BIOGRAPHICAL SKETCH

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NAME: Ralph E. Kleiner

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

POSITION TITLE: Associate 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 developed chemical biology strategies to investigate epitranscriptomic RNA modifications, RNA-binding proteins, and RNA dynamics. We have developed a chemoproteomic platform for profiling 'readers' of RNA modifications and characterized the effect of the mRNA modifications N^6-methyladenosine (m^6A) and N^1-methyladenosine (m^1A) on cellular protein-RNA interactions. We have also developed a small molecule-controlled RNA editing strategy, TRIBE-ID, to characterize dynamic RNA-protein interactions and biomolecular condensates in cells. Finally, a number of projects in the group rely upon the development and application of novel RNA metabolic labeling strategies based on the use of modified ribonucleosides and perturbations to enzymes in the nucleotide salvage pathway. These include strategies for live-cell RNA imaging, RNA synthesis and turnover monitoring, RNA structure probing with temporal resolution, and activity-based probing of RNA modifying enzymes. The subject of this proposal is based upon our work using activity-based probes to generate mechanism-based crosslinks between RNA modifying enzymes and their substrates. 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 GM152748
Kleiner (PI)
9/21/2024-8/31/2028
An RNA editing platform to investigate the dynamic RNA interactome

R01 GM132189
Kleiner (PI)
4/1/2019-3/31/2025
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

Citations:

1. Seo KW, Kleiner RE. (2023) Profiling dynamic RNA-protein interactions using small-molecule-induced RNA editing. Nat Chem Biol. 11, 1361-1371. PMID: 37349582
2. 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
3. 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
4. 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

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2024-present	Associate Professor of Chemistry, Princeton University, Princeton, NJ
2023-present	Associated Faculty, Omenn-Darling Bioengineering Institute, Princeton University, Princeton, NJ
2022-present	Consultant, Alida Biosciences, San Diego, CA
2017-present	Associated Faculty in Molecular Biology, Princeton University, Princeton, NJ
2016-present	Assistant Professor of Chemistry, Princeton University, Princeton, NJ
2014-2016	Revson Foundation Biomedical Fellow, The Rockefeller University, New York, NY
2011-2014	Damon Runyon Postdoctoral Fellow, The Rockefeller University, New York, NY
2005-2011	Graduate Research Fellow and Teaching Assistant, Harvard University, Cambridge, MA

Honors

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

C. Contributions 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. Yu NJ, Dai W, Li A, He M, Kleiner RE. Cell type-specific translational regulation by human DUS enzymes. bioRxiv. 2023 Nov 8:2023.11.03.565399. doi: 10.1101/2023.11.03.565399. PMID: 37965204.

    b. Ji J, Yu, NJ, Kleiner RE. Sequence- and Structure-Specific tRNA Dihydrouridylation by hDUS2. ACS Cent Sci. 2024 Mar 12; 10(4):803-812. Doi: 10.102/acscentsci.3c01382. PMID: 38680565.

    c. 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

    d. 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 diverse modified nucleosides into cellular RNA. Our work provides new approaches for studying RNA synthesis and turnover, RNA imaging, and RNA structure probing with temporal resolution. I supervised this work.

    a. Wang D, Tang Y, Li A, Sun L, Kleiner RE. Transcriptome-wide RNA structure probing with temporal resolution. bioRxiv. 2023 doi: https://doi.org/10.1101/2023.09.28.560059.

    b. 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. PMID: 35930766.

    c. 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. PMID: 32786764.

    d. 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. PMID: 30735369.

3. My laboratory has developed an RNA-protein interactomics strategy based upon small-molecule controlled RNA editing with adenosine deaminase enzymes (ADAR) to characterize dynamic RNA-protein interactions in living cells. We applied this approach to study RNA substrate interactions by the stress granule-localized protein G3BP1 during normal conditions and upon oxidative stress-mediated biomolecular condensation. We found that G3BP1 stabilizes its substrate RNAs. Our platform is broadly applicable to study dynamic RNA-protein interactions in cells. I supervised this work.

    a. Seo KW, Kleiner RE. (2023) Profiling dynamic RNA-protein interactions using small-molecule-induced RNA editing. Nat Chem Biol. 19, 1361-1371. PMID: 37349582

4. 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. Recent work has included studies of 8-oxoguanosine (8OG)-sensitive RNA-RBP interactions. I supervised this work.

a. Villers J, McCann Smith E, DeLiberto AN, Arguello AE, Nyaanga J, Kleiner RE. Chemoproteomic profiling of 8-oxoguanosine-sensitive RNA-protein interactions. Biochemistry. 2023 Dec 5;62(23):3411-3419. PMID: 38010074

b. 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. PMID: 33674589

c. 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. PMID: 31815430.

