# 3rd Symposium of Stanford RNA Medicine Program

## *Monday, April 14, 2025*

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| 8:30 - 9:00 am | Registration and Breakfast |
| 9:00 - 9:10 am | **WELCOME AND OPENING REMARKS**<br><br>**William Greenleaf, Ph.D.**<br>Director, RNA Medicine Program<br>Professor of Genetics<br>Stanford University School of Medicine |
| 9:10 – 9:50 am | **OPENING KEYNOTE**<br>Moderated by William Greenleaf, Ph.D.<br><br>**Drew Weissman, M.D., Ph.D.**<br>Director, Penn Institute for RNA Innovation<br>Roberts Professor of Vaccine Research<br>University of Pennsylvania<br>*Nucleoside-Modified mRNA-LNP Therapeutics* |
| 9:50 – 10:50 am | **SESSION 1: RNA THERAPEUTICS** |
| 9:50 – 10:10 am | **William Robinson, M.D., Ph.D**.<br>James W. Raitt, M.D. Professor<br>Division Chief, Immunology & Rheumatology<br>Stanford University School of Medicine<br>*EBV in Autoimmune Disease* |
| 10:10 – 10:30 am | **Fyodor Urnov, Ph.D.**<br>Professor, Molecular Therapeutics<br>Scientific Director, Innovative Genomics Institute (IGI)<br>University of California, Berkeley<br>*RNA Medicines On-Demand in CRISPR Space: a Gene-Editing Platform for Inborn Errors of Immunity* |
| 10:30 – 10:50 am | **Howard Y. Chang, M.D., Ph.D.**<br>CSO, Amgen<br>*Xist RNP in female biased autoimmunity* |
| 10:50 - 11:10 am | **Coffee Break** |
| 11:10 – 12:10 pm | **SESSION 1: RNA THERAPEUTICS - CONT.** |

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| 11:10 – 11:30 am | **Ravi Majeti, M.D., Ph.D.**<br>Virginia and D.K. Ludwig Professor<br>Professor of Medicine (Hematology)<br>Director, Institute for Stem Cell Biology and Regenerative Medicine<br>Stanford University School of Medicine<br>*Reprogramming Cancer into Antigen Presenting Cells for Cancer Vaccination* |
| 11:30 – 11:50 am | **Bali Pulendran, Ph.D.**<br>Professor, Pathology, Microbiology & Immunology<br>Director, Institute for Immunity, Transplantation & Infection<br>Stanford University School of Medicine<br>Systems biological assessment of human immunity to vaccination |
| 11:50 – 12:10 pm | **Robert Waymouth, Ph.D.**<br>Robert Eckles Swain Professor of Chemistry and Professor, by courtesy, of Chemical Engineering<br>New materials for RNA delivery: How to get it where it needs to go |
| | **Poster-Teaser** |
| 12:10 – 1:30 pm | **Lunch** |
| 1:10 – 1:25 pm | **INDUSTRY SPONSORED TALK** |
| | **Devan Shah**<br>Founder & CEO, RNAV8 Bio<br>mRNA Engineering Redefined |
| 1:30 – 2:50 pm | **SESSION 2: AI AND COMPUTATIONAL TECHNOLOGY** |
| 1:30 – 1:50 pm | **Liana Lareau, Ph.D.**<br>Assistant Professor, Bioengineering<br>University of California, Berkeley<br>Deep learning and deep sequencing for RNA design |
| 1:50 – 2:10 pm | **Le Cong, Ph.D.**<br>Assistant Professor, Pathology & Genetics<br>Stanford University School of Medicine<br>Accelerating RNA Medicines with CRISPR-GPT AI Agents integrating Expert LLMs and RNA Foundation Models |
| 2:10 – 2:30 pm | **Stephen Quake, Ph.D.**<br>Lee Otterson Professor, School of Engineering<br>Professor, Bioengineering, Applied Physics, and by Courtesy, Physics<br>Head of Science, Chan Zuckerberg Initiative |

Stanford University
A Decade of Molecular Cell Atlases

2:30 – 2:50 pm **William Greenleaf, Ph.D.**
Director, RNA Medicine Program
Professor of Genetics
Stanford University School of Medicine
Scaling quantitative measurements of RNA function

2:50 - 3:10 pm **Coffee Break**

3:10 – 4:10 pm **SESSION 3: RNA TECHNOLOGY**

3:10 – 3:30 pm **Ryan Flynn, M.D., Ph.D.**
Principal Investigator, Flynn Lab
Assistant Professor, Stem Cell & Regenerative Biology, Harvard University
Cell surface glycoRNA biology

3:30 – 3:50 pm **Eric T. Kool, Ph.D.**
George & Hilda Daubert Professor of Chemistry
Stanford University
Profiling the transcriptome interactions of drugs of unknown target

3:50 – 4:10 pm **Felix Horns, Ph.D.**
Core Investigator, Arc Institute
Assistant Professor, Department of Genetics
Stanford University School of Medicine
Harnessing RNA export to monitor and manipulate living cells

4:10 – 4:50 pm **CLOSING KEYNOTE**
Moderated by William Greenleaf, Ph.D.

