BIOGRAPHICAL SKETCH

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NAME: Ramanathan, Sharad

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POSITION TITLE: Llura and Gordon Gund Professor of Neurosciences and of Molecular and Cellular Biology, Professor of Applied Physics and of Stem cell and Regenerative Biology, Department of Molecular and Cellular Biology, Stem Cell and Regenerative Biology, School of Engineering and Applied Sciences, Harvard University, Cambridge, MA; Faculty Director of the Harvard IPS Core.

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)	END DATE MM/YYYY	FIELD OF STUDY
Indian Institute of Technology, Kanpur, India	MS	1992	Chemistry
Harvard University, Cambridge, MA	MA	1994	Physics
Harvard University, Cambridge, MA	PhD	1997	Chemical Physics
Institute for Theoretical Physics, Santa Barbara, CA	Postdoctoral Fellow	1999	Theoretical Physics

A. Personal Statement

My laboratory has been working in the field of Quantitative Biology since 2008. I was trained as a theoretical physicist and worked as a permanent member in the Theoretical Physics Department at Bell labs before moving my research focus to Biology. My lab brings together techniques from the physical and life sciences to address fundamental questions in Biology. We have developed computational, microscopy, microfluidics, modeling, experimental and theoretical tools to understand how complex molecular networks process signals and make decisions. The work in the lab builds on my expertise in quantitative measurement and modeling methods and focuses on understanding these complex biological circuits through experiments and mathematical modeling.

Relevant to this proposal, **(i)** my lab has established methods to work with human embryonic stem cells as well as human fetal tissue, modified the human genome to build tagged fluorescent stem cell lines for measuring the dynamics of development, standardized *in vitro* differentiation systems, applied single cell genomics, used sequencing in conjunction with viral barcoded libraries for lineage tracing, to uncover the early lineage decisions and the underlying gene regulatory networks during human cortical development (Yao 2017). **(ii)** We established new Bayesian computational techniques to analyze single-cell transcriptomics data from differentiating cells and tissues to infer cell types, sequence of lineage decisions, and critical genes driving these decisions (Furchtgott 2017). **(iii)** We developed a machine learning-based framework to identify how to couple pluripotent stem cell organoids such that every one of them broke anterior-posterior symmetry and developed a tailbud, elongated axially, and patterned to develop a neural tube with a single lumen. We also applied computational methods developed based on our previous work with Melton to map single-cell RNA-Seq data and *in situ* sequencing data from these organoids onto fetal tissue data to validate their cellular composition. This work overcame a critical barrier in stem cell biology: the large organoid-to-organoid variability in morphology and cellular composition, which has prevented the use of genetic perturbations to uncover new biology (Anand 2022). **(iv)** We extended this machine learning-based approach to determine how to differentiate epithelial pluripotent stem cell organoids into axially elongating structures with tailbuds that gave rise to a single lumen posterior neural tube flanked by paraxial mesoderm that then underwent somitogenesis. We mapped the expression data from these organoids onto fetal tissue using single-cell and *in situ* sequencing. We used this system to determine that diffusive FGF signaling governs the anterior movement of NOTCH activity oscillations along the PSM, using a combination of fluorescence imaging, chemical and CRISPR-dCas9-based perturbation, and modeling. For the first time, we could build hundreds of such organoids at a time, both from IPS and embryonic stem cell lines, that differentiated predictably, thus allowing for higher throughput genetic and chemical perturbations to extract the underlying mechanisms (Yaman 2022).

The current proposal further builds on our expertise in stem cell biology, molecular biology, genomics, computational methods, microfluidics, and imaging.

