated that this uORF functions as a translation repressor in both humans and mice. Further, we identified two RNA helicases, IGHMBP2 and DHX36, which interact with the INSR 5'-UTR and hypothesize that they regulate INSR expression by controlling the translational balance between INSR's uORF and main (m)ORF. To evaluate inhibition of INSR's uORF as a potential strategy to overexpress INSR we designed and characterized antisense oligonucleotides (ASOs) targeting the secondary structure near the uORF of human INSR. Through potential ASO-mediated structural modulation, we demonstrated upregulation of endogenous Insr protein expression without altering its mRNA level in HEK293T cells. Lastly, we generated a genetic loss of uORF function mouse model and human cell culture model to investigate the role of INSR's uORF on cellular and organismal well-being. Future studies will explore the role that mRNA structure plays in the translation repression conferred by INSR's uORF as well as determine its pathophysiological role.

P-103: **Sangeetha Selvam**

Lumicks C-Trap Dymo-300 at the RNA Institute

Sangeetha Selvam, Ken Halvorsen

The RNA Institute, University at Albany, State University of New York, NY 12222.

Lumicks C-Trap Dymo-300 facilitates high-resolution investigation of biomolecular interactions at the single-molecule level. This platform integrates optical tweezers with fluorescence microscopy to enable real-time observation, monitoring, and analysis of biomolecular interactions. The instrument supports diverse research areas including studies on RNA structure, RNA modifications, cellular processes, nucleic acid nanotechnology, viral infection dynamics, RNA repeat disorders and drug discovery strengthening the RNA Institute's mission to advance cutting-edge RNA research and innovation.

For training, reservations, and usage, please contact Ken Halvorsen (khalvorsen@albany.edu)

P-104: **Justa Sentre**

Temporal Control of CRISPER-Cas9 using Bioorthogonal click chemistry

Robillard M. S; Royzen M.

Department of Chemistry and RNA Institute, University at Albany

CRISPR-Cas9 is a gene editing technology used to treat genetic diseases, including cancer. It consists of single-guide RNA (sgRNA) that directs the Cas9 enzyme to cut a faulty gene within double-stranded DNA

In my presentation, I will discuss the synthesis of a 103 nucleotide (nt) pBR322 sgRNA modified with TZ to enhance Cas9 nuclease activity for double-stranded DNA cleavage in-vitro over a 16hr period. The modified sgRNA is tested at five different uridine residues position, U1 to U5, in the repeat and anti-repeat regions to identify the best modification position for optimal DNA cleavage This model illustrates a chemically controlled CRISPR/Cas9 system regulated through bioorthogonal “click-to-release” chemistry. In the inactive state, the CRISPR/Cas9 complex is rendered nonfunctional by chemical masking (cloaking) of the guide RNA, which prevents proper hybridization to the target DNA sequence and blocks Cas9-mediated cleavage. Upon addition of a trans-cyclooctene (TCO) trigger, a rapid and selective bioorthogonal reaction with a tetrazine (Tz) moiety occurs. This TCO–Tz ligation induces cleavage of the masking group (“click-to-release”), restoring the native structure of the guide RNA. As a result, the reactivated CRISPR/Cas9 complex binds the target DNA adjacent to the PAM sequence and generates site-specific double-stranded breaks.

This strategy enables temporal and chemical control over genome editing activity in cells. The model highlights the potential of bioorthogonal chemistry as a powerful tool for spatiotemporal regulation of gene-editing systems. The effectiveness of the modification will be analyzed using pymol.

Evidence of DNA cleavage will be confirmed using flow cytometry to determine the molecular weight of the target pBR322 gene in live HEK293 cells.

If this method does not succeed in synthesizing constitutively functional sgRNA containing Tz in the repeat:anti-repeat region computational studies will be done to determine a position that will.

