Smith Family Awards Program for Excellence in Biomedical Research
2017 Award Recipients

- **Aaron Hata, M.D., Ph.D.**
  Assistant Professor of Medicine at Harvard Medical School
  MGH Cancer Center

  "Determining the Impact of Therapy-Induced Mutations on Tumor Heterogeneity and Evolution of Acquired Drug Resistance"

  Key Words: Non-Small Cell Lung Cancer, Targeted Therapies, Tumor Evolution, Acquired Drug Resistance, APOBEC Mutations

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Targeted therapies that inhibit oncogenic kinases (TKIs) such as EGFR or ALK have significantly improved the survival of patients with lung cancers that harbor activating mutations in these genes. Unfortunately, patients eventually develop drug resistance leading to disease progression. It is now increasingly apparent that tumors are comprised of heterogenous populations of cancer cells that are capable of adapting and evolving in response to drug treatment. We recently showed that drug resistance in EGFR mutant lung cancers caused by acquisition of the gatekeeper EGFRT790M mutation can result from evolution of drug tolerant persister cells that acquire the mutation during therapy. Newly emerging data suggests that this process may be accompanied by other genomic changes including the appearance of specific mutational signatures, such as C-to-T substitutions characteristic of APOBEC enzymatic activity. We hypothesize that TKI treatment induces APOBEC-catalyzed cytidine deamination, leading to tumor genetic heterogeneity and facilitating the development of acquired resistance. In this project, we will integrate genomic analysis of tumors from EGFR mutant lung cancer patients with mechanistic laboratory studies using patient-derived cancer cell lines and patient-derived xenograft (PDX) mouse models in order to define the mutational signatures present in resistant tumors and determine the impact of TKI-induced mutational processes on the evolution of acquired drug resistance. These studies will yield fundamental biological insights into how TKI resistance evolves in oncogene-addicted cancers in general and will inform the development of novel therapeutic strategies to prevent this process.

- **Amity Manning, Ph.D.**
  Assistant Professor of Biology
  Worcester Polytechnic Institute

"Epigenetic Regulation of Mitotic Fidelity"

Key Words: Cancer, Mitosis, Centromere, Cohesion, CIN, Retinoblastoma, pRB

Function of the retinoblastoma tumor suppressor (pRB) is compromised in most cancers. Historically, the role of pRB as a tumor suppressor has been attributed to its function in regulating E2F-dependent transcription and cell cycle progression. Emerging data now demonstrate that pRB's cellular roles are more diverse than regulating the G1 to S phase transition of the cell cycle and more complex than regulating E2F-dependent transcription. Our work has helped defined a role for pRB in the regulation of mitotic progression and genome stability. We have shown that loss of pRB compromises epigenetic regulation of heterochromatin and centromeric structure, that these changes lead to defects in mitosis and increased DNA damage, and render cells sensitive to regulation of chromosome cohesion. While our data suggest this function of pRB is independent of its role in regulating E2F, the molecular mechanism by which pRB impacts chromatin structure and mitotic fidelity remains unclear. This proposal will utilize molecular, cellular, and imaging approaches to mechanistically define the role of the retinoblastoma protein in the regulation of centromeric heterochromatin and genome stability.

Chromosome instability (CIN), defined as frequent gains and losses of whole chromosomes, arises due to underlying defects in mitotic fidelity. Although CIN is prevalent in solid tumors and has been implicated in tumor evolution and drug resistance, conserved mutations that contribute to CIN in cancer have remained elusive. Given the high frequency at which the pRB pathway is corrupted in cancer, and this newly recognized role for pRB in regulating genome stability, mechanistic insight into pRB's role in regulating chromatin structure and mitotic fidelity is likely to be broadly relevant to CIN cancers. Through an understanding of changes that promote CIN, it is our long-term goal to provide insight into how CIN tumors may be targeted efficiently and specifically by novel therapeutics.

