BIOGRAPHICAL SKETCH

NAME: Bintu, Lacramioara

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

POSITION TITLE: Associate Professor of Bioengineering

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
Brandeis University, Waltham, MA	B.S.	05/2005	Physics, Mathematics, Neuroscience
University of California, Berkeley, CA	Ph.D.	12/2010	Physics
University of California, Berkeley, CA	Postdoctoral	06/2011	Quantitative Biology
California Institute of Technology, Pasadena, CA	Postdoctoral	12/2016	Biological Engineering

A. Personal Statement

The research goals of my lab are to uncover **general principles** of **gene and chromatin regulation** in human cells, especially during **innate immune responses**, and to build **better synthetic tools** for controlling gene expression and cell signaling. We use a host of experimental and mathematical methods:

- **High-throughput synthetic biology** with readouts based on next generation sequencing. These methods allow us to test the function of 10,000-100,000 of protein and DNA sequences inside living human cells in order to arrive at general principles.
- **Single-cell methods:** fluorescence microscopy imaging (including live time-lapse imaging of and in situ barcode sequencing), flow cytometry, and single-molecule footprinting (SMF) of accessible DNA. These methods allow us to analyze cellular responses that are heterogeneous, and test protein and DNA libraries with readouts on sub-cellular localization (imaging) or individual nucleosome and transcription factor occupancy on DNA (SMF).
- **Mathematical models** of gene regulation and cell signaling: kinetic models and analytic solutions to differential equations, stochastic simulations, and machine learning. We can use these models to analyze and predict performance of **synthetic biology parts and circuits**.

Building on these discoveries, we are developing **new tools for mammalian synthetic biology** with a focus on gene control and signal integration. Examples include fusions between programmable DNA binding domains and compact transcriptional effectors, and synthetic circuits that can measure and record temporal signals.

This interdisciplinary quantitative research is powered by my group of students, research assistants, and postdocs with diverse training in Physics, Computer Science, Biochemistry, Genetics, Biology, Bioengineering, and Medicine. My own training positions me ideally to lead an interdisciplinary team focused on these research goals. In addition to the research goals, I am committed to teaching and mentoring, with a focus on molecular and cellular engineering, effective communication, and collaborative science. Our research training, together with collaborations with Stanford colleagues from both engineering and medicine, and access to Stanford's technological resources, is enabling my lab to make major contributions to gene and chromatin regulation, functional genomics, and mammalian synthetic biology.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2025-present Associate Professor, Department of Bioengineering, Stanford University, Stanford, CA

2017-2025 Assistant Professor, Department of Bioengineering, Stanford University, Stanford, CA

2011-2016 Postdoctoral Fellow with Michael Elowitz, California Institute of Technology, Pasadena, CA

2011 Postdoctoral Fellow with Carlos J. Bustamante, QB3, University of California Berkeley, Berkeley, CA

2006-2010 Graduate Student Researcher with Carlos J. Bustamante, University of California, Berkeley, Berkeley, CA

2005-2006 Graduate Student Instructor, University of California Berkeley, Berkeley, CA

2004-2005 Research Assistant, with Jané Kondev, Brandeis University, Waltham, MA

2004, 2005 Research Assistant, with Rob Phillips, California Institute of Technology, Pasadena, CA

2003 Science Teacher in TOPS (Teaching Opportunities in Physical Sciences), MIT & Harvard

2002 Undergraduate Teaching Assistant for Modern Physics, Brandeis University, Waltham, MA

Other Professional Experience and Memberships

2021-present Member, Genome Technology Development Working Group, NHGRI, NIH

2022-present Co-founder and Scientific Advisor, Stylus Medicine

2021-2025 Co-chair of Imaging Working Group, 4D Nucleome, NIH

2022-2025 Co-chair of Single-Cell 4DNsc4ALL Working Group, 4D Nucleome, NIH

2021-2025 Member, Integrating Imaging and Omics Working Group, 4D Nucleome, NIH

2021-2024 Member, Real-time Chromatin Dynamics and Function Interest Group, 4D Nucleome, NIH

2024 Conference co-chair, Keystone Single-Cell Biology: Tissue Genomics, Technologies and Disease

2024 Conference co-chair, Quantitative biology to molecular mechanisms at EMBL Heidelberg

2024 Member of planning committee for the Advances in Genomic Technology Development (AGTD) Annual Meeting, through NHGRI, NIH