P-105: **Thandolwethu Shabanagu**

Nearest Neighbor Parameters for Estimating the Folding Stability of RNA sequences Including Pseudouridine

Elzbieta Kierzek [2], Olivia M. Hiltke [1], Megan Miaro [1], Sebastian Arteaga [3], Gregory S. Orf [3], Julia Stone [3], Brent M. Znosko [3], Ryszard Kierzek [2], and David H. Mathews [1]

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Nearest neighbor parameters are widely used in software for estimating the conformational stability of an RNA sequence folding into a specific structure. Folding stability for RNA folding with canonical nucleotides A, C, G, and U has been widely studied, but the same is not true for most modified nucleotides. In this work, we present a comprehensive set of nearest neighbor parameters for estimating the folding stability of RNA sequences including pseudouridine. These parameters are derived from 199 optical melting experiments for helices with pseudouridine-A and pseudouridine-G pairs and for loops with pseudouridine. On average, pseudouridine stabilizes the folding of helices and loops compared to U, although this effect is sequence-context dependent. These parameters improve the modeling of folding stability for RNA secondary structures containing pseudouridine. We demonstrate that these parameters successfully model a conformational shift for S. cerevisae U2 snRNA when two additional inducible pseudouridines are present. These parameters are freely available and incorporated into the RNAstructure software package.

P-106: **Sharon Shaughnessy**

Innate Immune Activation in Myotonic Dystrophy Type 1

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Over the last decades, many scientists have uncovered the link between the activation of innate immunity in the brain and the development of neurodegenerative diseases like AD, FTD, PD, and ALS. The innate immune response is not only a biomarker – it is shown to be a contributor to the development of neurodegenerative disease. Although myotonic dystrophy type 1 (the most common form of adult-onset muscular dystrophy) also has a neurodegenerative component, there is a lack of knowledge on how the brain pathology develops in this disease. The central hypothesis of this project is that innate immune activation, specifically type 1 interferon and its resulting inflammation, play a role in DM1 brain pathology. In order to determine whether signs of innate activation were present, I first looked at transcriptomic datasets from the DM1 brain. Upregulated genes in these datasets were highly enriched for involvement in interferon response and inflammation. I also analyzed DM1 plasma using a sensitive Luminex assay and found that interferon alpha was increased in DM1 patients. Although these changes were significant, determining whether they constitute a causative or treatable aspect of DM1 requires an appropriate model. I therefore examined the brain transcriptome of a prodromal DM1 mouse model to see if it replicated the immune changes seen in humans. Using single-nuclei sequencing of the frontal cortex, I observed upregulation of innate response genes in oligodendrocytes, which also express relatively high levels of the disease-causing transgene. My current work extends this analysis to two other DM1 mouse models with more severe symptoms of the disease. Outcomes that establish a link between innate immune activation and cognitive decline in DM1 will have implications for our understanding of DM1 pathology, and may provide new avenues for treatment.

P-107: **Shubhajit Singha**

Machine learning (ML)-assisted DNA origami shape sorting using fingerprinting nanosensors and feature engineering

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Reconfigurable DNA nanostructures have emerged as a promising research area with applications in drug delivery, molecular computing, biosensing, and stimuli-responsive soft nanomaterials. While significant progress has been made in creating novel DNA nanostructures and exploring their applications, comparatively little effort has focused on developing new methodologies to confirm their folding. Conventional imaging approaches typically rely on sophisticated microscopy techniques including atomic force and transmission electron microscopy. Alternative low-cost methods for verifying DNA nanostructure assembly and shape sorting are thus highly valuable. Here, we present a fingerprinting nanosensor array integrated with machine learning (ML) to distinguish between two DNA origami shapes (triangle and nanotube) and to differentiate them from an unfolded scaffold strand. The nanosensor array, consisting of eleven nano assemblies, termed nanosensors, is prepared by complexing graphene oxide nanosheets (nGO) with eleven fluorophore-labeled single-stranded DNA probes. Upon complexation the fluorescence of the DNA probes is quenched through graphene oxide–mediated fluorescence quenching. Adding the DNA nanostructures to each nanosensor displaced a fraction of the surface-adsorbed fluorescent DNA probes, producing unique fluorescence recovery signatures that were subsequently processed through feature engineering for accurate ML-assisted classification. Using this approach, we achieved 94% prediction accuracy in discriminating DNA origami triangle, DNA origami nanotube, and unassembled M13 scaffold. Our strategy provides a new and generalizable platform for shape sorting in DNA origami field, offering new avenues for high-throughput, label-free classification.