**Carolyn Bertozzi, Ph.D.**
Baker Family Director, Sarafan ChEM-H
Investigator, Howard Hughes Medical Institute
Professor of Chemistry, Stanford University
Therapeutic opportunities in glycoscience

**Poster-Teaser**

5:00 – 6:30 pm **POSTER SESSION AND DRINK RECEPTION**

## POSTER SESSION

**Poster Title:** Quantifying Data Distortion in Bar Graphs in Biological Research
**Author:** Teng-Jui Lin

**Affiliation:** Department of Chemical and Biomolecular Engineering, UC Berkeley

**Abstract:**
Over 88% of biological research articles use bar graphs, of which 29% have undocumented data distortion mistakes that over- or under-state findings. We developed a framework to quantify data distortion and analyzed bar graphs published across 3387 articles in 15 journals, finding consistent data distortions across journals and common biological data types. To reduce bar graph-induced data distortion, we propose recommendations to improve data visualization literacy and guidelines for effective data visualization.

**Poster Title:** Novel Age Reversal Targeted Therapy Using Apelin mRNA Nanotechnology
**Author:** Jayakumar Rajadas
**Affiliation:** Cardiovascular Institute, Stanford University

**Abstract:** Aging is accompanied by a systemic decline in physiological functions, a challenge that underlies many age-related disorders. Recent studies have identified apelin, an endogenous circulating peptide, as a critical factor whose diminished levels contribute to tissue degeneration across multiple organ systems. Accordingly, the exogenous delivery of apelin or agonists targeting its receptor, APJ, is emerging as a promising intervention to counteract a range of aging-related chronic diseases and promote overall healthy aging. However, traditional delivery methods have been limited by barriers such as the blood-brain barrier and rapid hepatic clearance. We previously developed an innovative intranasal formulation that delivers an apelin peptide efficiently to the brain, successfully treating a mouse model of Parkinson's disease. We have now created a novel apelin mRNA to achieve delivery to other tissues, which includes the use of a novel lipid nanoparticle formulation to prevent liver clearance. Our novel apelin mRNA and novel lipid nanoparticle formulation offer a powerful approach for overcoming age-related chronic diseases combined with a new tool to improve delivery of mRNA to non-liver tissues.

**Poster Title:** Constraints on codon choice identified using protein language models and genome wide screens
**Author:** Helen Sakharova
**Affiliation:** Lareau Lab, UC Berkeley

**Abstract:** Identical proteins can be encoded in DNA using different synonymous codons, which can have dramatic effects on the amount of functional protein produced. When choosing codons to optimize protein production, a standard approach is to replace slowly translated codons with synonymous, quickly translated codons. However, slow codons can have important functions, such as facilitating co-translational folding and localization. The exact rules governing codon choice are still poorly understood. We probe when and where codon choice is most strongly constrained using computational and experimental methods. Leveraging existing protein language models, we build a machine learning model to predict codon choice from amino acid sequence. The model effectively combines information about position and protein structure to learn subtle but wide-reaching constraints on codon choice in yeast. In parallel, we conduct a genome-wide screen in yeast to reliably identify synonymous variants that significantly affect fitness, using Cas9 retron editing to create thousands of synonymous codon substitutions. We identify individual synonymous variants that are likely to disrupt cotranslational localization or important mRNA structures. We find that the model predictions correspond to experimentally measured fitness effects of mutations that change slow codons to fast codons. By considering

both the small but general effects of codon choice that can be learned from evolution and the strong but highly specific effects determined via experiment, we expose biological constraints on codon choice.

**Poster Title:** irCLIP-RNP and Re-CLIP reveal patterns of dynamic protein assemblies on RNA
**Author:** Luca Ducoli
**Affiliation:** Department of Dermatology, Stanford University

