# 33<sup>rd</sup> Keck Annual Research Conference



October 20, 2023

<u>Conference Chairs</u> Wenbo Li, UT Health Science Center at Houston Monica Pillon, Baylor College of Medicine

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#### New Frontiers in RNA Biology and Therapeutics

#### **Conference Chairs**

Wenbo Li, PhD

Biochemistry and Molecular Biology UT Health Science Center at Houston Monica Pillon, PhD Biochemistry and Molecular Biology Baylor College of Medicine

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#### The Keck Center and Gulf Coast Consortia

#### for Quantitative Biomedical Sciences

#### The Keck Center

The Keck Center, established in 1990 with support from the W. M. Keck Foundation, celebrates its 33<sup>rd</sup> year of supporting predoctoral and postdoctoral trainees and their mentors. From the founding institutions, Baylor College of Medicine and Rice University, the Keck Center grew in its first 10 years to six major public and private institutions in the Houston/Galveston area, including University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, and The University of Texas MD Anderson Cancer Center. The Institute of Biosciences and Technology of Texas A&M Health Science Center joined in 2015. The Houston Methodist Research Institute joined in 2022. Guiding the formation of this collaboration was the realization that significant advances in the biological sciences, such as the DNA sequencing of the human genome, would be driven by the integration of biology and computer science. The partners realized, however, that most biological scientists were not prepared to capitalize on novel approaches to visualization, analysis and interpretation of experimental data made possible by rapid advances in computing technology. Moreover, most researchers in computer programming and analysis systems did not have adequate knowledge about biology and biological systems. The Keck Center was explicitly designed to bridge this gap between biological and computational sciences by fostering collaborations among scientists through specially designed research and training programs.

Building on its expertise in interdisciplinary, inter-institutional programs, the Keck Center's focus has evolved to the quantitative biomedical sciences. Participants are drawn from various disciplines such as biophysics, chemistry, bioengineering, neuroscience, computer science, biochemistry, genetics, physics, mathematics, data science, biomedical informatics, environmental health, biology and statistics. Currently, the Keck Center administers training programs in biomedical informatics and data science, molecular biophysics, pharmacological sciences, computational cancer biology, precision environmental health, antimicrobial resistance, cancer therapeutics, and infectious diseases.

#### **Gulf Coast Consortia**

In March 2001, the presidents of each of the six-member institutions of the Keck Center signed an unprecedented agreement establishing the Gulf Coast Consortia (GCC), explicitly designed to coalesce institutional strengths in order to:

- **1.** train new scientists at the intersection of biological sciences with quantitative and physical sciences
- 2. build cutting-edge research infrastructure and facilities
- **3.** cultivate a supportive atmosphere for the collaboration of basic and translational scientists, researchers, clinicians and students in both biological and non-biological fields
- 4. apply the resulting knowledge to prevent and treat diseases.

While the Keck Center serves as the training arm of the GCC, the research arm consists of individual, topic-focused research, including translational pain research, antimicrobial resistance, cellular and molecular biophysics, regenerative medicine, drug discovery and development, mental health research, single cell omics, immunology, translational imaging, artificial intelligence in health care, and theoretical and computational neuroscience. These consortia and newly forming clusters provide a supportive environment for the encouragement and development of research that might otherwise be beyond the reach of any one institution. New consortia form when faculty come together around a common interest, establishing a working vision and engaging a broad faculty community to pursue interinstitutional research, present conferences, acquire shared equipment and research cores and/or develop training, research or curriculum grants. https://www.gulfcoastconsortia.org/

#### **BIOLOGICAL SCIENCES**

Biophysics		]	MEDICINE
Computational & Structural Biology Bioengineering Neuroscience Genetics Environmental Health Microbiology/Virology Omics		Neuroscience Diagnostics Drug Discovery/Delivery Cancer Research Pain Research Mental Health Regenerative Medicine Immunology	
	Imaging Statistics Data Science Biomedical Informatics Artificial Intelligence Physics Chemistry Mathematics		

#### QUANTITATIVE SCIENCES

#### Acknowledgements

The Keck Center thanks the following for their generous support:

#### National Library of Medicine (NLM)

NLM Training Program in Biomedical Informatics and Data Science T15 LM007093 Program Director: Lydia E. Kavraki, PhD, Rice University

#### National Institute of General Medical Sciences (NIGMS)

*Training Interdisciplinary Pharmacology Scientists* T32 GM139801 Program Director: Carmen W. Dessauer, PhD, UT Health Science Center at Houston

Houston Area Molecular Biophysics Program T32 GM008280 Program Director: Theodore G Wensel, PhD, Baylor College of Medicine

**National Institute of Environmental Health Sciences (NIEHS)** *Training in Precision Environmental Health Sciences* T32 ES027801 Program Director: Cheryl L. Walker, PhD, Baylor College of Medicine

#### National Institute of Allergy and Infectious Diseases (NIAID) Molecular Basis of Infectious Diseases T32 AI055449 Program Director: Michael Lorenz, PhD, UT Health Science Center at Houston