P-108: **A.Serra Sonmez**

Peptide Design and Molecular Modeling of RNA-Binding Inhibitors for Myotonic Dystrophy

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Myotonic dystrophy (DM) is a multi-systemic neuromuscular disorder caused by expanded toxic RNA repeats that aberrantly sequester the RNA-binding protein Muscleblind-like 1 (MBNL1), leading to widespread splicing defects. Targeting this pathogenic RNA–protein interaction represents a promising therapeutic strategy. In this study, we designed and computationally evaluated peptide-based inhibitors aimed at preventing MBNL1 sequestration by toxic repeat RNAs. A total of eight peptides were investigated, comprising four cyclic and four linear peptides. Within each group, two peptides were identical except for a terminal phenylalanine (Phe) chirality, while the remaining two peptides shared a common scaffold but incorporated two point mutations. All peptide–RNA complexes were subjected to 150 ns molecular dynamics simulations using the Minerva high-performance computing platform. Structural stability, conformational dynamics, and binding energetics were systematically analyzed.

Our in silico results demonstrated that cyclic peptides exhibit strong agreement with available wet-lab binding data. Notably, peptide QZC12 showed the highest binding affinity toward toxic repeat RNA, consistent with experimental observations. Subtle yet statistically significant differences in binding affinity were detected among closely related cyclic peptides, and the simulations successfully captured the effects of single mutations and chirality on peptide conformation and RNA interaction. Although experimental validation for linear peptides is ongoing, computational analyses revealed distinct conformational behaviors and predicted differences in RNA binding affinity.

Overall, this work highlights the robustness of molecular dynamics simulations in predicting peptide–RNA interactions and elucidating how minor sequence and stereochemical variations influence binding. These findings support the rational design of peptide-based therapeutics for myotonic dystrophy and underscore the translational relevance of integrative computational approaches.

P-109: **Leah Sweeney**

Strand exchange in Switchback DNA monitored by Grating Coupled Fluorescent Plasmonic (GC-FP) biosensors

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Grating-coupled surface plasmon resonance, combined with fluorescence imaging (GC-FP) enables the high throughput detection of biological molecules in a microarray format. When fluorophores are positioned 20 to 200 nanometers above a metal film experiencing surface plasmon resonance, surface plasmon coupled emission (SPCE) amplifies fluorescence intensity, making the assay sensitive to minute changes in biological interactions. Previous work by our lab [1-3] and others have used these sensors in detecting disease biomarkers for COVID-19, Lyme disease, and other conditions. Integrating nucleic acid nanostructures into these GC-FP chips offers several advantages, including signal integration, rapid reconfiguration, and assay molecular self-assembly. In this work, we expand the use of the GC-FP platform to study biomolecular interactions in nucleic acid nanostructures.

The structure we are investigating is switchback DNA, a globally left-handed structure composed of two parallel DNA strands [4]. Switchback DNA can be conceptualized as a series of short conventional duplexes perpendicular to the helix axis linked by switchback turns. Here, we investigate the competition between conventional DNA duplexes and switchback structures by monitoring the displacement reaction of component DNA strands on GC-FP chips. In these experiments, the switchback structure is constructed from a biotinylated strand and a fluorophore-tagged switchback complement, allowing conjugation onto streptavidin spotted GC-FP chips. When a duplex complement is introduced, it displaces the switchback complement and results in the formation of a conventional duplex, turning off the SPCE signal. We also are investigating a duplex-to-switchback conversion by introducing mismatches within the duplex DNA, a biomolecular reaction that has not previously been shown.

P-110: **Tia Swenty**

Designing Superparamagnetic Iron Oxide Nanoparticles for sxRNA Delivery to 3D Cell Cultures

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Structurally interacting RNA (sxRNA) is an RNA switch technology that can be incorporated into mRNA to enable cell-specific translation of a gene of interest (GOI). Translation activation is controlled by a change in 3D confirmation in the 3' end, where a histone stem loop (HSL) forms when the sxRNA switch binds to its endogenous target. The stem loop binding protein (SLBP) then binds to the HSL and activates translation, using an analogous mechanism to the poly(A)-binding protein. Since sxRNA is exogenously transcribed, it must be protected and delivered to cell cultures for effective translation of the encoded GOI. Nucleic acid delivery systems are designed using multiple materials, each with its own benefits for delivering cargo to cells. Previously, sxRNA has been successfully delivered to 2D cell cultures using Lipofectamine reagents, demonstrating translational activation in both mono- and co-cultured cells; however, 3D cell cultures require a more robust delivery system to penetrate the extracellular matrix surrounding the cells. Here, we present using superparamagnetic iron oxide nanoparticles (SMNPs) as an mRNA delivery system to magnetically pull the mRNA into the vicinity of the cells. The SMNPs are functionalized with Polyethylenimine (PEI), a cationic polymer that interacts electrostatically with the negatively charged phosphorus backbone of mRNA. Thus far, we have successfully used SMNPs to deliver plasmid DNA and mRNA to 2D cell culture. Plasmid delivery was comparable to commercial transfection reagents while mRNA delivery showed promising results for future in vivo delivery aims.