1. Yao Z, Mich JK, Ku S, Menon V, Krostag AR, Martinez RA, Furchtgott L, Mulholland H, Bort S, Fuqua MA, Gregor BW, Hodge RD, Jayabalu A, May RC, Melton S, Nelson AM, Ngo NK, Shapovalova NV, Shehata SI, Smith MW, Tait LJ, Thompson CL, Thomsen ER, Ye C, Glass IA, Kayaks A, Yao S, Phillips JW, Grimley JS, Levi BP, Wang Y, Ramanathan S. A Single-Cell Roadmap of Lineage Bifurcation in Human ESC Models of Embryonic Brain Development. Cell Stem Cell. 2017 Jan 5;20(1):120-134. PubMed Central PMCID: PMC5261831.
2. Furchtgott LA, Melton S, Menon V, Ramanathan S. Discovering sparse transcription factor codes for cell states and state transitions during development Elife. 2017 Mar 15;6. doi: 10.7554/eLife.20488. PubMed PMID: 28296636; PubMed Central PMCID: PMC5352226.
3. Anand GM, Megale HC, Murphy SH, Weis T, Lin Z, He Y, Wang X, Liu J, Ramanathan S. Machine learning directed organoid morphogenesis uncovers an excitable system driving human axial elongation. [PREPRINT] bioRxiv. 2022:2022.05.10.491358. doi: 10.1101/2022.05.10.491358.
4. Yaman YI, Huang R, Ramanathan S. Coupled organoids reveal that signaling gradients drive traveling segmentation clock waves during human axial morphogenesis. [PREPRINT] bioRxiv. 2022:2022.05.10.491359. doi: 10.1101/2022.05.10.491359.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 -	Llura and Gordon Gund Professor of Neurosciences and of Molecular and Cellular Biology, Professor of Applied Physics and of Stem cell and Regenerative Biology, Harvard University, Cambridge, MA; Faculty Director of the IPS Core that serves Harvard University and all the affiliated hospitals, Harvard University, Cambridge, MA
2017 - 2021	Llura and Gordon Gund Professor of Neurosciences and of Molecular and Cellular Biology, Professor of Applied Physics and Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA
2015 - 2017	Llura and Gordon Gund Professor of Neurosciences and of Molecular and Cellular Biology, Professor of Applied Physics, Harvard University,
2013 - 2015	Gordon McKay Professor of Applied Physics and Professor of Molecular and Cellular Biology, Harvard University, Cambridge, MA
2011 - 2018	Visiting Scientist, Allen Brain Institute, Seattle, WA
2008 - 2013	Assistant Professor of Molecular and Cellular Biology and of Applied Physics, Harvard University, Cambridge, MA
2004 - 2008	Member of Technical Staff, Theoretical Physics Dept, Bell Laboratories, Lucent Technologies, Murray Hill, NJ, and Bauer Fellow, Harvard University Cambridge MA.
1999 - 2004	Member of Technical Staff, Theoretical Physics Dept, Bell Laboratories, Lucent Technologies, Murray Hill, NJ
1997 - 1999	1997-1999 Postdoctoral Fellow, University of California, Santa Barbara, Institute for Theoretical Physics, Santa Barbara, CA
1992 - 1997	Graduate Student, Harvard University, Physics Department, Cambridge, MA

C. Contribution to Science

1. Understanding Gene Regulatory Networks: My lab has dissected complex networks to understand how key nodes in the gene regulatory networks process these morphogen signals to make developmental decisions. Our work has focused on early germ layer patterning in mouse and human as well as cortical development in humans. My lab discovered that Oct4 and Sox2 are together key regulators of the earliest developmental decision of pluripotent cells and that their dynamics of expression were predictive of the eventual germ layer fate choice of the cell (Thomson 2011). Using a combination of computational and experimental methods we have uncovered the gene regulatory network underlying early neocortical development, developed new single-cell sequencing techniques to extract intact RNA and DNA from fixed, permeabilized, and antibody-

stained cells, and in doing so, have discovered progenitor cell types highly enriched in the developing human cortex (Thomsen 2016) and uncovered the cell types, sequence of cell fate decisions and gene regulatory network underlying early development of the ventral forebrain giving rise to inhibitory neuron (Close 2017). We also developed novel methods to analyze single-cell data to extract the relevant genes and cell types, a method that underlies several of our computational approaches (Melton, 2021)

Relevant to this proposal: these publications demonstrate our ability to combine computational, genomics, and stem cell biology to uncover mechanisms underlying early human and mouse development.

a. Melton S, Ramanathan S. Discovering a sparse set of pairwise discriminating features in high-dimensional data. Bioinformatics. 2021 Apr 19;37(2):202-212. PubMed Central PMCID: PMC8599814.
b. Close JL, Yao Z, Levi BP, Miller JA, Bakken TE, Menon V, Ting JT, Wall A, Krostag AR, Thomsen ER, Nelson AM, Mich JK, Hodge RD, Shehata SI, Glass IA, Bort S, Shapovalova NV, Ngo NK, Grimley JS, Phillips JW, Thompson CL, Ramanathan S, Lein E. Single-Cell Profiling of an In Vitro Model of Human Interneuron Development Reveals Temporal Dynamics of Cell Type Production and Maturation. Neuron. 2017 Nov 15;96(4):949. PubMed PMID: 29144976.
c. Thomsen ER, Mich JK, Yao Z, Hodge RD, Doyle AM, Jang S, Shehata SI, Nelson AM, Shapovalova NV, Levi BP, Ramanathan S. Fixed single-cell transcriptomic characterization of human radial glial diversity. Nat Methods. 2016 Jan;13(1):87-93. PubMed Central PMCID: PMC4869711.
d. Thomson M, Liu SJ, Zou LN, Smith Z, Meissner A, Ramanathan S. Pluripotency factors in embryonic stem cells regulate differentiation into germ layers. Cell. 2011 Jun 10;145(6):875-89. PubMed Central PMCID: PMC5603300.