*Texas Medical Center Training Program in Antimicrobial Resistance* T32 AI141349 Program Director: Anthony R. Flores, MD, PhD, UT Health Science Center at Houston

Cancer Prevention and Research Institute of Texas (CPRIT) Cancer Therapeutics Training Program RP210043 Program Director: Peter J.A. Davies, MD, PhD, Institute of Biosciences and Technology, Texas A&M University

### **Gulf Coast Consortia Member Institutions**





#### 33rd Keck Annual Research Conference Houston, TX October 20, 2023

The past decade has witnessed exponential growth of RNA research that demonstrated their essential roles as regulatory molecules, as tools for bioengineering, and as therapeutic targets. This year's conference focuses on major RNA discoveries with an emphasis on cutting-edge RNA research, engineering and RNA-based therapeutics. It brings together experts in the field of RNA biology to discuss the current advances in a broad range of important processes and the promise of RNA medicine. The conference and poster session will also highlight the outstanding research underway by predoctoral and postdoctoral trainees in the seven inter-institutional training programs administered by the GCC/Keck Center in the greater Houston area.

#### Chairs:

#### Wenbo Li, PhD

Associate Professor, Biochemistry and Molecular Biology, UTHealth-Houston

and

#### Monica Pillon, PhD

Assistant Professor, Biochemistry and Molecular Biology, Baylor College of Medicine

#### Friday, October 20, 2023

8:15-8:25	Welcome: Keck Center Overview and Conference Day Overview Monica Pillon and Wenbo Li
Session 1	
3:25-9:15	Keynote Speaker (40 Min + 10 Min Q&A)
	Haifan Lin, PhD, Professor of Cell Biology and Professor of Genetics; Director, Yale Stem Cell Center, Yale University
	The Piwi-piRNA Pathway: A New World of Genetic Regulation in the Germline
):15-9:55	Guest Speaker (30 min + 10 Min Q&A)
	Lydia Contreras, PhD, Professor of Chemical Engineering, University of Texas at Austin
	Mapping and Engineering Functions of Novel Regulatory RNA Networks
9:55-10:10	Keck Center Trainee Speaker (10 min + 5 min Q&A)
	Sara Abouelniaj, predoctoral trainee, Houston Area Molecular Biophysics Training Program
0:55-10:10	Keck Center Trainee Speaker (10 min + 5 min Q&A) Sara Abouelniaj, predoctoral trainee, Houston Area Molecular Biophysics Training Program

10:10-11:00 Poster Session and Coffee Break (50 Min)

11:00-11:50	Keynote Speaker (40 min + 10 min. Q&A) Samie Jaffrey, PhD, Professor of Pharmacology, Weill Cornell Medicine RNA Synthetic Biology: Probing and Controlling Cellular Function with Genetically Encoded RNA Devices
11:50-12:05	Keck Center Trainee Speaker (15 min) Jacob McPherson, PharmD, postdoctoral trainee, Training Program in Antimicrobial Resistance The Microbiome-Sparing Properties of a C. difficile-Selective Antibiotic with a Narrower Spectrum Activity than Anticipated via Antibiotic Resistant Commensal Microbiota
12:05-12:20	Keck Center Trainee Speaker (15 min) Chrystine Gallegos, predoctoral trainee, Training Interdisciplinary Pharmacology Scientists Program Effects of Satellite Glial Cell Stimulation on Isolated DRG Nociceptors
12:20-12:50	Lunch Break (30 min)
Session 2	
12:50-1:40	<b>Keynote Speaker</b> (40 min + 10 Min Q&A) <b>Jimena Giudice, PhD</b> , Associate Professor of Cell Biology and Physiology, University of North Carolina <i>Alternative Splicing of Clathrin Impacts Skeletal Muscle Structure and Function</i>
1:40-2:20	<b>Guest Speaker</b> (30 min + 10 min. Q&A); <b>Ken Yamada, PhD,</b> Assistant Professor, RNA Therapeutics Institute, UMass Chan Medical School <i>Extended Nucleic Acid (exNA): A Platform Technology for Enhancing siRNA Potency and</i> <i>Accumulation in vivo</i>
2:20-2:35	Keck Center Trainee Speaker (15 min) <b>Kenneth Trimmer, PhD</b> , postdoctoral trainee, Training in Precision Environmental Health Sciences Program <i>Spatial Single Cell Sequencing of Meiosis I Arrested Oocytes Reveals Acquisition of Maternal</i> <i>Transcripts from the Soma</i>
2:35-3:35	Poster Session and Coffee Break (60 min)
3:35-3:50	Keck Center Trainee Speaker (15 min) <b>Xuejiao Shirley Guo, PhD</b> , postdoctoral trainee, Cancer Therapeutics Training Program <i>Development of Selective ENL Inhibitors for Acute Myeloid Leukemia Therapy</i>
3:50-4:05	Keck Center Trainee Speaker 6 (15 min) Ivan Coronado, predoctoral trainee, NLM Training Program in Biomedical Informatics and Data Science Proxy Analysis of Neural Vascular System Using Retina Imaging and Deep Neural Networks
4:05-4:20	Keck Center Trainee Speaker 7 (15 min) Lois Armendariz, predoctoral trainee, Molecular Basis of Infectious Diseases Training Grant The Role of Mitochondria in Innate Immunity and Cancer
4:20-5:00	<b>Guest Speaker</b> (30 min + 10 min Q&A) <b>James Chappell, PhD</b> , Assistant Professor of Biosciences, Rice University <i>Genetically Engineering Microbial Consortia and Non-Model Microbes Using Programmable RNA</i> <i>Tools</i>
5:00-5:10	Awards and Closing Monica Pillion and Wenbo Li
5:10	Reception