P-111: **Hannah Talbot**

Isothermal assembly of DNA nanostructures in hydrated ionic liquids

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DNA nanostructures can be tailored to perform a wide variety of functions, with specific interest in drug delivery applications. Some aspects of DNA nanostructure assembly can hinder the ability of nanostructures to act as effective drug carriers. Typical assembly methods employ magnesium ions to stabilize the structure, which leave the structure susceptible to damage by enzymes in biological environments. Further, DNA nanostructure assembly typically involves a thermal annealing protocol in which DNA strands are heated in a specific buffer to a high temperature and cooled slowly at specific rates, preventing the encapsulation of temperature-sensitive guest molecules. In this work, we demonstrate the assembly of a wide variety of DNA nanostructures in an ionic liquid (choline dihydrogen phosphate) instead of magnesium, with enhanced biostability and improved functionality in biological systems. In addition, we show successful assembly of DNA nanostructures in ionic liquids at constant moderate temperatures, thus avoiding thermal annealing. Overall, this work develops a potential method to construct more biostable DNA nanostructures in simpler one pot process. Assembly of DNA nanostructures under isothermal conditions is desirable for scaffolding biomolecules and to reduce the need for thermal annealing instruments, allowing nanostructure preparation in low-resource settings.

P-112: **Gaëlle Talross, PhD**

lncRNAs and a metabolically regulated lncRNA-micropeptide: Insights from the fly olfactory system

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From humans to insects, it is increasingly clear that long noncoding (lnc)RNAs contribute to neural development, function, and evolution. lncRNAs often operate within complex networks that feature multiple redundancies. This creates challenges for the functional analysis of lncRNAs and highlights the pressing need for simple neuronal model systems.

We sought an optimal platform to evaluate the role of neuronal lncRNA: the fly olfactory system. This numerically simple system is highly sensitive and finely tunes its sensitivity according to the animal's internal state. The olfactory receptor neurons (ORNs) have been extensively characterized. Importantly, perturbations in this system often lead to clear and measurable phenotypes, which allows for effective functional assays to study the role of lncRNAs.

We have identified a diversity of lncRNAs in the fly's main olfactory organ, the antenna. We have generated a lncRNA-to-neuron map, which revealed that ORNs differ not only in receptor but also in lncRNA expression. Particularly striking, we identified species-specific lncRNAs enriched in pheromone-sensing neurons, suggesting a potential role in species recognition.

We compared the transcriptomes of starved and fed flies, and identified an Antenna-enriched RNA that is Upregulated by Starvation by ≥10-fold, which we renamed AnRUS. Intriguingly, AnRUS is also regulated by food-associated sensory cues. We found that AnRUS is a cytoplasmic lncRNA conserved by synteny across Drosophila species and exhibits rapid evolution in splicing, regulation, and sequence. AnRUS encodes a micropeptide that can be readily detected by antibody staining in pheromone-sensing olfactory sensilla — the specialized sensory hairs that house these neurons. Electrophysiological analysis in flies lacking ANRUS demonstrates that ANRUS acts in the starvation-dependent modulation of pheromone-sensing neurons, raising the possibility that the lncRNA-micropeptide contributes to behavioral prioritization between mating and food seeking.

Overall, our work lays a foundation to investigate the roles of other lncRNAs in olfactory function and broader neuronal modulation.

P-113: **Parmesh Thakoordial**

Dissecting transactivation by the ΔN-specific N-terminal domains of p63 and p73

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The p53 family of transcription factors have numerous functions in the body from tumor suppression, drivers of epidermal development, and neurogenesis. p63 is a master regulator of skin development and function. At least six isoforms of the p63 protein exist which vary in the specific protein domains they carry, including the two N-terminal domains, TAp63, which contains an N-terminal transactivation domain (TAD) or ΔNp63, which lacks this domain and has a 14 amino acid sequence in its place.