2019, 2022,2024 Conference organizer, International Mammalian Synthetic Biology Workshop

2018-2024 Conference organizer, International Conference on Epigenetics and Bioengineering (EpiBio), co-chair of organizing committee 2018&2022

2021 Member of the Planning Committee for the 4D Nucleome NIH Annual Meeting

2020 NIH Peer Review Committee: 4D Nucleome Program, ad hoc reviewer

2018-Present Member, Society for Biological Engineering

2008-Present Member, American Society for Cell Biology

Honors

2017-present Maximizing Investigators' Research Award (MIRA), NIGMS, NIH

2015-2020 Career at the Scientific Interface Award, Burroughs Wellcome Fund

2011-2014 Jane Coffin Childs Postdoctoral Fellowship

2011-2104 Beckman Institute Fellowship for equipment, California Institute of Technology

2011 Harold M. Weintraub Graduate Student Award for outstanding achievement during graduate studies, Fred Hutchinson Center

2006 Outstanding Graduate Student Instructor Award, University of California, Berkeley

2005 Doris Brewer Cohen Endowment Award for best senior thesis, Brandeis University

2005 Molly W. and Charles K. Schiff Memorial Award in Science, Brandeis University

2001-2005 Wien International Scholarship, Brandeis University

2004 Elihu A. Silver Prize for undergraduate research in science, Brandeis University

C. Contributions to Science

1. We developed new high-throughput methods for measuring and controlling gene expression and used them to discover and characterize new activation and repression domains from thousands of human and viral gene regulators. In collaboration with the Bassik lab, we developed an assay in which pooled libraries of protein domains are recruited to a reporter gene and transcriptional effects are measured with a sequencing readout. This approach has enabled us to measure transcriptional activation and repression strength for the majority of Pfam annotated domains from nuclear proteins, perform a deep mutational screen of the KRAB domain from CRISPRi – resulting in a mutant with increased stability and silencing, and discover new repressor and activator domains as short as 10 amino acids. We used our high-throughput approach to annotate activation and repression domains across most known human transcription factors and chromatin regulators (~2,000 proteins) and ~1500 viral proteins (including all proteins from human herpesviruses), and performed systematic mutations to extract the sequence rules necessary for their function. Additionally, we discovered a set of bifunctional domains that can both activate and repress transcription. Together, these results establish a compendium of human and viral transcriptional effector domains and their sequence determinants, accelerating research across diverse areas, including gene regulation, developmental biology, immunology, and systems and synthetic biology. In addition, the methods we developed can be readily extended to study other processes with established fluorescent reporters: DNA repair, RNA regulation, or protein signaling.

    a. Tycko J, DelRosso N, Hess GT, Aradhana, Banerjee A, Mukund A, Van MV, Ego BK, Yao D, Spees K, Suzuki P, Marinov GK, Kundaje A, Bassik MC†, Bintu L†. (2020). High-throughput discovery and characterization of human transcriptional effectors. *Cell*, S0092-8674(20)31541-5. PMCID: PMC8178797.

    b. DelRosso N, Tycko J, Suzuki P, Andrews C, Aradhana FN, Mukund A, Liongson I, Ludwig C, Spees K, Fordyce P, Bassik MC, Bintu L. (2023) Large-scale mapping and mutagenesis of human transcriptional effector domains. *Nature 616, 365–372.* PMCID: PMC10484233.

    c. Ludwig CH, Thurm AR, Morgens DW, Yang KJ, Tycko J, Bassik MC, Glaunsinger BA, Bintu L. (2023) High-throughput discovery and characterization of viral transcriptional effectors in human cells. *Cell Systems*, 14(6), 482-500. PMCID: PMC10350249.

    d. Tycko J*, Van MV*, Aradhana, DelRosso N, Yao D, Xu X, Ludwig C, Spees K, Liu K, Hess GT, Gu M, Mukund AX, Suzuki PH, Kamber RA, Qi LS, Bintu L#, Bassik MC#. Development of compact transcriptional effectors using high-throughput measurements in diverse contexts. *Nature Biotechnology*, doi: 10.1038/s41587-024-02442-6 Epub 2024 Nov 1.