# The Piwi-piRNA Pathway: A New World of Genetic Regulation in the Germline



# Haifan Lin, PhD

Professor Yale Stem Cell Center and Department of Cell Biology Yale University School of Medicine https://medicine.yale.edu/profile/haifan-lin/

Dr. Lin's work is focused on the self-renewing mechanism of stem cells, using Drosophila germline stem cells, mouse germline stem cells, mouse embryonic stem cells, Hydra, and planarian stem cells as models. He also studies germline development and stem cell-related cancers. Dr. Lin received his B.S. degree from Fudan University (1982) and his Ph.D. degree from Cornell University (1990). Following his postdoctoral research at the Carnegie Institution of Washington, he joined the faculty of Duke University Medical School in 1994, where he rose to the rank of Full Professor. He founded and directed the Duke Stem Cell Research Program (2005-2006) and moved to Yale in 2006 to establish and direct the Yale Stem Cell Center, building it from just two labs to currently one of the largest stem cell research organizations in the world with 100-member labs.

Dr. Lin is President (2022-2023) of the International Society for Stem Cell Research (ISSCR) and serves on the Medical Advisory Board of New York Stem Cell Foundation (2009-present). Dr. Lin has been serving on Editorial Boards of *Cell Stem Cells* (2007-), *Cell Research* (2010-), *Stem Cell Reports* (2013-), *National Science Review* (2013-), and *Science China* (2013-). Dr. Lin received many awards and honors, and is a Member of US National Academy of Sciences (2018-), a Member of American academy of Arts and Sciences (2018-), and a Fellow of the American Association for the Advancement of Science (2010-).

**Abstract:** Small non-coding RNAs play key roles in gene regulation that defines cell fate. In 1998, we discovered the Argonaute (Ago) protein family that are key components of small RNA pathways and are essential for stem cell self-renewal in both animal and plant kingdoms. Within this protein family, the Ago subfamily proteins are ubiquitously expressed. They bind to siRNA and miRNA and function in the RNAi and miRNA pathways. However, the Piwi subfamily is mostly expressed in the germline and primitive stem cells. In 2006, we and other found that Piwi proteins bind to millions of species of piRNA that are mostly 24-32 nucleotides in length and correspond to all types of genomic sequences. Our recent work indicates that the Piwi-piRNA pathway plays key roles at both epigenetic and post-transcriptional levels in genome-wide regulation of protein-coding genes, transposons, pseudogenes, and numerous genomic loci that encode diverse types of non-coding RNAs. In this talk, I will focus on how the Piwi-piRNA pathway mediates the post-transcriptional regulation of mRNA and lncRNA by transposons and pseudogenes as well as centromere function. This post-transcriptional regulation represents a new paradigm of genetic regulation that functionally connects all major constituents of the genome.

# Mapping and Engineering Functions of Novel Regulatory RNA Networks



# Lydia Contreras, PhD

Professor Department of Chemical Engineering University of Texas at Austin <u>https://sites.utexas.edu/contreraslab/</u>

Dr. Lydia Contreras is the Paul D. and Betty Robertson Meek Centennial Professor in Chemical Engineering and Vice Provost for Faculty Diversity, Equity, and Inclusivity at the University of Texas at Austin. She received a Bachelor of Science in Engineering degree in Chemical Engineering from Princeton University (2003) and a Ph.D. in Chemical and Biomolecular Engineering from Cornell University (2008). Dr. Contreras was an NIH Postdoctoral Fellow (Infectious Diseases) in the Division of Developmental Genetics and Bioinformatics at the Wadsworth Center-New York State Department of Health. She was a Visiting Postdoctoral Fellow in the Dept. of Biochemistry with Max F. Perutz Labs in Vienna, Austria. Dr. Contreras is a 2023 Fellow of the American Academy of Microbiology and has received numerous awards in biotechnology and bioengineering.

**Abstract:** Bacterial regulatory RNAs enable dynamic responses to stresses caused by changes in environmental conditions. These global regulators enable responses to diverse and rapidly changing environmental stimuli by affecting vast networks of targets at, frequently, multiple biological levels. Given their relevance to pathogenesis and their potential to manage global regulatory networks that affect biological production of industrially relevant compounds, understanding their functions is a goal in both medicine and metabolic engineering. While advances in omics-based technologies has led to the discovery of hundreds of sRNAs, their identification and characterization has lagged. Even in the model gram-negative organism, *Escherichia coli*, only ~40% and 60% of sRNAs have had at least one transcriptional regulator or post-transcriptional target confirmed, respectively. Additionally, the mechanistic roles of RNA-binding proteins in sRNA regulation remains elusive. This gap can be partly attributed to the selective nature of corresponding high-throughput techniques that typically rely on traditional sRNA features, such as basic promoter-based transcriptional regulation and dependence on RNA binding proteins for target activity. Additionally, lowly expressed sRNAs in commonly tested conditions are oftentimes missed.

