BIOGRAPHICAL SKETCH

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NAME: Ralph E. Kleiner

eRA COMMONS USER NAME (credential, e.g., agency login): rkleiner

POSITION TITLE: Assistant Professor of Chemistry; Associated Faculty in Molecular Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Princeton University, Princeton, NJ	A.B.	06/2005	Chemistry
Harvard University, Cambridge, MA	Ph.D.	05/2011	Chemistry
The Rockefeller University, New York, NY	Postdoctoral	08/2016	Chemistry and Cell Biology

A. Personal Statement

I have been studying nucleic acid chemistry since I began my doctoral training with Prof. David Liu at Harvard University in 2005. During this period, I developed and applied DNA-templated chemical transformations to generate sequence-encoded artificial polymers and synthetic small-molecule libraries, and discovered novel small-molecule inhibitors of Src kinase and Insulin-Degrading Enzyme. This experience complemented my postdoctoral training with Prof. Tarun Kapoor at The Rockefeller University, during which I used chemoproteomic strategies to investigate phosphorylation-dependent protein-protein interactions in the DNA damage response. As an Assistant Professor, my lab has combined chemistry, biochemistry, cell biology, proteomics, and transcriptomics to investigate epitranscriptomic RNA modifications– post-transcriptional modifications found on mRNA that are hypothesized to regulate gene expression. We have developed a chemoproteomic platform for profiling 'readers' of RNA modifications and characterized the effect of N^{6}-methyladenosine and N^{1}-methyladenosine on cellular protein-RNA interactions. Further, we have developed an activity-based platform to profile RNA modifying enzymes in living cells which has led to identification and characterization of novel RNA modifying enzymes in human cells. We have also developed strategies for the metabolic incorporation of modified ribonucleosides into cellular RNA based upon overexpression and engineering of kinases in the nucleotide salvage pathway, which has led to new methods for live live-cell RNA imaging and for monitoring RNA synthesis and turnover. Finally, we have developed a small-molecule controlled RNA editing strategy to decipher cellular RNA-protein interactions, which is the subject of this proposal. I am well positioned to lead this multi-disciplinary proposal due to my expertise in RNA chemical biology, and my laboratory's track record of developing and applying cutting-edge approaches at the interface of chemistry and biology to address challenging questions in RNA biology and biomedical research.

Ongoing and recently completed projects that I would like to highlight include:

R01 GM132189
Kleiner (PI)
4/1/2019-3/31/2024
Chemical Approaches to Illuminate the Epitranscriptome

NSF CAREER MCB 1942565
Kleiner (PI)
12/15/12/15/2019-11/30/2024
A Chemoproteomic Strategy to Decipher Epitranscriptomic Pyrimidine Modifications

Gordon and Betty Moore Foundation
Kleiner (Co-PI)
1/1/2019-12/31/2022
Electron Transfer Through Entrained DNA Strands

Sloan Foundation Research Fellowship
Kleiner (PI)
9/15/2019-9/14/2021
Chemical Approaches to Illuminate Nucleic Acid Biology

Citations:

1. Wang D, Shalamberidze A, Arguello AE, Purse B, Kleiner RE. (2022) Live-cell RNA imaging with metabolically incorporated fluorescent nucleosides. J Am Chem Soc. 144, 14647-14656. PMID: 35930766
2. Arguello AE, Li A, Sun X, Eggert TW, Mairhofer E, Kleiner RE. (2022) Reactivity-dependent profiling of RNA 5-methylcytidine dioxygenases. Nat Commun. 3, 4176. PMID: 35853884
3. Dai W, Li A, Yu NJ, Nguyen T, Leach RW, Wuhr M, Kleiner RE. (2021) Activity-based RNA-modifying enzyme probing reveals DUS3L-mediated dihydrouridylation. Nat Chem Biol. 17, 1178-1187. PMID: 34556860
4. Zhang Y, Kleiner RE. (2019) A Metabolic Engineering Approach to Incorporate Modified Pyrimidine Nucleosides into Cellular RNA. J Am Chem Soc. 141, 3347-3351. PMID: 30735369

B. Positions and Honors

Positions and Employment

2005-2011	Graduate Research Fellow and Teaching Assistant, Harvard University, Cambridge, MA
2011-2014	Damon Runyon Postdoctoral Fellow, The Rockefeller University, New York, NY
2014-2016	Revson Foundation Biomedical Fellow, The Rockefeller University, New York, NY
2016-present	Assistant Professor of Chemistry, Princeton University, Princeton, NJ
2017-present	Associated Faculty in Molecular Biology, Princeton University, Princeton, NJ

Honors

2012	Damon Runyon Cancer Research Foundation Postdoctoral Fellowship
2014	Revson Foundation Fellowship in Biomedical Science
2016	Damon Runyon Dale F. Frey Award for Breakthrough Scientists
2017	Sidney Kimmel Foundation Scholar Award
2019	Alfred P. Sloan Foundation Research Fellow
2019	National Science Foundation CAREER award
2023	Kavli Fellow

C. Contribution to Science

1. My laboratory has developed a reactivity-based approach to profile RNA modifying enzymes in their native context, known as RNA-mediated activity-based protein profiling (RNABPP). We have applied this strategy to characterize multiple classes of pyrimidine-modifying enzymes in human cells enabling the profiling of m^5C and m^5U methyltransferases, m^5C dioxygenases, and dihydrouridine synthases (DUS). Our work provides a new approach for studying RNA modifying enzymes in their native context and sheds new light on the biological role and distribution of multiple RNA modification in mammals. I supervised this work.