2. Signaling pathways and embryonic patterning: We studied how WNT signaling results in the early patterning of the embryo during germ layer differentiation. We were the first to show (my contributions were in modeling, microscopy, and data analysis in this project) that there could be Turing-like mechanisms for patterning early vertebrate embryos (Müller 2012). We determined how pluripotent stem cells respond to signals and make fate decisions. We used a combination of single-cell sequencing, Bayesian ensemble modeling, and perturbation experiments to uncover the sequence of cell states along the developmental trajectory to the different germ layer progenitors (Jang 2017). We were also the first to show that BMP and TGF beta receptors are localized in the pre-gastrulation mouse embryos and human embryonic stem cells, which in conjunction with embryo geometry leads to robust patterning (Zhang 2019). We addressed the question of the competence of stem cells to respond to signals and adopt a fate, and how this competence window is set. Using computational methods and novel cell lines to prospectively identify cells before and after they commit the neural lineage, we uncovered the underlying network that can modulate the competence window to respond to BMP along the neural ectodermal developmental trajectory to differentiate into mesendodermal progenitors. We did so using RNA and ATAC seq, mathematical analysis, time-lapse microscopy, and genetic perturbation experiments (Valcourt 2020). Together with the discovery and characterization of gene regulatory networks, the study of signaling pathways allow us to understand how multipotent cells make fate decisions.

   Relevant to this proposal: these publications show our expertise in the signaling pathways involved in early development, particularly how these signals lead to patterning, as well as in working with different model organisms and being able to perform challenging molecular manipulations in early embryos.

   a. Müller P, Rogers KW, Jordan BM, Lee JS, Robson D, Ramanathan S, Schier AF. Differential diffusivity of Nodal and Lefty underlies a reaction-diffusion patterning system. Science. 2012 May 11;336(6082):721-4. PubMed Central PMCID: PMC3525670.
   b. Jang S, Choubey S, Furchtgott L, Zou LN, Doyle A, Menon V, Loew EB, Krostag AR, Martinez RA, Madisen L, Levi BP, Ramanathan S. Dynamics of embryonic stem cell differentiation inferred from single-cell transcriptomics show a series of transitions through discrete cell states. Elife. 2017 Mar 15;6 PubMed Central PMCID: PMC5352225.
   c. Zhang Z, Zwick S, Loew E, Grimley JS, Ramanathan S. Mouse embryo geometry drives the formation of robust signaling gradients through receptor localization. Nat Commun. 2019 Oct 4;10(1):4516. PubMed Central PMCID: PMC6778081.

d. Valcourt JR, Huang RE, Kundu S, Venkatasubramanian D, Kingston RE, Ramanathan S. Modulating mesendoderm competence during human germ layer differentiation. Cell Rep. 2021 Nov 9;37(6):109990. PubMed Central PMCID: PMC8601596.

3. MAPK Signaling and Decision Making: An important question is how biological circuits listen to and process environmental signals. To answer this question, my lab dissected signaling networks to understand how they process information (McClean 2007, Hersen 2008, Nachman 2007), how they achieve signaling specificity and avoid cross talk (McClean 2007, Mody 2009), and how they integrate signals for a long period before making developmental decisions (Nachman 2007). We have also studied how these same signaling pathways evolved and if we can exploit their evolutionary history to change their wiring (Mody 2009). We developed microscopy, microfluidics, image processing techniques, and computation and modeling to address these questions. We focused on the MAP kinase pathways and used S.cerevisae for experiments. The MAP Kinase pathway plays a crucial role in normal development and disease. Therefore, understanding the pathways' dynamics and protein architecture is essential.

   a. Mody A, Weiner J, Ramanathan S. Modularity of MAP kinases allows deformation of their signaling pathways. Nat Cell Biol. 2009 Apr;11(4):484-91. PubMed Central PMCID: PMC3374951.
   b. Hersen P, McClean MN, Mahadevan L, Ramanathan S. Signal processing by the HOG MAP kinase pathway. Proc Natl Acad Sci USA. 2008 May 20;105(20):7165-70. PubMed Central PMCID: PMC2386076.
   c. Nachman I, Regev A, Ramanathan S. Dissecting timing variability in yeast meiosis. Cell. 2007 Nov 2;131(3):544-56. PubMed PMID: 17981121.
   d. McClean MN, Mody A, Broach JR, Ramanathan S. Cross-talk and decision making in MAP kinase pathways. Nat Genet. 2007 Mar;39(3):409-14. PubMed PMID: 17259986.