To better characterize non-traditional and elusive still sRNAs networks that can provide novel insight into molecular arrangement and functionality, we have devised integrated multi-tiered approaches over a variety of cellular conditions. In this talk, we will describe our recent advances in developing high throughput approaches that allow for the simultaneous in vivo characterization of functional regions within RNA molecules and multiple modalities that could enable different functions in complexes with proteins. We will describe how RNA insights obtained from these synthetic probing approach can be used in the basic characterization of newly discovered RNAs and in the discovery of novel RNA mechanisms that can provide unique benefits to synthetic biology. The talk will also highlight our use of these methods in conjunction with new biophysical model and machine learning approaches for expanding our understanding of sRNA-regulation.

# RNA Synthetic Biology: Probing and Controlling Cellular Function with Genetically Encoded RNA Devices



### Samie Jaffrey, PhD

Professor Department of Pharmacology Weill Cornell Medicine https://www.jaffreylab.org/

Samie Jaffrey graduated from the Massachusetts Institute of Technology in 1992, and received his M.D. and Ph.D. from the Johns Hopkins University School of Medicine in 1999 where he studied mechanisms of nitric oxide signaling with Dr. Solomon H. Snyder. Dr. Jaffrey started his own lab at Weill Cornell Medical College in 2001 which focuses on identifying novel RNA regulatory pathways the control protein expression in normal cellular function and in disease processes. He is currently the Greenberg-Starr Professor of Pharmacology. Dr. Jaffrey is a member of several advisory boards and serves as a consultant to several institutions, and, in 2014, he was awarded the American Society for Biochemistry and Molecular Biology's Young Investigator Award for his advancements in RNA technology and mRNA regulation.

Abstract: RNA synthetic biology involves designing novel RNAs that can be expressed in cells in order to either image cellular processes or to manipulate cellular function and physiology. In this talk, we will describe new tools that enable RNA aptamers and RNA devices to be potent intracellular regulators of cellular function. We will describe new fluorogenic aptamer technologies for imaging RNA and RNA devices in cells. We will also describe RNAs that bind and regulate protein function, including novel RNAs that control specific cellular processes. We describe the Tornado ('Twister-optimized RNA for durable overexpression') expression system that allows heterologously expressed RNAs to circularize in cells. We will show how the Tornado system overcomes the problem of RNA instability, thus allowing RNA-based tools to accumulate to levels that are needed for them to affect cell physiology. We will also show how RNA synthetic biology systems can be controlled by small molecules, thus allowing these systems to be regulated in a tunable manner to control the magnitude of the cellular effects and to allow reversibility. We will discuss how these RNA synthetic biology concepts enable the design of novel regulatory RNAs and their potential uses in regulating cell biology and for therapeutic development.

# Alternative Splicing of Clathrin Impacts Skeletal Muscle Structure and Function



### Jimena Giudice, PhD

Associate Professor Department of Cell Biology and Physiology, University of North Carolina https://www.med.unc.edu/cellbiophysio/directory/jimena-giudice-phd/

Dr. Guidice received her Ph.D. in biological chemistry from the University of Buenos Aires in Argentina. Her postdoctoral training was in Molecular and Developmental Biology at Baylor College of Medicine in Houston. Dr. Guidice also completed Postdoctoral Fellowships at the American Heart Association and The Pew Charitable Trusts. Dr. Guidice's laboratory focuses on how alternative splicing regulates the expression of trafficking and membrane dynamics proteins in normal development and diseases. A second angle of the lab is how alternative splicing impacts on the functions of these proteins and thus in internal cell architecture and physiology. They are studying the crosstalk between alternative splicing and membrane trafficking and its contribution to the maturation and maintenance of the myocyte exquisite architecture and functions. The lab utilizes a broad spectrum of approaches ranging from molecular biology, cell biology, and physiology techniques including animal models.

**Abstract:** Alternative splicing is a posttranscriptional mechanism that produces multiple protein isoforms from single genes. Coordinated splicing networks regulate organ development and tissue identity and genes encoding trafficking proteins are highly alternatively spliced. In particular, alternative splicing of the clathrin heavy chain (Cltc) gene regulates the expression of specific variants during striated muscle development. CLTC is the main driver of clathrin mediated endocytosis (CME); but also regulates the formation and maintenance of the costamere in skeletal muscles. Exon 31 is skipped in neonates but included in adulthood exclusively in the heart, skeletal muscles, and brain, with skeletal muscle being the tissue with highest levels of inclusion. Furthermore, we identified the two RNA-binding proteins (PTBP1, QKI) that antagonistically regulate Cltc splicing in muscle cells: PTBP1 represses exon 31 inclusion and QKI promotes its inclusion.

