

**Smith Family Awards Program for Excellence in Biomedical Research
2016 Award Recipients**

• **Jerry Chen, Ph.D.**

Assistant Professor of Neurobiology
Department of Biology
Boston University

“Circuit Mechanisms for Long-Range Communication in the Neocortex”

Key Words: Cortex, Behavior, Whiskers, Sensorimotor, Decision making, Imaging, Oscillations

A key aspect of cognitive function is the capacity for the brain to function as a whole. In order to behaviorally adapt to changing environmental conditions, the brain needs to dynamically route information to different areas and coordinate the exchange of information between areas according to cognitive demands. However, the mechanisms underlying this are unclear as it has been difficult to observe large-scale brain activity across areas with sufficient resolution to observe individual neurons and their connected partners.

In my lab, we use a novel "multi-area" two-photon microscope that I have previously developed to perform simultaneous functional imaging of neuronal activity with cellular resolution across cortical areas. By combining multi-area two-photon calcium imaging with anatomical tracers in the mouse sensorimotor whisker system, we have begun to investigate the flow of information between connected neocortical areas with unprecedented scope and detail. Here, we propose to identify circuit mechanisms that enable dynamic and coordinated information exchange across the neocortex during behavior. This will be investigated through an integrated approach using viral-based anatomical methods to map local and long-range inputs of specific cortico-cortical pathways and combined functional measurements using simultaneous micro-electro-corticography recordings, multi-area two-photon calcium imaging, and optogenetic perturbation in the awake, behaving mouse. We will explore potential mechanisms governing long-range cortical communication through the following aims:

Aim 1: We will test whether cortical areas are segregated into distinct local subnetworks in order to facilitate information flow across cortical areas.

Aim 2: We will test whether the exchange of information between reciprocally connected cortical areas is coordinated by top down regions that provide common input to those areas.

Aim 3: We will test whether coherent oscillatory activity across neuronal populations can serve as a physiological mechanism to support information flow across cortical areas in a pathway-specific manner.

- **Aleksandar Kostic, Ph.D.**

Assistant Investigator

Section of Pathophysiology and Molecular Pharmacology

Joslin Diabetes Center

“Immunological Investigation of Human Type 1 Diabetes–Associated Microbiomes in the Gnotobiotic Autoimmune Diabetic Mouse”

Key Words: Microbiome, gut, immunology, microbiology, mouse models, gnotobiotic mice, type 1 diabetes, autoimmunity, metagenomics, computational biology, bioinformatics, microbial isolation

Type 1 diabetes (T1D) is a complex disease with no single cause; a combination of interacting factors contributes to overall risk for developing T1D. Among the strongest factors is the genetics of the individual. But just as important (or perhaps more important) is the environmental exposures of the individual at an early age. My post-doctoral work thus far has characterized the developing infant gut microbiome in a longitudinal, frequently-sampled, multi-omic analyses of birth cohorts at risk for T1D. We found that this T1D-associated microbiota may produce a gut mucosal inflammatory stimulus that moves the systemic immune response towards islet autoimmunity in a genetically-prone individual. In the proposed study, I will bring findings from my human studies to the germ-free non-obese diabetic (NOD) mouse to further define the role of the microbiome in T1D.

We will isolate and identify unique bacterial strains from the mouse and human-associated T1D gut microbiome, perform whole-genome sequencing on isolates with the strongest T1D association, and design simplified communities that are predicted to either induce or prevent diabetes in a germ-free NOD mouse upon colonization. We will colonize germ-free NOD mice with these simplified communities of T1D-associated microbes predicted to either induce or prevent diabetes in NOD mouse. This enables the dissection of the role of the microbiome in T1D using a simplified system in which we know every microbial gene that is introduced via the simplified microbiome.

This work opens the opportunity for the discovery and eventual editing of microbial and mouse genetic elements linked to the diabetes phenotype in order to solidify mechanisms by which the microbiome impacts disease. Targeting these mechanisms therapeutically represents a new path towards the treatment of type 1 diabetes.

- **Philip Kranzusch, Ph.D.**

Assistant Professor of Microbiology and Immunobiology

Department of Cancer Immunology and Virology

Dana-Farber Cancer Institute

“Mechanism of Activation of a New Human RNA Second Messenger Signaling Enzyme”

Key Words: Biochemistry, RNA Second Messenger, Innate Immunity, Cell Signaling, Development, Cancer Immunotherapy

RNA second messenger molecules are a newly described form of signal amplification in humans, but their role is thus far limited to the immune response of adult cells. The long-term goal of my lab is to understand the function of RNA synthase enzymes in human biology and disease, and to discover new classes of RNA second messenger products. As a critical first step to reach this goal, our proposal will focus on the lead candidate RNA synthase in humans, and use this previously uncharacterized enzyme as a paradigm to understand the molecular rules controlling RNA synthase activation and pathway specificity.