2. We used single-cell fluorescence imaging measurements, and mathematical modeling to show that recruitment of different chromatin regulators at a target gene controls its expression in an all-or-none, recording the duration of a signal as the fraction of cells in the population that silence the gene transiently or permanently. Our single-cell measurements of gene expression as a function of time allowed us to propose a unified model of epigenetic control. In this model, chromatin regulation can move a gene among three states: active, reversibly silent, and irreversibly silent. Different chromatin regulators are associated with different rates between the three states, and as such allow for different types of signal integration. We have recently shown that compact nanobodies against endogenous chromatin regulators can also be used to silence gene expression and induce epigenetic memory, and their behavior can be described using the same 3-state model. For example, an antiDNMT1 nanobody works in synthetic circuits as a timer: it takes the duration of a signal and converts it linearly to a fraction of cells in the population that silence the target gene; because of its good epigenetic memory mediated by DNA methylation, it also records the signal to memory. Our mathematical model can accurately predict the behavior of this compact timer even across pulsed signals. Our systematic single-cell experimental approach and theoretical framework provide a useful way of thinking about chromatin regulators, especially in the context of synthetic mammalian engineering and systems biology. I started this work while I was a postdoc with Michael Elowitz (Caltech), and continued it in my lab.

a. Bintu, L.*, Yong, J.*, Antebi, Y.E., McCue, K., Kazuki, Y., Uno, N., Oshimura, M., Elowitz, M.B. (2016). Dynamics of epigenetic regulation at the single-cell level. *Science,* 351(6274):720-4. PMCID: PMC5108652.

b. Van MV, Fujimori T, Bintu L. (2021). Nanobody-mediated control of gene expression and epigenetic memory. *Nature Communications*, 12(1):1-2. PMCID: PMC7822885.

c. Mukund A, Bintu L. (2022). Temporal signaling, population control, and information processing through chromatin-mediated gene regulation. *Journal of Theoretical Biology* Feb 21;535:110977. doi: 10.1016/j.jtbi.2021.110977. PMCID: PMC8757591.

d. Mukund AX, Tycko J, Allen SJ, Robinson SA, Andrews C, Ludwig CH, Spees K, Bassik MC, Bintu L. (2023) High-throughput functional characterization of combinations of transcriptional activators and repressors. *Cell Systems*, Aug 2:S2405-4712(23)00186-2. PMCID: PMC10642976.

3. We measured the dynamics of chromatin-mediated spreading of gene silencing, activation and epigenetic memory in single cells as a function of time. These data showed that KRAB-mediated silencing spreads between neighboring genes very fast, in a distance dependent manner. KRAB is repressive domain commonly used in perturbation tools such as CRISPRi. We also showed that genetic insulators, together with promoters, act as dynamic elements associated with increased rates of gene reactivation rather than acting as absolute barriers against heterochromatin spreading. As a result, even though insulators can slow down background transgene silencing, they are not efficient in preventing spreading of silencing associated with a strong repressor such as KRAB that engages positive feedback through histone methylation. This work is important for understanding transgene gene silencing, building multi-gene mammalian synthetic circuits, and interpreting data from perturbation tools such as CRISPRi, as highlighted in the perspective we wrote with many members of the international mammalian synthetic biology community (Cabera et al below).

   a. Lensch S, Herschl MH, Ludwig CH, Sinha J, Hinks MH, Mukund A, Fujimori T, Bintu L. (2022). Dynamic spreading of chromatin-mediated gene silencing and reactivation between neighboring genes in single cells. *eLife*, *11*, p.e75115. PMCID: PMC9183234.

   b. Sinha J, Nickels JF, Thurm AR, Ludwig CH, Archibald BN, Hinks MM, *Wan J*, Fang D, Bintu L. The H3.3 K36M oncohistone disrupts the establishment of epigenetic memory through loss of DNA methylation, *Molecular Cell*, 2024 Oct 17;84(20):3899-915. PMCID: PMC39368466

   c. Cabera A*, Edelstein HI*, Glykofrydis F*, Love KS*, Palacios S*, Tycko J*, Zhang M*, Lensch S, Shields CE, Livingston M, Weiss R. Zhao H, Haynes KA, Morsut L, Chen YY, Khalil AS, Wong WW, Collins JJ, Rosser SJ, Polizzi K, Elowitz MB, Fussenegger M, Hilton IB, Leonard JN, Bintu L, Galloway KE, Deans TL. The sound of silence: Transgene silencing in mammalian cell engineering (2022). *Cell Systems*, 13(12):950-73. PMCID: PMC9880859.

   d. *Fujimori T*, Rios-Martinez C, Thurm AR, Hinks MM, Doughty BR, Sinha J, Le D, Hafner A, Greenleaf WJ, Boettiger AN, Bintu L. Single-cell chromatin state transitions during epigenetic memory formation, bioRxiv 2023. PMCID: PMC10592931.