**Abstract:**
RNA binding proteins (RBPs) control varied processes, including RNA splicing, stability, transport, and translation. Dysfunctional RNA-RBP interactions contribute to the pathogenesis of human disease. However, characterizing the nature and dynamics of multiprotein assemblies on RNA has been challenging. To address this, non-isotopic ligation-based ultraviolet crosslinking immunoprecipitation was combined with mass spectrometry (irCLIP-RNP) to identify RNA-dependent associated proteins (RDAPs) co-bound to RNA with any RBP of interest. irCLIP-RNP defined landscapes of multimeric protein assemblies on RNA, uncovering previously unknown patterns of RBP-RNA associations, including cell-type-selective combinatorial relationships between RDAPs and primary RBPs. irCLIP-RNP also defined dynamic RDAP remodeling in response to epidermal growth factor (EGF), uncovering EGF-induced recruitment of UPF1 adjacent to HNRNPC to effect splicing surveillance of cell proliferation mRNAs. To identify the RNAs simultaneously co-bound by multiple studied RBPs, a sequential immunoprecipitation irCLIP (Re-CLIP) method was also developed. Re-CLIP confirmed binding relationships seen in irCLIP-RNP and identified HNRNPC and UPF1 RBP co-binding on RND3 and DDX3X mRNAs. irCLIP-RNP and Re-CLIP provide a framework to identify and characterize dynamic RNA-protein assemblies in living cells.

**Poster Title:** A modular strategy for engineering RNA-activated reporters and enzymes
**Author:** Ana Novacic
**Affiliation:** Stanford University

**Abstract:** To facilitate the detection, imaging, and interactome mapping of cellular RNAs of interest, our objective is to engineer effector proteins that are natively in the OFF state but switch to the ON state when interacting with target RNA molecules. Such a design effectively achieves zero- or minimal-background RNA tracking and interactome mapping in living cells. We developed a generalizable allosteric module that shows RNA-mediated activation and demonstrate its utility in high-sensitivity RNA imaging, proximity labeling, and protein relocalization. This methodology should enable RNA biologists to tap into the vast array of highly optimized protein reporters and enzymes while bypassing the high background associated with conventional RNA targeting mechanisms. I will describe our engineering progress, early applications of the technology, and remaining hurdles.

**Poster Title:** A novel exonuclease mediates 3’end maturation of human telomerase RNA
**Author:** Mennatallah Albarqi
**Affiliation:** SCI/Hematology, Stanford University

**Abstract:** Proper processing of human telomerase non-coding RNA (hTR) is essential for telomerase function and telomere maintenance. Telomerase consists of hTR, telomerase reverse transcriptase (TERT), and additional proteins. hTR precursors undergo 3’end trimming to form

mature hTR. Mutations that alter expression of TERT, hTR, or the enzymes that process hTR can lead to telomere shortening and diseases associated with stem cell failure. hTR processing occurs in part through the opposing actions of the non-canonical poly(A) polymerase PAPD5 and the nuclear deadenylase PARN. While PAPD5-mediated oligoadenylation leads to hTR degradation, PARN-mediated trimming  mediates maturation of hTR. Surprisingly, proper hTR processing is restored in the absence of both PARN and PAPD5. To determine which exonucleases are involved in hTR 3'end maturation, we performed an siRNA screen followed by 3'RACE-seq. We identified ISG20L2 as a novel exonuclease that contributes to 3'end maturation of hTR. We found that knockdown of ISG20L2 reduces levels of mature hTR and results in accumulation of extended hTR transcripts. We also found that knockdown of ISG20L2 further reduces mature hTR in the absence of PARN. Interestingly, overexpression of ISG20L2 rescues hTR maturation defects and further elongates telomeres in PARN KO cells. Overall, our studies reveal that ISG20L2 promotes human telomerase RNA maturation through multiple pathways. Further investigation of these pathways will improve our understanding of telomerase regulation and reveal potential therapies that targets telomerase.

**Poster Title:** Trias: An Encoder-Decoder Model for Generating Synthetic Eukaryotic mRNA Sequences and Studying Codon Usage Rules
**Author:** Marjan Faizi
**Affiliation:** UC Berkeley

**Abstract:** The genetic code is degenerate, with most amino acids encoded by multiple synonymous codons. Codon usage varies across species, influenced by differences in tRNA abundance. Fast codons correspond to abundant tRNAs and enable efficient translation, while slow codons are linked to scarce tRNAs and slower translation. Optimizing protein output by using fast codons seems favorable, however, slow codons play critical roles in specific mRNA regions, influencing co-translational folding and proper protein function. Predicting optimal codon sequences is therefore challenging, and current methods rely on hand-crafted features like codon frequency or GC content, missing broader patterns in codon usage. To address this, we developed Trias, a sequence-to-sequence language model trained on millions of mRNA coding regions. Trias captures both local and global sequence dependencies, learning codon usage rules directly from data to generate species-specific codon sequences. These outputs align with biological constraints and correlate strongly with mRNA stability, ribosome load, and GFP expression. Trias also outperforms existing commercial tools in generating sequences that match high-expression codon variants. This highlights Trias as a powerful, data-driven approach for codon optimization, with applications in recombinant protein production and mRNA-based therapeutics.