Initially, it was suggested that ΔN isoforms repress transactivation; however, recent work demonstrates that the ΔN domain of p63 is likely a TAD. TADs are composed of clusters of aromatic residues near acidic residues, and the ΔN domain of p63 is roughly composed of 20% acidic and 30% hydrophobic amino acids. Previous studies from our group have shown that deletion of the ΔN domain of p63 or mutation of hydrophobic residues substantially reduces transactivation.

Here we perform a scanning alanine mutagenesis screen and further examine 14 naturally occurring variants in the human ΔN domain of p63 using a reporter gene assay to understand how these mutations impact transactivation. Our findings indicate that mutations of the first three evolutionarily conserved amino acids in the ΔN domain of p63 and several natural human variants affecting acidic and hydrophobic residues significantly decreases transcriptional activation. We also used evolution informed mutagenesis to identify key amino acids in the ΔN domain of p73 that drive both shared and unique activities relative to the ΔN domain of p63.

Our data suggests key functional residues in isoform-specific domains of p63 and p73 that drive shared and gene-specific activities and provide insight into gene variant associated gene activities that may be relevant across several human disorders.

P-114: **Hannah Theriault**

Characterizing the role of viral genotype and tick population in Powassan-tick interactions

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Powassan virus (POWV) is a tick-borne flavivirus capable of causing encephalitic disease. Historically, human infections were reported irregularly, but cases have increased in frequency and range within the Northeast and Upper Midwestern United States. POWV is divided into two lineages, lineage I and II (Deer tick virus [DTV]), which share ~84% nucleotide identity, and are maintained through separate enzootic transmission cycles. DTV is primarily vectored by Ixodes scapularis, where up to 5% of ticks isolated in parts of New York, Connecticut, Wisconsin, and Minnesota test positive. Because of this, POWV is considered an emerging public health threat. POWV is believed to be maintained in strict transmission foci, infrequently migrating between different geographic regions. Due to this focal nature, transmissibility may be influenced by the specific pairing of tick and virus genotype. This is similar to tick-borne encephalitis virus (TBEV), which is divided into five closely related subtypes endemic in distinct foci in Europe and Asia. Previous studies have shown that strain-specific differences in TBEV subtypes are linked to differences in virulence. We hypothesize that POWV circulating within enzootic transmission foci in the Northeast and Midwest are subject to distinct selective and stochastic pressures, driving genetic diversification and phenotypic variability. We have begun to test this hypothesis by assessing population-specific differences in infection and replication using genetically and geographically distinct DTV isolates and field-derived I. scapularis from areas within New York and Minnesota. A panel of genetically unique, low-passage DTV isolates were found to have significant differences in in vitro replicative fitness in a mammalian cell line. Additionally, results in I. scapularis suggest differences in viral replication and infection are influenced by both population and viral strain. Further results from these studies will clarify how genotype-by-genotype interactions could give rise to phenotypic variants of POWV that differ in competence and virulence.

P-115: **Catherine Tremblay**

RNA Sequence Mapping via Endonuclease Digestions and LC-MS Analysis Using a Novel Informatics Workflow

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The recent development and regulatory approval of COVID-19 mRNA vaccines, together with growing interest in mRNA-based therapeutics, have propelled mRNA molecules to the forefront of the biopharmaceutical industry. The resulting need for rapid product development has necessitated development of analytics for the precise characterization of mRNA critical quality attributes (CQAs), including sequence integrity. This study highlights recent advances in analytical methodologies aimed at improving LC–MS sequence coverage for mRNA characterization through the combined use of endonuclease digestion strategies and a dedicated software tool for comprehensive mRNA sequence mapping.

An mRNA construct (1,919 nucleotides) derived from the firefly luciferase (Fluc) sequence was custom synthesized by GenScript (Piscataway, NJ) using in vitro transcription (IVT). It was enzymatically digested using four ribonucleases: RNase T1, hRNase4, and two recently introduced enzymes, MC1 and Cusativin, each exhibiting distinct cleavage specificities. The resulting oligonucleotide fragments were separated by ion-pair reversed-phase (IP-RP) chromatography employing mobile phase containing 10 mM dipropylamine (DPA) and 40 mM hexafluoroisopropanol (HFIP). High-resolution (~100,000) negative-ion ESI-MSE (data-independent acquisition) spectra were collected for both precursor ions and their corresponding fragment ions using a QTOF mass spectrometer. Data-processing and sequence assignment were performed using “MAP Sequence”, a recently developed software tool designed to accurately correlate precursor ions with their collision-induced dissociation (CID) fragments from oligonucleotide digests.