To investigate the functional roles Cltc splicing in skeletal muscles, we are using our CRISPR mouse model where we blocked endogenous Cltc splicing transition: homozygous (HO) mice express exclusively the short isoform throughout development while wild type (WT) mice exhibit the normal splicing transitions. HO mice exhibited increased skeletal muscle mass and myofiber cross-sectional area that are accompanied by alterations on the AMPK-signaling pathway and an increase in ubiquitination in muscle tissues. Whereas RNA-sequencing revealed minimal differences in gene expression between skeletal muscles of WT and HO adult littermates, mass spectrometry identified robust differences in protein expression between genotypes. HO muscle showed an upregulation of proteins associated with CME and ion transport and a downregulation of structural proteins important for muscle contraction. Consistent with the decreased expression levels of structural proteins in HO skeletal muscle, transmission electron microscopy (TEM) images revealed disrupted sarcomeres in HO muscle. TEM studies also showed that mitochondria, transverse tubules, and neuromuscular junctions were structurally abnormal in HO mice. Collectively, our data suggest that Cltc splicing promotes proper skeletal muscle structure and function.

### Extended Nucleic Acid (exNA): A Platform Technology for Enhancing siRNA Potency and Accumulation in vivo



### Ken Yamada, PhD

Assistant Professor RNA Therapeutics Institute UMass Chan Medical School https://profiles.umassmed.edu/display/25584404

Ken Yamada, PhD, works on the chemical biology of nucleic acids, including DNA and RNA, and shorter oligonucleotides. He received his PhD under the supervision of Professor Mitsuo Sekine at the Tokyo Institute of Technology, where he studied a variety of novel chemically modified nucleoside analogues. As a postdoctoral fellow at McGill University, Dr. Yamada became involved in chemical biology and the development of antisense oligonucleotides. He began his academic career as an assistant professor in IMRAM at TOHOKU University, then left to train in Professor Anastasia Khvorova's oligonucleotide therapeutics research lab in the RNA Therapeutics Institute at the University of Massachusetts Chan Medical School. In the Khvorova Lab, Dr. Yamada invented a transformative novel oligo backbone chemistry, which has grown to be the platform technology in the lab. Dr. Yamada was promoted to assistant professor in 2021.

Abstract: Modulation of backbone structures of siRNA has profound impact on pharmacokinetics and pharmacodynamics profile. In the RNA therapeutics field today, chemically stabilized synthetic RNA without compromising interactions of siRNA molecules with RISC-forming proteins are required for prolonged therapeutic effects. Phosphorothioate (PS) is the only backbone modification that significantly stabilize oligonucleotide and used extensively in FDA-approved oligonucleotide drugs and multiple drug candidates in late-stage of clinical trials. However, a certain amount of metabolites of PS backbones is observed in vivo, which motivated us to develop nucleotide analogues that further increases the metabolic stability of PS backbones to have much longer duration of effect than up-to-date structurally optimized siRNAs. In this study, we developed a metabolically stable nucleoside analogue, extended Nucleic Acid (exNA). exNA contains single carbon extended backbone structure designed as a "pseudo" epigenetic modification, which slightly modulates local molecular structure to modulate RNA protein interactions. We discovered that just a few exNA modifications at 3'-terminus of the siRNA guide strand (3'-exNA cluster) in combination with PS backbone provides an order of magnitude of longer half-life against 3'-exonuclease without compromising siRNA RISC function. This resulted in giving an improved plasma PK profile (~6 fold increase in AUC), tissue accumulation (3-15 fold) and longer duration of effect in multiple tissues such as Kidney, Muscle, Heart, Liver and Fat in mice. This enhanced potency was also highly reproducible in the context of CNS with multiple targets. The data presented here would be a demonstration of novel and widely applicable platform technology for future RNAi-based therapeutics.

### Genetically Engineering Microbial Consortia and Non-Model Microbes Using Programmable RNA Tools



### James Chappell, PhD

Assistant Professor Department of Biosciences Rice University https://www.chappell-lab.org/

James Chappell received his PhD in Molecular Biosciences from Imperial College London. In March 2023, he won a National Science Foundation CAREER Award to develop RNA programming methods to improve human health and the environment. In the CAREER research, Chappell's lab will focus on creating a new method for genetically programming microbial communities that live in soil, wastewater and other messy environments.

The goal of the Chappell lab is to forward the ability to understand and engineer the bacteria domain of life. Central to this is the ability to control how cells express their genetic code. The lab focuses on understanding how the biomolecule RNA can be designed to create synthetic regulators of gene expression—allowing for the manipulation of natural cellular processes to elicit deeper biological understanding and for the engineering of new synthetic cellular functions. As such the lab focuses both on the creation of new gene regulatory tools and their application. The main areas of research focus are currently: (1) Creation of synthetic RNA regulators of gene expression. (2) Deciphering the portability of RNA regulators across the bacteria domain of life. (3) Creation of synthetic genetic circuits capable of performing signal processing. (4) Applying RNA-based tools for functional genomics.