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. PMID: 29140688.

5. My laboratory has studied the mechanism of action of the chemotherapeutic Pt drug oxaliplatin. We uncovered a new synergy between the ability of oxaliplatin to induce nucleolar stress and inhibit RNA pol I transcription in the nucleolus and its activation of the DNA damage response (DDR) through ATM/ATR kinases. Our work informs further development of Pt drugs and mechanistic links between DDR signaling and the nucleolus. I supervised this work.

   a. Nechay M, Wang D, Kleiner RE. Inhibition of nucleolar transcription by oxaliplatin involves ATM/ATR kinase signaling. Cell Chem Biol. 2023, 30, 906-919. PMID: 37433295.

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

BIOGRAPHICAL SKETCH

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NAME: Jonathan Bouvette

ORCID: https://orcid.org/0000-0003-3550-5319

POSITION TITLE: Manager of Biomolecular Electron Microscopy

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
University of Montreal, Montreal, Canada	B.Sc	05/2010	Biochemistry
University of Montreal, Montreal, Canada	Ph.D	09/2018	Biochemistry

A. Personal Statement

As core facility manager in Biomolecular Electron Microscope, I specialize in Cryo-Electron Microscopy and Tomography (Cryo-EM and Cryo-ET). In this capacity, I advise students, postdocs and professors on their projects with the aim of answering their specific biological question efficiently. With a robust background in biochemistry, system administration and programming, I am involved from project conceptualization, sample preparation and optimization, data acquisition and processing. I successfully managed multiple projects and contributed to the Cryo-EM aspects in multiple publications. My research focuses on method development in Cryo-EM and Cryo-ET, aiming to lower the barrier of entry in Cryo-EM and Cryo-ET through automation. These projects have fostered multiple international connections with both academic and industrial partners with whom I am actively collaborating. I am driven in using my expertise and network to push as many projects as possible to success.

B. Positions, Scientific Appointments, and Honors

Positions

2023-Present Manager of Biomolecular Electron Microscopy, Department of Molecular Biology, Princeton University, Princeton, NJ

2018-2023 Postdoctoral Fellow, National Institute of Environmental Health Sciences (NIEHS), NIH, Research Triangle Park, NC

Honors

2022 Microscopy and Microanalysis, Robert P. Apkarian Memorial Scholarship

C. Contributions to Science

1. My main project in Cryo-Electron microscopy (Cryo-EM) method development is the SmartScope software. SmartScope revolutionizes cryo-EM by automating and standardizing specimen evaluation, eliminating tedious manual screening. Powered by advanced deep-learning technology, SmartScope identifies and classifies imaging-ready features with precision, streamlining your workflow and ensuring

consistent results.With its intuitive web interface, SmartScope provides real-time remote control of your microscope and access to annotation tools. Plus, manual annotations can be integrated to continuously improve performance.Designed to bring cryo-EM to the non-microscopist, SmartScope lowers the barrier of adoption, making this powerful technology accessible and efficient for researchers at all levels. SmartScope is free and open-source, empowering the scientific community to collaborate and innovate together.

   a. **Bouvette J**, Huang Q, Riccio AA, Copeland WC, Bartesaghi A, Borgnia MJ (2022). Automated systematic evaluation of cryo-EM specimens with SmartScope. eLife, https://doi.org/10.7554/eLife.80047

2. BISECT introduced a transformative approach to cryo-tomography, setting a new standard by demonstrating for the first time that it is possible to acquire data with beam-image-shift with high-resolution information preserved. This method expands the number of areas imaged at each stage position and incorporating geometrical constraints for precise targeting which accelerates data collection speed by up to an order of magnitude.Moreover, our novel per-tilt astigmatic CTF estimation and data-driven exposure weighting methodologies further elevate the final map resolution, enabling clear resolution of individual side chains in low molecular weight targets (~300 kDa) at 3.6 Å resolution. BISECT represents a substantial advancement in cryo-tomography methodologies. Since publication, similar algorithm was added to the Thermo Fisher software Tomo5 and improved by the PACEtomo package.
   a. **Bouvette J**, Liu H, Du X, Zhou Y, Sikkema AP, Da Fonseca J, Klemm B, Huang RK, Schaaper RM, Borgnia MJ, Bartesaghi A (2021). Beam image-shift accelerated data acquisition for near-atomic resolution single-particle cryo-electron tomography. Nature Communications. 12:1957

