Smith Family Foundation: Odyssey Award

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2019 Award Recipients

- **Shantanu Jadhav, Ph.D.**
  Assistant Professor of Psychology & Neuroscience
  Brandeis University

  "Neural Coordination Mechanisms for Memory Function and Dysfunction"

  Key Words: Memory, Hippocampus, Prefrontal Cortex, Behavior, Neural circuits, Rodents, Physiology, Neural synchronization, Causal manipulation, Optogenetics, Cognitive disorders

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Memory is key for our most fundamental cognitive abilities, and memory deficits are a common feature of neuropsychiatric disorders. The ability to learn new experiences and recall memories of these experiences to guide daily behavior is a remarkable capacity of the brain. The hippocampus is a key structure in the mammalian brain responsible for these memory abilities. Decades of work has uncovered cellular and synaptic mechanisms in hippocampal circuits that underlie memory processes. Despite this progress, it still remains a challenge to treat memory disorders. One of the major reasons is that we still lack a systems-level understanding of memory; namely, how is activity coordinated in large networks of neurons in hippocampal and associated cortical circuits in order to store and recall information that is the substrate of our memories. Recently, physiological mechanisms that synchronize activity in neural circuits have been uncovered, and importantly, these neural synchronization (or, coordination) mechanisms are linked to normal memory function. In particular, we have discovered that a process called "memory replay" is exquisitely synchronized between the hippocampus and prefrontal cortex, the executive function center of the brain. Preliminary evidence suggests that this hippocampal-prefrontal neural coordination plays a role in memory. Our central hypothesis is that neural coordination at the network level is necessary for memory storage and recall, and breakdown of this coordination is a major contributor to memory disorders. In this research proposal, we aim to gain a mechanistic and causal understanding of the role of hippocampal-prefrontal neural coordination in memory, and develop new techniques to modulate this synchronization as a novel target to rescue memory deficits in models of cognitive dysfunction. We will 1) establish links between neural coordination mechanisms and memory using rodent behavior and in vivo physiology; 2) develop novel closed-loop feedback techniques using optogenetics that can detect and modulate neural coordination in behaving animals online; 3) investigate if restoring neural coordination in rodent models of cognitive disorders can rescue memory function. This research will thus address the "missing link" at the neural coordination level for understanding and targeting neural mechanisms of memory.

- **Maofu Liao, Ph.D.**
  Assistant Professor of Cell Biology
  Harvard Medical School

"Elucidating the Molecular Mechanism of ABCA7, a Membrane Transporter associated with Alzheimer's Disease"

Key Words: ABCA7, Alzheimer's disease, Neurodegenerative diseases, Cryo-EM, ABC transporter, Nanodisc

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Neurodegenerative diseases affect millions of people worldwide, and there is currently no way to slow disease progression or cure the disease. Alzheimer's disease (AD) is one of the most common neurodegenerative diseases, representing approximately 60-70% of dementia cases. More than five million Americans live with AD, and the number increases at an accelerated rate. Alzheimer's association reports that, if there are no effective therapies by 2050, the number of AD patients and medical care will increase to 13.8 million and 1.1 trillion dollars, respectively.

A large number of genetics studies, mouse model analyses and cell-based assays have established that loss-of-function variants in ABCA7 are associated with increased risk of AD. ABCA7 belongs to the family of ATP-binding cassette (ABC) transporters, which utilize the energy from ATP hydrolysis to move various substances cross the cellular membrane. Previous studies show that ABCA7 translocates lipid molecules and affects multiple aspects of cellular lipid homeostasis. However, the precise function of ABCA7 at the molecular level is poorly defined. It remains unclear what lipids ABCA7 preferentially translocates, how AD-related coding variants affect ABCA7 function, how ABCA7 couples ATP hydrolysis to lipid transport. Thus, there is a remarkable unmet need of understanding the structure and mechanism of ABCA7.

In this proposed project, we will elucidate the molecular mechanism of ABCA7 and gain insights into how the variants in ABCA7 affect the protein structure and function. Specifically, we will 1) purify human ABCA7 and characterize its activity in lipid membranes; 2) determine high-resolution cryo-EM structure of ABCA7 in lipid nanodiscs; and 3) characterize the function and structure of AD-related genetic variants of ABCA7. The results from this study will fundamentally advance our understanding of the molecular basis underlying the involvement of ABCA7 in the pathological pathway of AD, and may provide guidance for developing novel AD treatments via stimulation of ABCA7.

- **Sarah Slavoff, Ph.D.**
  Assistant Professor of Chemistry
  Yale Cancer Center

  "Non-Canonical Translation of Small Open Reading Frames in the Human Genome"