The best-characterized example of RNA second messenger signaling is the human innate immunity enzyme cGAS (cyclic GMP-AMP synthase). cGAS is a switch-like enzyme that recognizes cytosolic DNA and is then activated to synthesize a small RNA that potently induces an immune response. Surprisingly, our previous results reveal that cGAS is part of a broad family of potential signaling enzymes, and many RNA second messenger pathways remain to be discovered. Potential RNA synthases include orphan immunity receptors as well as genes implicated in developmental disease. We hypothesize that these uncharacterized enzymes produce novel RNA second messengers to control immunity and developmental tissue patterning.

The proposed research plan will combine a direct biochemical approach with a high-throughput genetic screen to identify new RNA second messenger signaling cascades. Using *in vitro* reconstitution and structural biology, we will determine the mechanism of activation of a previously undiscovered RNA second messenger signaling pathway (Aim 1). As a complementary strategy, we will create a genetic-based screen to unlock the activity of uncharacterized RNA synthases and allow direct discovery of new classes of RNA second messenger products (Aim 2).

- **Gene-Wei Li, Ph.D.**

Helen Sizer Career Development Assistant Professor of Biology
Massachusetts Institute of Technology

“Breaking the Robustness of Bacterial Translation”

Key Words: Translation, Bacterial Physiology, Genomics, Genetic Interactions

Translation of mRNA is arguably the most complex molecular process that is present across all domains of life. Although many of its key components have been characterized in detail by biochemical and structural studies, a critical systems-level understanding of translation in vivo is missing due to the sheer number of core and accessory players involved. A growing body of evidence suggests that bacterial translation is remarkably robust against perturbations to the cell—layers of genes with overlapping functions protect many aspects of translation, and the few features that lack this redundancy are targets of known antibiotics.

In the proposed study, I will interrogate the redundant factors that control translation initiation and ribosomal function. Many of these factors are among the most highly expressed during rapid growth, but the deletions confer no phenotype. Using both the Gram-negative bacterium *E. coli* and the Gram-positive bacterium *B. subtilis* as model organisms, my objectives are to define the extent and nature of functional redundancy and expose weaknesses in these central processes. To do so, I will begin dissecting the networks by leveraging three enabling concepts and technologies: i) a large compensatory induction as a signature of overlapping gene functions upon the loss of its member, ii) a combinatorial knockdown method based on CRISPR interference to quantify high-order genetic interactions, and iii) a high-resolution assay for monitoring the kinetics of translation in vivo at the genomic scale. This set of tools will enable systematic mapping of the backup systems responsible for maintaining translation initiation and ribosomal functions. Overall, a better characterization of the strengths and weaknesses of bacterial translation will have broad implications for designing points of attack against infections and provide guiding principles for understanding human diseases associated with protein synthesis defects.

- **Maofu Liao, Ph.D.**

Assistant Professor of Cell Biology
Harvard Medical School

“Cryo-EM Studies of Multidrug Resistance Conferred by Human ABC Transporters”

Key Words: cryo-EM, multidrug resistance, ABC transporter, Nanodisc

Multidrug resistance is a common cause for the failure of cancer chemotherapy and has become a serious health concern. One major cause is correlated with the ATP-binding cassette (ABC) multidrug transporters that are overexpressed in tumor cells and pump out a wide variety of chemotherapeutic drugs. Developing cancer drugs against these molecular pumps has great potential to dramatically improve the outcome of cancer treatment. To achieve this goal, it is essential to obtain the structural information of these transporters with substrates and in different functional states. However, this has not been achieved, mainly due to the limitations of traditional structural methods, which often require isolation of transporters away from their lipid environments and thus prevent proper transporter-substrate interactions. Therefore, new approaches are needed.

Here we propose to reconstitute ABC transporters into lipid nanodiscs to mimic their native lipid environments, and use single particle cryo-electron microscopy (cryo-EM) to characterize the transporter structures. We will first focus on P-glycoprotein (Pgp), the most prevalent multidrug transporter. The findings from this project will fundamentally advance our understanding of multidrug resistance driven by ABC transporters, and enable drug development to combat multidrug pumps.

Aim 1: Determine overall architecture of Pgp in the context of lipid bilayers. We will purify the Pgp proteins and incorporate them into nanodiscs. Cryo-EM will be used to determine Pgp structures at subnanometer resolution.

Aim 2: Delineate conformational rearrangements of Pgp during its functional cycle. We will use cryo-EM to determine the structures of Pgp in different ATP hydrolysis states, thus obtaining the long-sought-after complete picture of conformational rearrangements.

Aim 3: Reveal structural basis of Pgp-mediated drug recognition and translocation. We will determine ~3 Å resolution cryo-EM structures of Pgp bound with different chemotherapeutics and build atomic models to reveal the structural bases of substrate specificity and cross-membrane translocation.