**Abstract:** Microbial consortia are found almost everywhere (*e.g.*, in soils, rivers, and guts and on skin, minerals, and built materials) and underlie processes critical to sustainable agriculture, waste treatment, the longevity of materials, and health. Current microbiome engineering approaches primarily rely on domestication, whereby individual species are isolated, genetically manipulated in the lab, and then reintroduced back into microbiomes. However, this approach can be arduous and incompatible with the large fraction of uncultured microbes found in native microbiomes. The overarching goal of my group is to establish a framework to create genetic programs that are efficiently transferred into natural consortia, can be precisely controlled, and can actuate useful genetically-encoded functions in these communities (*e.g.*, biosensing, antibiotic, production of metabolites). To achieve this, we innovate RNA technologies that allow us to efficiently transfer, control, and actuate programs in native consortia and non-model microbes for diverse applications in biotechnology and biomedicine.

### Trainee Speaker Abstracts (In order of appearance) Graphene Grids: Cleaner with a Longer Shelf Life



# Sara Abouelniaj

PhD Student Materials Science and Nanoengineering Rice University Houston Area Molecular Biophysics Training Program

Sara graduated with a Biochemical and Biophysical sciences degree in 2018. She got her Master's in Business Administration (MBA) in 2019 while working full-time as a lead in a department at a pharmaceutical company. She is pursuing her Ph.D. in Material Science and Nanoengineering from Rice with Dr. Yimo Han and is co-advised at Baylor College of Medicine by Dr. Zhao Wang. This is her second year with the Houston Area Molecular Biophysics Trainee Program as she is working on bridging the gap between material sciences and biochemistry.

**Abstract**: Cryo-EM has become one of the most powerful and sought techniques for revealing the high-resolution structures of biological macromolecules. Graphene grids aim at preserving thin and uniform ice while also significantly improving protein absorption and sample preparation as well as data acquisition in cryo-EM. However, making graphene grids remains challenging for structural biology labs. In addition, we noticed that the graphene grids in the market generally are of low quality and cleanliness.

Here, we performed a time study to test the cleanliness and coverage of the graphene grids. The results showed us the possibility of keeping the graphene grids cleaner for longer by keeping the PMMA (Poly(methyl methacrylate)). We were also able to have a good estimate for the shelf life of the grids. Our work proved that our method of making graphene grids provided clean grids every time as well as verifying that keeping the PMMA layer can be beneficial in preserving the grids as well as saving time producing the grids.

# The Microbiome-Sparing Properties of a C. difficile-Selective Antibiotic with a Narrower Spectrum Activity than Anticipated via Antibiotic Resistant Commensal Microbiota



### Jacob McPherson, PharmD

Postdoctoral Fellow Pharmacological and Pharmaceutical Sciences University of Houston Training Program in Antimicrobial Resistance Jacob McPherson earned his B.S. in Cell & Molecular Biology from the University of Texas at Austin, his PharmD from the University of Houston College of Pharmacy, and is currently completing a PhD in Pharmacology with the University of Houston Department of Pharmacological and Pharmaceutical Sciences. Jacob is interested in the application of drug-receptor theory, cryo-electron microscopy, and comparative genomics to improve treatments for infectious diseases. His current work studies the impact of antibiotics on the human intestinal microbiome of *Clostridioides difficile* infections. He is currently under the mentorship of Dr. Kevin Garey at the UHCOP, Dr. Julian Hurdle at the Texas A&M Institute of Biosciences and Technology, and Dr. Matthew Baker at UT Health McGovern Medical School. His goal is to understand the impact of antibiotics on the human microbiome through structural pharmacology.

**Abstract**: *Clostridioides difficile* infection (CDI) is a CDC urgent threat level pathogen due to its high burden of disease and limited number of treatment options. To address this clinical need, we are studying the efficacy of a novel antibiotic, ibezapolstat (IBZ), a PolC-type DNA polymerase III (PolC) inhibitor for the treatment of CDI. PolC is responsible for leading-strand synthesis in DNA replication of the phylum Bacillota that includes *C. difficile*, but not Actinomycetota, Bacteroidota, or Pseudomonadota. Hence, this knowledge provides the foundation for a microbiome-sparing antibiotic that targets *C. difficile*, a PolC+ Bacillota pathogen that spares the other beneficial microbiome bacteria.

During clinical trials, we found that IBZ has a narrower spectrum than anticipated, sparing more commensal sub-taxa of Bacillota than we initially anticipated. The reason certain sub-taxa of PolC+ Bacillota are IBZ non-susceptible remains unknown and forms the basis of my current research. Specifically, we observed increased relative abundance in the beneficial commensals Lachnospiraceae and Oscillospiraceae in CDI patients treated with IBZ, sub-taxa known to suppress CDI recurrence through bile salt and short-chain fatty acid metabolism. We hypothesize these commensal Bacillota sub-taxa are IBZ non-susceptible due to PolC structural variants in IBZ drug-binding residues. My research will investigate the structural and kinetic basis of IBZ non-susceptibility in these commensal Bacillota sub-taxa through cryogenic electron microscopy and rapid quench-flow kinetics, respectively. The implications of this research will support the future development of microbiome-sparing antibiotics, the importance of variant residues in drug-target interactions, and enzyme kinetics at millisecond resolution.