Predicted oligonucleotide masses were matched against experimental MS1 and MS2 spectra from data-independent acquisitions. Generation of unambiguous, unique digestion products is essential to minimize dependence on MS/MS fragmentation for resolving assignment ambiguities. LC–MS analyses of Fluc mRNA digests generated using the four ribonucleases were compared in terms of sequence coverage. MC1 and Cusativin enzymes produce longer and more distinctive digestion products due to their unique cleavage specificities and controlled missed-cleavages, leading to increased sequence coverage. The combined Fluc mRNA sequence coverage achieved with four ribonucleases was 95%.

P-116: **Sweta Vangaveti**

Modeling RNA, Proteins, and Disease Pathways Through Molecular Simulations and Machine Learning

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We combine bioinformatics, molecular simulations, and machine learning to study the dynamic interactions that drive biological processes. Research projects in the lab span investigation of DNA/RNA structures and modifications, protein–ligand interactions, and small molecule drug discovery and delivery, using molecular docking and molecular dynamics simulations. We also work on machine learning frameworks that integrate multi omic datasets for deeper insights into disease pathways. In addition, we are developing workflows that use computer vision to automatically analyze patient derived videos to quantify symptoms and evaluate therapeutic responses. If you're interested in biophysics, drug design, AI for biology, or translational applications, our lab bridges all these worlds. Stop by to explore how we use computation to turn molecular motion and biological data into new discoveries!

P-117: **Phillip Weeber**

Investigating the Effects of Antisense Transcription Using NET-seq

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Contrary to the textbook model of bacterial transcription, many promoters are positioned inside genes, often in the antisense orientation. Antisense transcription has the potential to regulate expression of overlapping genes through a variety of mechanisms, including transcription interference, i.e., clashes between RNA polymerases transcribing in the opposite direction. In addition, overlapping sense and antisense promoters may lead to competition for access to the same DNA region, potentially reducing transcription from one or both promoters. However, despite its prevalence, the extent of interference caused by antisense transcription initiation or elongation is poorly understood.

In this study, we artificially induced strong antisense transcription using plasmid-driven overexpression of σ^{32} at 30°C, a condition that minimizes endogenous σ^{32} activity. By comparing NET-seq profiles from cells expressing σ^{32} to an empty-vector control, we directly test whether elevated antisense transcription from σ^{32}-dependent promoters is sufficient to measurably alter transcription of the corresponding sense genes. We analyzed transcript levels of genes that are downstream and antisense to σ^{32}-dependent promoters, as well as genes where the promoter overlaps and is antisense to a σ^{32}-dependent promoter. This strategy allows us to probe whether antisense transcription is merely tolerated by the transcription machinery or whether it can be used in a regulatory manner.

We were able to identify one example each of transcripts that may be downregulated through transcriptional interference, or competition for promoters. However, more follow-up testing is necessary to confirm antisense transcription as the mechanism of regulation. When looking at widespread gene regulation, antisense transcription initiation or elongation do not seem to have a broad interference effect, indicating that more work needs to be done to determine if antisense transcription serves a regulatory purpose or is simply the result of pervasive transcription.