- **Noah Palm, Ph.D.**

Assistant Professor of Immunobiology

Yale University

“A Universal Genetic Toolkit to Illuminate Host–Microbiota Interactions”

Key Words: Microbiota, Microbiome, Immune

The trillions of bacteria that constitutively colonize our intestines (our gut microbiota) play a central role in almost all immune-mediated diseases and disorders. However, the specific molecular mechanisms by which gut microbes interact with and influence the host immune system remain largely unknown. We hypothesize that uncovering these mechanisms will be critical to revealing how gut microbial communities impact human health and disease—i.e., cracking the microbiota composition code. A foremost obstacle to achieving this goal is a lack of robust tools, such as CRISPR, to genetically manipulate gut microbes of diverse phylogenies. Solving this problem would enable elucidation of the full spectrum of mechanisms that mediate host–microbiota interactions, reveal potential targets for therapeutic manipulation, and open the door to the development of genetically-engineered gut microbes as a new therapeutic paradigm.

Here, we propose to develop a novel toolkit to enable facile genetic modification of gut microbes from seven common bacterial phyla that colonize the gut. This system, which we term UMaMI, will enable sequence-specific insertion into genomic DNA independently of homologous recombination. We will then use UMaMI to perform genome-wide forward genetic screens that will reveal the molecular mechanisms by which two immunologically-important and phylogenetically-disparate microbes colonize the gut and interact with the immune system. Analogous to the revolutionary application of CRISPR to gene targeting in eukaryotic cells, we believe that UMaMI will transform the study of host–microbiota interactions by enabling rapid and reliable gene-editing at an unprecedented scale. More broadly, the approaches developed here will be generally applicable to understanding the impact of the microbiota on a wide range of human traits and eventually may allow us to 'crack' the microbiota composition code.

- **Silvia Rouskin, Ph.D.**

Whitehead Fellow

Whitehead Institute for Biomedical Research

“Identifying the Factors Required for mRNA Localization during Metazoan Development”

Key Words: development, oogenesis, RNA structure, mRNA localization

mRNA localization is a universal cellular process whereby mRNAs are trafficked to distinct locations in the cell. Decades of research in model organisms have shown that specific mRNA structures are critical for mRNA localization during embryonic development, and that altered mRNA structures are sufficient to cause a variety of pathologies. Furthermore, defects in mRNA localization are associated with a variety of genetic diseases, such as Spinal Muscular Atrophy, Fragile X Syndrome, and Spinocerebellar Ataxia 8. However, the basic mechanisms underlying the transport of mRNAs to distinct subcellular destinations are poorly understood. Moreover, our understanding of the functions of RNA structure is derived largely from a handful of cases that have been examined through years of painstaking molecular biology and in vitro biochemistry.

The goals of the proposed research are to: 1) measure the structures of hundreds of mRNAs simultaneously in vivo, even when a given mRNA might adopt several alternate or competing structures, 2) identify biologically relevant mRNA structures and changes in mRNA structure during oogenesis, and 3) identify the specific RNA binding proteins that recognize functional structures to direct mRNA localization.

To accomplish these aims, I have extended a transcriptome-wide RNA structure probing method I developed during my graduate work, DMS (dimethyl-sulfate)-seq, to the single molecule level, and established protocols for RNA structure probing inside a functional *Drosophila* ovary. We will apply this new technology together with Mass Spectrometry to identify the sequence and structural features that target mRNAs to specific subcellular locations as well as the proteins that read out the mRNA localization features. Collectively, these aims will greatly enhance our knowledge of RNA-based regulation, improve our understanding of how these mechanisms are disrupted in disease, and open new avenues for design of therapeutic approaches.

- **Mansi Srivastava, Ph.D.**

Assistant Professor

Department of Organismic and Evolutionary Biology

Harvard University

“Cellular Dynamics and Regulation of Pluripotent Stem Cells *in vivo*”

Key Words: Regeneration, Development, Stem cells, Pluripotency, Evolution, Acoels, Planarians

Though great advances have been made in uncovering some of the molecular pathways that maintain pluripotency and regulate differentiation in vertebrate cells in culture, the processes that would allow pluripotent stem cells to survive and replace tissue in the context of whole animals are unknown. The long-term goal of this project is to obtain a mechanistic understanding of how pluripotent stem cells are controlled *in vivo* such that they can regenerate any missing cell type in an adult animal. Some invertebrate species that are capable of extensive regeneration have pluripotent stem cells in adult animals and therefore can serve as effective models for uncovering the *in vivo* control of pluripotent cells.

Studies of *Schmidtea mediterranea*, the well-established planarian model system, have revealed some mechanisms that control their stem cells ("neoblasts") but many major questions remain unanswered. It is unknown if individual neoblasts are pluripotent in the context of wild-type animals and if so, how neoblasts are specified during development, how they are maintained in a pluripotent state, and how their behaviors (e.g., proliferation, renewal, and differentiation) are dynamically regulated to accomplish complete regeneration. The fates of single neoblasts cannot be visualized directly in planarians because their embryos are not currently amenable to lineage tracing. Therefore, our proposal leverages a new model system, *Hofstenia miamia*, which regenerates extensively, has neoblasts, and produces abundant accessible embryos that are amenable to manipulation. We aim to determine the fates and behaviors of *Hofstenia* neoblasts and identify genes that control these processes. This work will reveal the basic cellular and molecular principles that govern stem cell-mediated tissue replacement in the context of whole animals.