4. I used in vitro single-molecule methods and kinetic mathematical modeling to determine the direct effect that nucleosomes have on transcription as well as the effect that transcription has on nucleosomes *in vitro*. We found that the polymerase acts as a ratchet, waiting for the nucleosomal DNA to fluctuate free from histones in order to advance. The fate of the histones depends on the speed of the polymerase: for slow polymerases, the histones can be transferred behind the polymerase via a DNA loop; fast polymerases ratchet their way through so quickly that the transfer fails to happen, and the histone octamer dissociates from DNA. These findings act as a knowledge scaffold for the more complex *in vivo* scenarios that involve a larger number of transcription factors and chromatin regulators. I performed this research as a PhD student in Carlos Bustamante's Laboratory at the University of California, Berkeley.

   a. Hodges, C.*, Bintu, L.*, Lubkowska, L., Kashlev, M., Bustamante, C. (2009). Nucleosomal fluctuations govern the transcription dynamics of RNA polymerase II. *Science*, *325*(5940), 626-628. PMCID: PMC2775800.

b. Bintu, L.*, Kopaczynska, M.*, Hodges, C., Lubkowska, L., Kashlev, M., Bustamante, C. (2011). The elongation rate of RNA polymerase determines the fate of transcribed nucleosomes. *Nature structural & molecular biology*, *18*(12), 1394-1399. PMCID: PMC3279329.

c. Zamft, B., Bintu, L., Ishibashi, T., Bustamante, C. (2012). Nascent RNA structure modulates the transcriptional dynamics of RNA polymerases. *Proceedings of the National Academy of Sciences*, *109*(23), 8948-8953. PMCID: PMC3384149.

d. Bintu, L.*, Ishibashi, T.*, Dangkulwanich, M., Wu, Y.Y., Lubkowska, L., Kashlev, M., Bustamante, C. (2012). Nucleosomal elements that control the topography of the barrier to transcription. *Cell*, *151*(4), 738-749. PMCID: PMC3508686.

5. Developed biophysical models for predicting gene expression based on concentrations and binding affinities of transcription factors, and tested them using single-molecule footprinting. I started modeling gene expression using statistical mechanics while I was an undergraduate in the groups of Jané Kondev (Brandeis University) and Rob Phillips (Caltech); this work is now included in the Physical Biology of the Cell textbook. Recently, in collaboration with the Greenleaf lab, we have extended these statistical mechanical models to include nucleosomes, and connected them to kinetics of gene activation. We explicitly tested these models by measuring the probability of individual microstates using single-molecule footprinting of transcription factors, nucleosomes, and transcription start sites in live cells. We were able to show that transcription factors cooperate in their ability to bind and drive transcription by recruiting chromatin remodelers that decrease nucleosome occupancy at nearby sites, including other transcription factor binding sites. More importantly, being able to accurately measure individual microstates in the partition function associated with different DNA states enables us to develop a physics-based predictable framework for gene regulation.

   a. Bintu, L., Buchler, N. E., Garcia, H. G., Gerland, U., Hwa, T., Kondev, J., Phillips, R. (2005). Transcriptional regulation by the numbers: models. *Current opinion in genetics & development*, *15*(2), 116-124. PMCID: PMC3482385.

   b. Bintu, L., Buchler, N. E., Garcia, H. G., Gerland, U., Hwa, T., Kondev, J., Kuhlman, T., Phillips, R. (2005). Transcriptional regulation by the numbers: applications. *Current opinion in genetics & development*, *15*(2), 125-135. PMCID: PMC3462814.

   c. Doughty BR*, Hinks MM*, Schaepe JM*, Marinov GK, Thurm AR, Rios-Martinez C, Parks BE, Tan Y, Marklund E, Dubocanin D, Bintu L†, Greenleaf WJ†. Single-molecule chromatin configurations link transcription factor binding to expression in human cells. *Nature* 636: 745–754, 2024

Complete List of Published Work in MyBibliography:
https://pubmed.ncbi.nlm.nih.gov/?term=Bintu+L&sort=pubdate