- **Babak Momeni, Ph.D.**
  Assistant Professor of Biology
  Boston College

"Elucidating the Molecular Mechanisms of Microbial Interactions Using Tn-Seq"

Key Words: Microbial communities, Interspecies interactions, Transposon-sequencing, Infections, Microbiota, Interaction mechanisms

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Microbes that affect our health often exist in communities while interacting with other microbes. These interactions can impact our health. In the human nasal cavity, for example, harmless resident microbes are shown to inhibit the colonization of pathogens. Identifying microbial interactions, thus, opens new ways to prevent or treat infections.

Uncovering the mechanisms of microbial interactions remains a major challenge. Coculture studies can show phenomenologically whether interaction partners have beneficial or detrimental effects on one another. However, controlling infections often requires a more mechanistic understanding of how those interactions take place. Unfortunately, because of the diversity of possible interactions, previous mechanistic studies are often rare and on a case-by-case basis.

To determine how microbial species interact, we propose a systematic method based on transposon insertion sequencing (Tn-Seq). In this method, we correlate mutations in genes to the impact of those genes on interspecies interactions by quantifying mutants' fitness in the presence of interaction partners. By linking genes to their role in interactions, this will allow us to form a gene-interaction map that reveals molecular mechanisms of interactions.

To assess the applicability of the proposed method, we will set up proof-of-principle experiments using *E. coli* lab strains with known engineered interactions. We will identify candidate mutants that either strengthen or weaken the influence of interaction between the two strains. We will compare the identified genes with the ones known to be involved in the defined interaction. We will then validate the findings by directly constructing mutants of the identified genes to compare with the original strain. These experiments aim to evaluate the capabilities and limitations of our Tn-Seq based method.

The proposed project establishes a broadly applicable method to examine interactions between microbial species. This paves the way for future projects on polymicrobial infections or human-associated microbiota.

- **W. Benjamin Rogers, Ph.D.**
  Assistant Professor of Physics
  Brandeis University

"Developing DNA Nanotechnology Tools for Probing RNA Secondary Structure and RNA-Protein Interactions"

Key Words: DNA Nanotechnology, DNA, RNA, Secondary Structure, RNA-Protein Interactions, Self-Assembly, Virus

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RNA molecules are vital regulators of cell biology and their three-dimensional structures are essential to how they work. Having the ability to intentionally interfere with the structure of RNAs could therefore hold immense potential for the study of their function, as well as the development of molecular medicine and other biotechnological applications. One way to do this is to bind short sequences of synthetic nucleic acids to specific sites on a RNA molecule. But designing oligonucleotides that bind rapidly and with high affinity to a RNA target remains a challenge.

In my previous work, I combined experiment and theory to explore how interactions between nucleic acids could control self-assembly. Here my group will extend this approach to tackle a new challenge—understanding and designing oligonucleotides to bind to RNA molecules. Specifically we aim to:

(1) Determine kinetic and thermodynamic barriers to binding an oligonucleotide to nucleic-acid secondary structures. My group is working to greatly expand the fundamental understanding of the mechanisms, thermodynamics, and kinetics of binding oligonucleotides to nucleic-acid targets having known secondary structures.

(2) Characterize how multiple oligonucleotides cooperate to bind to secondary-structure motifs. Many RNAs are unsuitable targets for RNAi and antisense therapy since their secondary structures prevent binding. Using *in vitro* methods, we explore how cocktails of oligonucleotides remodel tight secondary structures and lower barriers to binding, which could greatly expand the space of molecules that can be targeted.

(3) Disrupt RNA secondary structures in a viral genome and study their role in capsid assembly. We will generate, evaluate, and refine design rules using the MS2 RNA viral genome, and test whether specific RNA secondary structures are essential for capsid assembly. This work also lays the foundation for a new possibility—developing therapies for human viruses with single-stranded genomes (e.g. HIV and influenza) by disrupting structure-dependent processes essential to their life cycle.