3. My general expertise in Cryo-EM has led me to make contribution to multiple projects. I have mainly helped and advised in the main steps of the Cryo-EM pipeline: sample optimization, data collection and data processing.
   a. Riccio AA, Brannon AJ, Krahn JB, **Bouvette J**, Williams JG, Borgnia MJ, Copeland WC (2024) Coordinated DNA polymerization by Polγ and the region of LonP1 regulated proteolysis, Nucleic Acids Research, gkae539, https://doi.org/10.1093/nar/gkae539
   b. Appel DC, Bermek O, Dandey VP, Wood M, Viverette E, Williams JG, **Bouvette J**, Riccio AA, Krahn, JM, Borgnia MJ, Williams SR (2023). Sen1 architecture: RNA-DNA hybrid resolution, autoregulation, and insights into SETX inactivation in AOA2, Molecular Cell, doi:10.1016/j.molcel.2023.09.024
   c. Riccio AA, **Bouvette J**, Krahn JM, Perera P, Williams JG, Longley MJ, Dutcher R, Borgnia MJ, Copeland W. C. (2022). Structural insight and characterization of Human Twinkle Helicase in mitochondrial disease. PNAS, 119 (32) e2207459119.
   d. Riccio AA, **Bouvette J**, Longley MJ, Krahn JM, Borgnia MJ, Copeland WC (2022). Method for the structural analysis of Twinkle mitochondrial DNA helicase by cryo-EM, Methods 2022 Jun 30;S1046-2023(22)00152-9.
   e. Sim√µes V, Harley L, Cizubu BK, Zhou YE, Pajak J, Snyder NA, **Bouvette J**, Borgnia MJ, Arya G, Bartesaghi A, Silva GM (2022) Redox sensitive E2 Rad6 controls cellular response to oxidative stress via K63 ubiquitination of ribosomes, Cell Rep. 2022 May 24;39(8):110860.
   f. Kwon DH, Zhang F, Suo Y, **Bouvette J**, Borgnia MJ, Lee SY (2021) Heat-dependent opening of TRPV1 in the presence of capsaicin, Nature Structural and Molecular Biology. 28(7):554-563
   g. Zhou Y, Kastritis PL, Dougherty SE, **Bouvette J**, Hsu AL, Burbaum L, Mosalaganti S, Pfeffer S, Hagen WJH, Förster F, Borgnia MJ, Vogel C, Beck M, Bartesaghi A, Silva GM (2020). Structural impact of K63 ubiquitin on yeast translocating ribosomes under oxidative stress. PNAS, 117(36), 22157–2216

4. During my Ph.D. I have worked on RNA-protein interaction. Mainly in the micro-RNA maturation pathway. I have contributed to new methods in the field to improve purification methods for biochemical studies as well as key contribution in the micro-RNA maturation regulatory pathways.
   a. Dadhwal G, Sam H, **Bouvette J**, El-Azzouzi F, Dagenais P, Legault P (2024). Substrate promiscuity of Dicer toward precursors of the let-7 family and their 3'-end modifications, Cell Mol Life Sci, 23;81(1):53. doi:10.1007/s00018-023-05090-2

b. **Bouvette J**, Korkut DN, Fouillen A, Amellah S, Nanci A, Durocher Y, Omichinski JG, Legault P (2018). "High-yield production of human Dicer by transfection of human HEK293-EBNA1 cells grown in suspension." BMC Biotechnol. 18(1):76
c. Di Tomasso G, Salvail-Lacoste A, **Bouvette J**, Omichinski JG, Legault P (2014). "Affinity purification of in vitro transcribed RNA with homogeneous ends using a 3'-ARiBo tag." Methods Enzymol 549: 49-84.
d. Desjardins A, **Bouvette J**, Legault P. (2014) "Stepwise assembly of multiple Lin28 proteins on the terminal loop of let-7 miRNA precursors" Nucleic Acids Res 42(7): 4615-4628.
e. Desjardins A, Yang A, **Bouvette J**, Omichinski JG, Legault P (2012) "Importance of the NCp7-like domain in the recognition of the let-7g precursor miRNA by the pluripotency factor Lin28" Nucleic Acids Research. 40(4): 1767-177