P-118: **Kennedi Weston**

Age-Associated microRNA and mRNA Expression Changes in Salivary Gland Tissue

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Aging leads to irreversible functional decline across tissues, including cellular senescence and altered intercellular communication. In salivary glands, these changes contribute to salivary gland hypofunction (SGH), which can arise from age-related degeneration, fibrosis, radiation therapy, and autoimmune disease such as Sjögren's Disease (SjD). These factors collectively reduce salivary flow, alter glandular morphology and structure, and promote pro-inflammatory and pro-fibrotic microenvironments. At the molecular level, microRNAs (miRNAs) and messenger RNAs (mRNAs) regulate pathways associated with cellular senescence, the senescence-associated secretory phenotype (SASP), chronic inflammation, and extracellular matrix (ECM) remodeling. Dysregulation of these RNA molecules may drive tissue dysfunction in aged submandibular glands (SMGs). However, the mechanisms underlying age-related SGH, particularly the interplay between mRNAs, miRNAs, and senescence-SASP signaling in aged SMGs, remain poorly understood. To address this gap, total RNA was isolated from young (3-month-old) and aged (22.5-month-old) female C57BL/J6 mice to purify mRNAs and miRNAs for microarray analyses. The goal was to identify age-associated transcripts involved in senescence, inflammation, and ECM remodeling in aged SMGs. mRNA microarray and ShinyGO analyses revealed decreased expression of ECM-related components, including collagens, MMPs, and fibroblast growth factors (FGFs), which are essential for ECM organization and assembly. In contrast, proinflammatory genes associated with NFκB, TNF, TGFβ, and B-and T-cell receptor signaling pathways were elevated, consistent with chronic inflammation, fibrosis, and SASP activation. miRNAs predicted to regulate these genes were identified, revealing possible miRNAs driving reduced ECM integrity and enhanced inflammatory signaling. Together, these findings provide valuable insights into age-and disease-related pathways and putative miRNA-based therapeutic targets to mitigate salivary gland dysfunction.

P-119: **Peng Yao, PhD**

Drugging the Heart with RNA: From Engineering to the Clinic

Aab Cardiovascular Research Institute, Department of Medicine and Biochemistry & Biophysics

University of Rochester School of Medicine and Dentistry

The Center for RNA Biology

The Center for Biomedical Informatics

Cardiovascular disease (CVD) is a growing global epidemic and a major economic burden; in the United States alone, one in four individuals will develop heart failure (HF). Despite major advances, current HF therapies largely target neurohormonal signaling and hemodynamics, reducing mortality by only 3–4%, and HF remains responsible for ~45% of cardiovascular-related deaths. Cardiac hypertrophy and fibrosis are central pathological drivers of HF progression, yet no approved therapies directly target these processes. Developing strategies that modulate cardiac gene expression to prevent or reverse maladaptive remodeling represents a critical unmet need.

RNA therapeutics provide a promising opportunity to restore cardioprotective gene function by increasing protein synthesis. However, existing approaches face major limitations: AAV-mediated gene therapy is constrained by toxicity and an inability to re-dose, while lipid nanoparticle (LNP)–based mRNA delivery lacks cell-type specificity within the cardiovascular system. To overcome these barriers, our laboratory is developing RNA-based platforms that enable potent, safe, and titratable enhancement of endogenous protein synthesis. We have established a novel antisense oligonucleotide (ASO) platform that selectively increases mRNA translation by targeting defined structural elements within transcripts. Using this approach, we initially focused on an “undruggable” cardiac transcription factor, demonstrating that ASOs targeting a conserved double-stranded RNA element enhance protein synthesis, modulate cardiomyocyte hypertrophy in human ESC-derived cardiomyocytes, and improve outcomes in preclinical models of heart failure. This platform is now being expanded to address cardiometabolic syndrome upstream of CVD and HF by targeting distinct mRNAs across multiple organs, supported by the Empire Discovery Institute–Novo Nordisk LeapRx program, in which subject-matter experts at Novo Nordisk identified high-priority therapeutic targets.

Because delivery remains a central challenge for RNA therapeutics, our research also aims to bridge a key academic–industrial gap by developing RNA aptamers capable of efficiently entering specific cardiac cell types and delivering conjugated RNA payloads. In parallel, we are engaged in translational efforts with a preventive cardiologist collaborating with CRISPR Therapeutics and the Cleveland Clinic to establish URMC/Strong Memorial Hospital as a site for an upcoming Phase 1b/2 CTX310 LNP–mRNA–CRISPR–Cas9 clinical trial. This effort builds on a landmark Phase 1a trial published recently in The New England Journal of Medicine, demonstrating that in vivo CRISPR-mediated knockout of ANGPTL3 safely and effectively lowers circulating lipids when delivered to the liver. Together, our work seeks to define new RNA engineering strategies that overcome barriers to delivery and specificity, enabling precise modulation of protein synthesis in cardiac cells. These advances have the potential to extend the therapeutic reach of RNA-based therapeutics beyond the liver and to open new avenues for treating cardiovascular disease.