4. Controlling and Recording from Simple Neural Networks: A fundamental question in biology is how the dynamics of complex networks control behavior. Our approach to answering this question is to understand the network well enough to identify its key nodes and take control of it (Guo 2009, Kocabas 2012, Lee 2019). We have established novel computational methods of rapidly deconstructing to identify critical nodes in a complex network whose dynamics allow us to understand the network's computation (Lee 2019).

   We have applied these methods to understand the neural circuits of *Caenorhabditis elegans*. We developed novel microscopy techniques using mirror arrays to achieve all-optical electrophysiology: we could optically activate a specific neuron from among a field of neurons expressing light-gated channels, and simultaneously measure the activity patterns in downstream neurons using GCaMP (Guo 2009). We have improved the microscope to perform all-optical electrophysiology in a freely moving animal, allowing us to control neural activity using optogenetics, monitor calcium activity in the neuron of choice under a 63x objective, and monitor the behavior of the animal (Lee 2019). We have further identified vital neurons in the nervous system of this animal and stimulated the correct activity patterns in them optically to make the animal navigate in any direction we wanted (Kocabas 2012).

   Relevant to this proposal: we developed novel optical tools for targeted optogenetic excitation.

   a. Lee JB, Yonar A, Hallacy T, Shen CH, Milloz J, Srinivasan J, Kocabas A, Ramanathan S. A compressed sensing framework for efficient dissection of neural circuits. Nat Methods. 2019 Jan;16(1):126-133. PubMed Central PMCID: PMC6335042.
   b. Kocabas A, Shen CH, Guo ZV, Ramanathan S. Controlling interneuron activity in Caenorhabditis elegans to evoke chemotactic behaviour. Nature. 2012 Oct 11;490(7419):273-7. PubMed Central PMCID: PMC4229948.
   c. Guo ZV, Hart AC, Ramanathan S. Optical interrogation of neural circuits in Caenorhabditis elegans. Nat Methods. 2009 Dec;6(12):891-6. PubMed Central PMCID: PMC3108858.

5. Modeling Complex Physical Systems: As a graduate student I worked on studying very complicated dynamical systems including the dynamics of sound moving through dirty solids. To model such systems on a computer requires solving an Avagadro’s number of equations which is technically extremely challenging. We developed novel mathematical methods to model these complex systems using tools from theoretical

physics. Through my work, we could predict the existence of a novel kind of sound wave that ran along the edge of a crack front, and such waves also had a serious impact on earthquake dynamics (Fisher 1997, Ramanathan 1997). The waves I predicted using mathematics were discovered (Ramanathan 1997) a few years later experimentally. The work here was supervised by my thesis advisor but was primarily mine. The success during my Ph.D. allowed me to be recruited to a permanent position at Bell Labs. There I developed algorithms to rapidly transmit information through communication networks (Basu 2003) that could also deform their topology in response to different demands (Basu 2004).

Relevant to this proposal: we have established expertise in using models to make predictions about complex systems with a large number of variables and in analyzing information flow through complex networks.

a. Basu A, Boshes B, Mukherjee S, Ramanathan S. Network Deformation: traffic-aware algorithms for dynamically reducing end-to-end delay in multi-hop wireless networks. Proceedings of IEEE ACM SigComm. 2004; :100-113. Available from: https://dl.acm.org/doi/10.1145/1023720.1023731
b. Basu A, Lin A, Ramanathan S. Routing using potentials: Dynamic Traffic Aware Routing Algorithms. Proceedings of IEEE ACM SigComm. 2003; :37-48.
c. Ramanathan S, Fisher DS. Dynamics and instabilities of planar tensile cracks in heterogeneous media. Physical review letters. 1997; 79(5):877-80.
d. Fisher D, Dahmen K, Ben-Zion Y. Statistics of Earthquakes in simple models of heterogeneous faults. Phys. Rev. Lett.. 1997; 78:4885-4888.

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