Smith Family Foundation: Odyssey Award

A program of the Richard and Susan Smith Family Foundation

2023 Award Recipients

- **Alan Brown, Ph.D.**
  Assistant Professor
  Harvard Medical School

  "The Molecular Organization of a Liquid-like Biomolecular Condensate"

  Key Words: Biomolecular condensates, Membraneless organelles, Phase separation, Tomography, FIB milling, Cryo-EM, Dyneins, Axoneme, Cilia, Ciliopathies

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"Biomolecular condensates" are theorized to carry out specialized functions in the cell, yet their existence and functional significance remain disputed as they challenge traditional concepts of cellular organization. High-resolution, three-dimensional images of condensates within cells are necessary to either validate or debunk their existence. However, this is yet to be achieved as the successful visualization and characterization of native condensates requires relatively stable condensates that contain large and identifiable protein complexes. These desirable traits are found in DynAPs – recently discovered liquid-like condensates believed to function as assembly factories for axonemal dyneins. In Aim 1, we propose to use DynAPs as a model system to determine whether condensates have a defined molecular organization. Our approach will involve cutting-edge technologies including CRISPR/Cas9-based gene editing, cryo-fluorescence microscopy, focused ion beam milling, and cryo-electron tomography to visualize fluorescently labeled DynAPs within cells. In Aim 2, we will exploit our gene-edited cell lines to determine the structural basis for axonemal dynein assembly. We will stall dynein assembly at different stages of biogenesis using dynein assembly factor (DNAAF) mutants. Cryo-EM on the stalled intermediates will reveal the principles and stepwise assembly mechanism of axonemal dyneins, clarify DNAAF functions, and reveal how mutations in DNAAFs might cause ciliopathies.

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2023 Award Recipients

- **Joseph Davis, Ph.D.**
  Assistant Professor of Biology
  Massachusetts Institute of Technology

  "Visualizing Protein Complexes At The Membrane"

  Key Words: Cryo-EM, Cryo-ET, Membrane protein complexes, Structural biology

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Cells utilize membranes and membrane-associated proteins in essential functions including metabolism (mitochondria), intercellular signaling (plasma membrane), and protein turnover (autophagosomes and lysosomes). Great advances have been made to enable the determination of high-resolution structures of integral membrane proteins, which can be readily solubilized in detergent and reconstituted in nanodiscs. Such methods have not however been extended to key protein complexes that associate with these membranes but do not bear true trans-membrane domains. Instead, most researchers treat such peripherally associated membrane proteins as soluble complexes, washing them away from their membranes and determining structures of the isolated complexes.

Here, we aim to understand how the membrane itself impacts the structure and function of these complexes by developing and utilizing novel cryoEM "membrane grids". Using this technique, we expect our resulting structures will teach us how these flexible proteins bend and adapt to bind to membrane surfaces, how they physically move to manipulate these membranes, and how these motions impact their function. With such tools, we hope the field can shed light on this "dark matter" of structural biology.

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2023 Award Recipients

- **Paul Greer, Ph.D.**
  Assistant Professor of Molecular Medicine
  University of Massachusetts Chan Medical School

"Elucidating Common Mechanisms Across Neurodegenerative Disorders"

Key Words: Microglia, Neuroimmunology, MS4A Receptors, Neurodegeneration, Alzheimer's Disease, ALS, Multiple Sclerosis, Parkinson's Disease, Huntington's Disease, Frontotemporal Dementia

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We have generated exciting data that reveal that deleting a specific Ms4a gene family member, Ms4a6c, significantly improves behavioral and cellular phenotypes in mouse models of AD, FTD, ALS, and MS suggesting that loss of function of Ms4a genes is beneficial in these diseases (Luu et al., manuscript in preparation, Mocarski et al., manuscript in preparation). However, the phenotypic rescue observed upon Ms4a deletion is incomplete, and we have generated pilot data suggesting that others of the 17 Ms4a family members may also participate in modulating neurodegeneration. We have generated a novel mouse genetic reagent in which all Ms4a genes are deleted and obtained pilot data suggesting that these mice have much stronger phenotypic rescue in neurodegenerative models. In the first part of this proposal, we will test this hypothesis. In the second part of the proposal, we will elucidate the mechanisms by which Ms4a genes regulate neurodegenerative processes. Together, these experiments will both increase our understanding of the etiology behind neurodegenerative diseases as well as take us a significant step closer toward leveraging Ms4a genes as a novel therapeutic strategy for treating neurodegeneration.

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2023 Award Recipients

- **Ruaidhri Jackson, Ph.D.**
  Assistant Professor of Immunology
  Harvard Medical School

"Interchromosomal mRNA Chimerism in Innate Immunity"

Key Words: mRNA splicing, transcriptomics, inflammation.

The mammalian transcriptome is a home of genomic dark matter. Using direct, long read RNA sequencing, we have uncovered an entirely new class of chimeric transcripts expressed in innate immune cells whose sequences derive from 2 distinct genes on entirely different chromosomes. Functional investigation of a lead candidate has uncovered that at least one of the identified chimeric mRNAs is stable, differential expressed in polarized macrophages, can encode chimeric protein and is functionally important during inflammation. However, the widespread functional and physiological relevance of this class of transcript is still unknown, as too are the mechanisms underlying their unique biosynthesis. In this proposal we aim to first screen the functionality of the plethora of chimeric mRNAs in macrophages with loss of function approaches. We further aim to identify the mechanisms governing chimeric mRNA formation, if de novo transcription and spliceosome factors are required, what proteins regulate this process, if interchromosomal interactions support this phenomenon, and finally, if specific nucleotide sequence motifs can facilitate mRNAs to participate in chimerism. Taken together, this proposal will illuminate the functionality of a novel class of mRNAs in innate immunity and uncover the general principles and molecular mechanisms underpinning interchromosomal RNA chimerism in mammals.

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2023 Award Recipients

- **Mohsan Saeed, Ph.D.**
  Assistant Professor, Biochemistry
  Boston University School of Medicine

  "Deciphering the Immune Evasion Mechanisms of Arboviruses in Mosquitoes"

  Key Words: Arboviruses, Viral Proteases, Mosquito Immunity, Immune Antagonism, Arbovirus-Resistant Mosquitoes

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Mosquito-borne arboviruses claim over one million human lives each year and are considered a global health priority due to frequent resurgence of activity and unprecedented geographical expansion in recent decades. In the absence of vaccines and targeted treatments, the design of strategies to control arboviruses at the mosquito level is imperative. This project is based on the premise that an in-depth understanding of mechanisms by which arboviruses establish life-long infection in mosquitoes can inspire the design of powerful approaches to reduce viral transmission to humans. I will use an advanced technique, which I recently developed, to investigate the molecular details as to how arboviruses disarm mosquitoes' antiviral defense systems and establish persistent infection. Specific Aim 1 will generate a comprehensive list of proteins cleaved during arboviral infection of mosquito cell lines and live mosquitoes. Specific Aim 2 will reveal the contribution of protein cleavages to ablation of the mosquito immune system. These studies will open up new lines of investigation into viral persistence and mosquito biology and facilitate the design of transgenic mosquitoes unable to harbor and/or transmit infections. It will also accelerate a discovery pipeline that can then be extended to other insect-borne pathogens such as Plasmodium and Borrelia.