P-120: **Clara Yeboah**

MicroRNA (miRNA) as a biomarker for Myotonic Dystrophy Type 1 (DM1)

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Myotonic dystrophy type 1 (DM1) is a complex neuromuscular disease that requires reliable, accessible biomarkers for effective management. While alternative splicing changes in skeletal muscle tissue have been extensively characterized in the disease, their analysis requires an invasive procedure, and obtaining multiple biopsies to track disease progression is difficult. To address the need for more accessible biomarkers in DM1, we have focused on microRNAs (miRNAs), that play a crucial role in muscle pathophysiology and regulate gene expression related to muscular dystrophies. Previous studies in the field have identified several miRNAs, such as miR-206, as differentially expressed in DM1 skeletal muscle and blood compared to controls. Here, we aim to identify miRNA-based biomarkers of DM1 disease and test their response to potential therapeutic interventions.

To validate miR-206, we first measured its expression levels using digital PCR in DM1 and control cell lines, including DM1-MyoD fibroblasts and primary myoblasts that contain either 1,317 or 2,900 CTG repeats. To test miR0-206 response to treatment, we used either vorinostat (SAHA) or modified polycyclic compounds (MPCs), previously shown to rescue DM1 alternative splicing defects. Lastly, to identify novel miRNAs dysregulated in patient-derived cell lines, we sequenced small RNAs from two DM1 cell lines with either 430 or 2900 CTG repeats. Our data shows that DM1 cells have elevated miR-206 expression compared to control cells, consistent with previous findings. We also show for the first time that miR-206 expression was rescued with treatments that also rescued splicing. We are currently analysing sequencing data to identify functional miRNAs that are specifically deregulated in DM1 patient-derived cell lines, which could be used as biomarkers of DM1 disease progression.

Identifying unique dysregulated miRNAs in DM1 patient-derived cells (moderate vs. severe disease) and healthy control cells will also reveal regulatory axes that are crucial for easy monitoring of disease progression and therapeutic interventions.

P-121: **Ahmed Zegher**

Dynamic competition between nuclear PIA-RNA stabilization and decay shapes mitochondrial activity

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Life has adapted to exist in extreme environments, such as elevated pH, extreme temperatures, and environments with a lack of oxygen. The biomolecules made by organisms that lives in extreme environments must adapt to remain folded and functional. Recent computational studies have shown that certain classes of RNAs adapt in thermophilic organisms through strengthening GC content in regions of simple secondary structure. The work presented herein determines the effects of high temperature adaptation, through strengthened secondary structure, using minimal hammerhead ribozymes as a model system. Two model hammerhead ribozymes were designed using characteristics of naturally occurring mesophilic hammerhead ribozymes and thermophilic hammerhead ribozymes. The thermophilic ribozyme construct has increased GC base pairing content in regions of secondary structure. The kinetics of self-cleavage for each ribozyme was studied over the temperature range at which the ribozymes are active. Ribozymes were designed to be a two-piece system, with separate enzyme and substrate strands. The substrates were labeled with fluorescein on the 5' end and kinetics was studied was by taking time points throughout the reaction, separating the substrate and products on a gel, and the bands were detected with a ChemiDoc imaging system. The results show that in the hammerhead ribozyme, adaptation through increasing GC content does increase thermal stability, but that comes with a functional penalty and a slower rate of self-cleavage.

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Topoisomerase Inhibitors Worsen Mis-regulated Splicing in Myotonic Dystrophy Models: Clinical and Mechanistic Implications

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Myotonic dystrophy type 1 (DM1) is caused by toxic expanded CUG repeat RNA that disrupts MBNL-dependent alternative splicing. In a small-molecule screen performed in DM1 fibroblasts, we identified compounds that worsened splicing abnormalities rather than rescuing them. These effects were confirmed in DM1 and DM2 myotubes, where the majority of active compounds were topoisomerase inhibitors, a drug class widely used in cancer therapy. Given reports suggesting increased cancer risk in DM1, we examined the effects of the FDA-approved topoisomerase I inhibitors topotecan and irinotecan. Both agents exacerbated MBNL-regulated splicing defects in DM1 and DM2 myotubes while sparing non-MBNL-dependent splicing events. Treatment was associated with reduced MBNL transcript levels and a downward trend in MBNL protein abundance, along with decreased expression of DMPK and CNBP1. These findings are notable in light of clinical reports and GeneReviews recommendations describing intolerance to topotecan in DM1 patients. Together, our data suggest that topoisomerase inhibitors can aggravate core molecular features of myotonic dystrophy, with potential implications for cancer treatment in affected individuals.