    a. Arguello AE, Li A, Sun X, Eggert TW, Mairhofer E, Kleiner RE. Reactivity-dependent profiling of RNA 5-methylcytidine dioxygenases. Nat Commun. 2022 Jul 19; 13(1):4176. doi: 10.1038/s41467-022-31876-2. PMID: 35853884

b. Li A, Sun X, Arguello AE, Kleiner RE. Chemical Method to Sequence 5-Formylcytosine on RNA. ACS Chem Biol. 2022 Mar 18; 17(3):503-508. doi: 10.1021/acschembio.1c00707. Epub 2022 Feb 25. PMID: 35212224.

c. Dai W, Li A, Yu NJ, Nguyen T, Leach RW, Wuhr M, Kleiner RE. Activity-based RNA-modifying enzyme probing reveals DUS3L-mediated dihydrouridylation. Nat Chem Biol. 2021 Nov 17(11):1178-1187. doi: 10.1038/s41589-021-00874-8. Epub 2021 Sep 23. PMID: 34556860

2. My laboratory has developed an approach for incorporating artificial nucleosides into cellular RNA. We performed protein engineering on enzymes in the pyrimidine salvage pathway to alter their substrate specificity and enable the incorporation of azido-containing nucleosides into cellular RNA. Our work provides new approaches for studying RNA synthesis and turnover in living cells and a general approach for the incorporation of modified pyrimidine nucleosides into RNA. I supervised this work.

   a. Wang D, Shalamberidze A, Arguello AE, Purse B, Kleiner RE. Live-cell RNA imaging with metabolically incorporated fluorescent nucleosides. J Am Chem Soc. 2022 Aug 17; 144(32):14647-14656. Pubmed PMID: 35930766.

   b. Wang D, Zhang Y, Kleiner RE. Cell- and Polymerase-Selective Metabolic Labeling of Cellular RNA with 2'-Azidocytidine. J Am Chem Soc. 2020 Aug 26;142(34):14417-14421. PubMed Central PMCID: PMC7720414.

   c. Zhang Y, Kleiner RE. A Metabolic Engineering Approach to Incorporate Modified Pyrimidine Nucleosides into Cellular RNA. J Am Chem Soc. 2019 Feb 27;141(8):3347-3351. PubMed PMID: 30735369.

3. My laboratory has developed a chemical proteomics approach to profile 'readers' of modified RNA. We applied this strategy to investigate proteins that interact with N^6-methyladenosine (m^6A)-modified RNA in human cells thereby discovering new m^6A 'readers' as well as proteins that are repelled by this modification. Our findings generated novel biochemical hypotheses for the role of m^6A in cells and provide a powerful tool to study other RNA modifications. We have also extended our strategy to characterize readers of N^1-methyladenosine (m^1A) as well as to profile m^6A readers in D. melanogaster. I supervised this work.

   a. Kan L, Ott S, Joseph B, Park ES, Dai W, Kleiner RE, Claridge-Chang A, Lai EC. A neural m6A/Ythdf pathway is required for learning and memory in Drosophila. Nat Commun. 2021 Mar 5;12(1):1458. PubMed Central PMID: 33674589

   b. Seo KW, Kleiner RE. YTHDF2 Recognition of N^1-Methyladenosine (m^1A)-Modified RNA Is Associated with Transcript Destabilization. ACS Chem Biol. 2020 Jan 17;15(1):132-139. PubMed Central PMCID: PMC7025767.

   c. Arguello AE, Srikumar T, Kleiner RE. A Photocrosslinking-Based RNA Chemical Proteomics Approach to Profile m^6 A-Regulated Protein-RNA Interactions. Curr Protoc Nucleic Acid Chem. 2018 Dec;75(1):e69. PubMed PMID: 30408339.

   d. Arguello AE, DeLiberto AN, Kleiner RE. RNA Chemical Proteomics Reveals the N^6-Methyladenosine (m^6A)-Regulated Protein-RNA Interactome. J Am Chem Soc. 2017 Dec 6;139(48):17249-17252. PubMed PMID: 29140688.

4. My laboratory has developed a promixity labeling strategy based upon small-molecule controlled RNA editing with adenosine deaminase enzymes (ADAR) in order to characterize dynamic RNA-protein interactions in living cells. We applied this approach to characterize RNA substrate interactions by the stress granule-localized protein G3BP1 during normal conditions and upon oxidative stress. I supervised this work.

   a. Seo KW, Kleiner RE. Profiling dynamic RNA-protein interactions using small molecule-induced RNA editing. *bioRxiv* https://doi.org/10.1101/2022.06.30.498348

5. As a postdoctoral fellow, I developed chemical proteomics methods to study protein-protein interactions, with a particular emphasis on phosphorylation-dependent interactions involved in the DNA damage response. This work led to the identification of a new pathway for the repair of DNA double-strand breaks in heterochromatin and provided methods for characterizing protein-protein interactions in cells. I led these projects.

    a. Kleiner RE, Hang LE, Molloy KR, Chait BT, Kapoor TM. A Chemical Proteomics Approach to Reveal Direct Protein-Protein Interactions in Living Cells. Cell Chem Biol. 2018 Jan 18;25(1):110-120.e3. PubMed Central PMCID: PMC5775914.
    b. Kleiner RE, Verma P, Molloy KR, Chait BT, Kapoor TM. Chemical proteomics reveals a γH2AX-53BP1 interaction in the DNA damage response. Nat Chem Biol. 2015 Oct;11(10):807-14. PubMed Central PMCID: PMC4589150.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1vCbfkvaSSfkV/bibliography/public/