- **Sichen Shao, Ph.D.**
  Assistant Professor
  Harvard Medical School

"Mechanistic Dissection of Tail-Anchored Membrane Protein Sorting"

Key Words: Membrane protein biogenesis, Protein targeting and sorting, Protein quality control, In vitro reconstitution, Structural biology, Neurodegeneration

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Newly-synthesized proteins should have protected time to mature, but be degraded if they fail. Mistakes at any point produce aberrant proteins linked to numerous genetic, neurodegenerative, and aging diseases such as cystic fibrosis and ALS. How cells accurately triage nascent proteins between disparate fates is poorly understood, primarily due to the lack of biochemically-defined systems that permit mechanistic dissection of these reactions.

My lab aims to understand the molecular mechanisms that determine the fate of new proteins. We use tail-anchored (TA) membrane protein sorting as a model to study protein triage. TA proteins have an aggregation-prone transmembrane domain that must be chaperoned in the cytosol and inserted into the correct cellular organelle membrane to carry out essential functions. How do TA proteins sort between biosynthesis at different organelles and degradation? To address this, I recently reconstituted TA protein triage through the TRC, a three-chaperone complex that either targets TA proteins to the endoplasmic reticulum for maturation, or tags mislocalized proteins for degradation in the cytosol. Here, we propose to obtain structural information of this triage reaction and explore how different organelles selectively recognize TA proteins. This will reveal fundamental mechanistic principles of protein triage reactions that are critical for maintaining cellular physiology and preventing disease.

Aim 1. Capture structural snapshots of TA protein triage. Molecular structures of triage factors in different functional conformations are necessary to understand how they sort substrates to the appropriate fate. We will combine in vitro reconstitution with high-resolution electron cryo-microscopy (cryo-EM) to determine structures of the TRC trapped at sequential stages of TA protein triage.

Aim 2. Reconstitute the mechanism of TA protein discrimination at mitochondria. TA proteins must sort correctly to various organelles. We will develop new biochemical tools to identify mitochondrial receptors and mechanisms that differentiate between ER and mitochondrial TA proteins.

- **Bryan Spring, Ph.D.**
  Assistant Professor of Physics
  Northeastern University

  “Peering into Cancer Stem Cell Niches to Guide Suppression of Multiple Signaling Loop Pathways”

  Key Words: Cancer, Cell signaling, Microscopy, Fluorescence, Protein engineering

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Despite advances in cytoreductive surgery, chemotherapy and targeted therapies, survival of glioblastoma (GBM), a malignancy of the brain, remains dismal due in part to tumor heterogeneity and complex molecular signaling loops among specific cancer cell phenotypes and their stromal signaling partners. Cancer cell–stromal cell niches (a specialized local microenvironment that regulates cell phenotype) promote GBM tumor growth, cancer cell survival and invasion. Presently, the tumor microenvironment is either modeled in vitro or probed by ex vivo biochemical and immunostain imaging assays to identify key cell types and signaling mediators involved in these cellular niches. Due to a lack of tools, it is presently not possible to record hetero-cellular signaling cascades in living systems. This proposal boldly and ambitiously develops new imaging technology that will enable multiplexed visualization of these coordinated signaling events with high spatiotemporal resolution in patient-derived xenograft mouse models of human GBM. First, we introduce new concepts to synthesize modular Förster resonance energy transfer (FRET) protein biosensors for secreted factors and intracellular kinases that carry out the mechanics of signal transduction. These biosensors are injected into the blood stream for specific tumor cell-type-targeted delivery, where the sensor domains escape from endosomes and are released within the cytosol to report selectively on the expression and activation of specific cytokines and kinases. Second, new instrumentation concepts are introduced to achieve multiplexed, quantitative FRET imaging microendoscopy building on our recent progress in developing miniature, cellular-resolution fluorescence imaging instrumentation capable of multiplexed molecular imaging of tumors deep within the body. Current preclinical and clinical imaging technologies cannot probe these microscopic cellular niches, a blind spot in our present approach to cancer therapy. We anticipate that this new paradigm for visualizing cancer-stromal cell signaling will guide development of novel multi-molecular targeted therapies—overcoming cell signaling network cross talk and compensation—to suppress signaling loops that promote treatment resistance, escape and recurrence.