# Effects of Satellite Glial Cell Stimulation on Isolated DRG Nociceptors



### **Chrystine Gallegos**

PhD Student Neuroscience UT Health Graduate School of Biomedical Sciences Training Interdisciplinary Pharmacology Scientists

Chrystine completed her B.A. in Kinesiology Sports Medicine at Rice University and M.S. in Biomedical Sciences at MD Anderson UT Health Graduate School of Biomedical Sciences and is now a fourth year Ph.D. student in the Neuroscience and Molecular Translational Biology Graduate Programs at MD Anderson UT Health GSBS. Her research interests focus on identifying mechanisms of chronic pain after spinal cord injury (SCI). Her goal is to better understand how SCIinduced changes in satellite glial cells (SGCs) affect nociceptors in the dorsal root ganglion (DRG). Chrystine is mentored by Dr. Carmen Dessauer and co-advised by Dr. Edgar T. Walters.

**Abstract**: Nociceptors in the dorsal root ganglion (DRG) are persistently altered after spinal cord injury (SCI), exhibiting reduced sensitivity to opioids and increased excitability which contributes to chronic pain. Satellite glial cells (SGCs) within the DRG are also functionally altered after SCI, yet they have been largely overlooked with respect to pain research. We hypothesize that SGCs are stimulated by inflammatory molecules in the body persistently upregulated after SCI which alter neuronal responses. We have used high content microscopy from pure primary cultures of isolated SGCs or mixed cells from rat DRGs to explore cellular responses and signaling changes after treatments with inflammatory factors. We are currently examining the effect of SCI on SGC activation, the sensitivity of SGCs and neurons to cytokines, and whether SCI-induced neuronal hyperexcitability and opioid effects require SGC activation and signaling.

### Spatial Single Cell Sequencing of Meiosis I Arrested Oocytes Reveals Acquisition of Maternal Transcripts from the Soma



### Kenneth Trimmer, PhD

Postdoctoral Fellow Genetics MD Anderson Cancer Center Training in Precision Environmental Health Sciences Program

Kenneth Trimmer obtained his B.S. from the University of Maryland Baltimore County and his PhD from the UT MD Anderson UTHealth Graduate School of Biomedical Sciences. He is currently a postdoctoral fellow in the lab of Dr. Swathi Arur at MD Anderson Cancer Center in the Department of Genetics. Kenneth is interested in developing and leveraging tools to study the impact of nutritional availability on female reproductive output and oocyte quality. He is in his second year in the TPEHS fellowship training program and is co-mentored by Dr. Cheryl Walker.

**Abstract**: Maternal RNAs are stored from minutes to decades in oocytes throughout meiosis I arrest. For long, studies have suggested that during this arrest oocytes are transcriptionally quiescent. Recent reports suggest that nascent transcription in oocytes leads to generation of maternal transcripts. Whether arrested oocytes launch nascent transcription in response to environmental or hormonal signals while maintaining the meiosis I arrest remains undetermined. We test this by integrating spatial extraction, single cell RNA deep sequencing, RNA Velocity and RNA fluorescence in-situ hybridization (RNA-FISH) on *C. elegans* oocytes of different ages during meiosis I arrest. We discovered a population of transcripts that increases as the arrested meiosis I oocyte ages but ruled out extracellular signaling, through ERK MAPK, and nascent transcription as a mechanism for this increase. Instead, we report transcript acquisition from neighboring somatic cells as a mechanism for the increase in transcripts during meiosis I arrest. These analyses provide a new view of the RNA landscape at a single-cell resolution of a meiosis I arrested oocyte and as it prepares for oocyte maturation and embryogenesis.

# Development of Selective ENL Inhibitors for Acute Myeloid Leukemia Therapy



### Xuejiao Shirley Guo, PhD

Postdoctoral Fellow Chemistry Texas A&M University The Cancer Therapeutics Training Program

Shirley Guo received a B.S. in Pharmaceutical Engineering from Beijing University of Chemical Technology in 2013, an M.S. degree in Pharmaceutical Engineering from Tianjin University in 2016, and her Ph.D. in Biomedical Sciences from City University of Hong Kong in 2020. She is currently a postdoctoral fellow in the Texas A&M Drug Discovery Laboratory working with Dr. Wenshe Ray Liu. Her research is centered around improving treatments for Acute Myeloid Leukemia (AML), focusing on the development of selective inhibitors and PROTACS for AML therapy.