  Key Words: Genomics, Proteomics, Biochemistry

Over the past decade, new technologies have enabled the discovery that regions of the human genome once thought to be non-coding actually produce thousands of functional polypeptides encoded in small open reading frames (smORFs) that have been invisible to geneticists – until now. It is now of paramount importance to understand the mechanism and regulation of smORF translation in cells. The standard mechanism of eukaryotic protein translation initiation is well understood, from mRNA cap binding by translation initiation factors to recognition of the first AUG codon and ribosome assembly. However, it has also been known for decades that the translation of hundreds of human mRNAs is regulated by non-canonical initiation during (patho)physiological processes including cellular stress responses, cell division and apoptosis. Evidence is accumulating that some smORFs undergo this cap-independent translation, potentially implicating them in these cellular processes, but a complete understanding of the scope and mechanism of cap-indepdendent smORF translation is currently lacking. This is due in part to technical limitations in current experimental methods for (1) promotion of cap-independent translation in cells, which requires exposure to (patho)physiological stimuli such as viral infection, and (2) detection of smORFs via ribosome footprinting, which can be prone to false positives and do not always correlate with production of detectable polypeptide products. We propose a novel approach to enable unbiased, sensitive and specific global profiling of non-canonical smORF translation in human cells. In Aim 1, we will establish a method for specific and unbiased isolation of cap-independent translation in cells via chemical knock out of a translation initiation factor required for mRNA cap binding. In Aim 2, we will combine ribosome footprinting and deep sequencing with mass spectrometry-based chemoproteomic quantitation of nascent smORF translation under cap-independent conditions. These technologies will enable establishment of a high-confidence, global catalog of smORFs that can undergo cap-indepdent translation, and subsequently inform identification of consensus RNA sequence motifs and/or modifications required for this process. In the long term, understanding the mechanism of smORF translational regulation may shed light on the functions of smORFs, and will broaden our understanding of the scope of cap-independent translation in human cells.

- **Jessica Whited, Ph.D.**
  Assistant Professor
  Brigham and Women's Hospital

"Transmission of Amputation Information Throughout the Body in Axolotl"

Key Words: Regenerative Biology, Limb, Axolotl, Injury, Systemic Responses, Proteomics

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Axolotl salamanders are master regenerators and can fully regenerate entire limbs throughout life. The initial responses to an amputation injury are very poorly understood but clearly are critical for the success of the entire process; defining them will offer unique insights into regenerative biology and, ultimately, medicine. Most effort in this field has focused on cellular and molecular events that happen near the site of injury, where the limb will regenerate. However, we recently discovered that amputation of a single limb stimulates cell cycle re-entry throughout the body, within uninjured limbs and even internal organs. How systemic activation occurs and how it is related to local regeneration remain outstanding questions. We hypothesize that amputation induces factor(s) in the circulation that stimulate tissue-resident progenitor cells throughout the body to synthesize DNA and that this is a necessary first step toward eventual conversion of the response to a local event. The long-term objective of this work is to understand exactly how axolotls regenerate limbs and whether there is a two-step mechanism in which broad progenitor activation becomes refined to the site of injury, perhaps by the wound epidermis. The long-term implications of the work is to provide a framework for tackling the deficits seen in mammals following amputation; since a similar systemic response to more limited injuries has been reported in mammals (Rodgers et al., 2014), it is possible that mammals do execute the first step but fail to convert the response to local tissue regeneration.

Specific Aims

Aim 1: To create several key genetic resources that will enable the identification and purification of cells that respond to the systemic activation signal, to understand the spatial and temporal kinetics of their activation and their global gene expression profiles, and to test their differentiation capabilities in vitro and in vivo.

Aim 2: To use proteomics to identify the signaling pathways that may underlie cell cycle re-entry in tissues distant to, and proximal to, amputated limbs. In parallel, we will test eight pathways that, based on data in other systems, may be considered candidates for mediating systemic activation response in axolotls.

- **Wesley Wong, Ph.D.**
  Assistant Professor of Biological Chemistry and Molecular Pharmacology and of Pediatrics, HMS
  Boston Children's Hospital

"Single-Molecule Protein Identification with Mechanical Nanocalipers"

Key Words: Single-molecule, Proteomics, DNA nanotechnology, Force spectroscopy

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The ability to rapidly identify of proteins with single-molecule sensitivity would greatly advance both basic science and clinical practice, from helping untangle molecular mechanisms underlying cellular processes, to facilitating new approaches for drug discovery and diagnostics. While mass spectrometry has demonstrated substantial utility in protein analysis, its inability to identify low-abundance proteins, and their post-translational modifications, stands in stark contrast with our analogous ability to use deep sequencing to identify low-abundance DNA molecules. A protein-fingerprinting method that operates on single molecules with high throughput could overcome current sensitivity limitations of protein identification.

Our approach to meet this challenge — mechanical nanocalipers — draws from recent developments in DNA nanotechnology and single-molecule manipulation. First, target protein molecules are labeled with DNA handles on specific residues of interest (e.g. lysines). Subsequently, nanocalipers enable identification of a single protein by measuring distances between its amino (N) terminus and its labeled residues. A nanocaliper consists of a target protein linked on its amino (N) terminus to a bead through a branched DNA adaptor and linked on its carboxy (C) terminus to a coverslip through a linear DNA adaptor. Hybridization between a randomly selected residue handle and the branched adaptor loops out the intervening protein region, decreasing the distance between bead and coverslip; measuring the extent of this constriction yields the distance between that residue and the N terminus. By repeatedly releasing and grabbing one new handle at a time, each caliper can generate a fingerprint sufficient for unique identification of the target. Leveraging our expertise in parallel single-molecule force spectroscopy, thousands to millions of mechanical nanocalipers on a single coverslip will be operated in parallel to achieve sufficient throughput for clinical applications.

First, we will develop the mechanical nanocaliper construct, and demonstrate single-molecule profiling of post-translational modifications for proteins related to Alzheimer's disease using optical tweezers. Next, we we will improve the throughput of our approach by adapting our nanocalipers to a parallel force spectroscopy platform. This approach could lead to a high-throughput single-molecule proteomics platform that will provide an unprecedented view into biological function and diseases states, for clinical and research applications.