Abstract: Acute myeloid leukemia (AML) is the second most diagnosed and the deadliest subtype of leukemia. Recently genetic loss-of-function studies have demonstrated that a human YEATS domain-containing protein named eleven-nineteen-leukaemia (ENL) functions as a transcriptional coactivator and is essential for the proliferation of AML that harbors oncogenic multiple lineage leukemia (MLL) rearrangements. We previously synthesized a series of small molecule inhibitors that displayed significant and specific inhibitory effects against the ENL YEAST domain. In the current work, we report the development of a novel NanoBRET system that allows the analysis of cellular permeability, potency, selectivity, and stability of synthesized ENL inhibitors for their prioritization for further characterizations. Followed by in vitro metabolic stability and cell growth inhibition studies, we narrowed down to a potent and specific ENL YEATS domain inhibitor 13 with both high in vitro metabolic stability and strong anti-proliferation ability on MLL-fusion leukemia cell lines. A mouse pharmacokinetic (PK) analysis showed that at an oral dose of 20 mg/kg compound 13 had 60.9% bioavailability and 2.6 h mean residence time. With these favorable PK characteristics, compound 13 exhibited remarkable anti-AML activity and prolonged survival in vivo. Cumulatively, the current study has prioritized compound 13 as a promising drug candidate to disrupt the pathogenic functions of ENL for the AML treatment.

# Proxy Analysis of Neural Vascular System Using Retina Imaging and Deep Neural Networks



### **Ivan Coronado**

PhD Student Biomedical Informatics The University of Texas Health Science Center at Houston National Library of Medicine (NLM) Training Program in Biomedical Informatics and Data Science Ivan Coronado earned his Bachelor of Science and Master of Engineering in Electrical Engineering from Texas A&M University. As a fourth-year Biomedical Informatics Ph.D. student at UTHealth, his research delves into the design and application of machine learning to retinal imaging analysis, aiming to uncover novel indicators of systemic cardiovascular disease. Guided by the expertise of Dr. Luca Giancardo and Dr. Sunil Sheth, Ivan is dedicated to bridging the gap between technology and medicine, creating transformative diagnostic tools.

**Abstract**: Fundus photography presents a non-invasive, easy-to-acquire method for visualizing the retina, a layer at the back of the eye. Scientific evidence has indeed established connections between retinal structures and broader systemic diseases. However, the act of quantifying and measuring these retinal features remains laborious, error-prone, and demands specialized expertise. Advanced machine learning techniques based on deep learning tend to function as a "black box", hindering insights into pivotal structures and their diagnostic links, and making the incorporation of clinically relevant knowledge challenging. In response, we propose vasculature-focused machine learning techniques to surpass these inherent challenges. By repurposing machine learning methods used for segmenting retinal vasculature (U-Net) and synthesizing perfusion visualizations within the retina (cGAN), we have consolidated methods that emphasize the vascular attributes in retinal images while retaining the advantages of data-driven approaches. Our findings suggest that machine learning models which prioritize the vascular features of the retina may offer some advantages in detecting and analyzing systemic diseases linked to vascular abnormalities.

### The Role of Mitochondria in Innate Immunity and Cancer



### **Lois Armendariz**

PhD Student Biochemistry and Cell Biology Rice University Molecular Basis of Infectious Diseases Program

Lois Armendariz is currently a 3rd-year Biochemistry and Cell Biology PhD candidate at Rice University studying the role of mitochondria in innate immunity and cancer. She is in her second year of the NIAID T32 Molecular Basis of Infectious Diseases Program under the direction of Dr. Michael Lorenz. Her mentor is Dr. Natasha Kirienko. Lois' project aims to elucidate the relationship between lipid metabolism and mitochondrial surveillance in pathological states including infection with Pseudomonas aeruginosa.

Abstract: The present leading causes of death in the U.S. include cancer and antimicrobial- resistant infections. It is imperative that we understand the underlying mechanisms driving the pathology of these diseases in order to develop effective treatments. Recent studies have shown that cancer cells heavily rely on production of mitochondrial reactive oxygen species (ROS) and  $\alpha$ -ketoglutarate as well as glucose and glutamine for survival. Targeting these substances readily kills certain types of cancer cells, which leads to the notion that mitochondria could be used as targets for cancer treatments.

The model nematode *Caenorhabditis elegans* is a powerful tool for high-throughput drug screening as well as for hostpathogen interactions studies. We screened 45,000 compounds in *C. elegans* for activation of mitophagy (autophagic degradation of mitochondria) and identified 8 compounds. In vitro studies showed that compounds of the PS127 family significantly upregulate ROS production in acute myeloid leukemia (AML), activating multiple cell death pathways specifically in cancer, but not in healthy cells. Cheminformatics on PS127 family molecules have suggested mechanisms of action for these compounds that are currently under study.

Mitochondria in healthy cells also play important roles in innate immunity and host defense against infection. Research in *C. elegans* showed a relationship between lipid metabolism and mitochondrial surveillance in response to *Pseudomonas aeruginosa* liquid-based infection (LK-Pa). We identified 22 lipid metabolism genes that are indispensable for survival in LK-Pa, of which four are required for activation of the evolutionarily-conserved ESRE (ethanol and stress response element) mitochondrial surveillance pathway. This work establishes the involvement of lipid metabolism in defense response against Gram-negative bacterial pathogens and it sets the groundwork for the discovery of candidate genes critical for host defense.

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