Program in Cellular and Molecular Medicine
at Boston Children's Hospital

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

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APRIL 2025 NEWSLETTER

About PCMM

The Program in Cellular and Molecular Medicine (PCMM) is a research program at Boston Children's Hospital (BCH) recognized worldwide for its discoveries that increase the body's ability to fight disease and to heal. The breakthroughs of PCMM scientists are greatly increasing our understanding of the influence of immune defense and inflammation on medical discovery, healthcare, and disease management. Additionally, other primary areas of research include a) adhesion molecules and inflammation, b) stem cells, c) structural biology, and d) new technology development and usage. Our investigators are academically affiliated with Harvard Medical School. In this first issue of the PCMM newsletter, we summarize the news from the past year and will present monthly updates from now on. Please contact Colin Smith (colin.smith@childrens.harvard.edu) and Vera Gaun (vera.gaun@childrens.harvard.edu) for questions and suggestions for future newsletters.

In this issue

Research Highlights

In or out of the loop: A tale of two immunoglobulin loci

Almost 50 years ago, it was discovered that the immune system can reshuffle gene fragments in antibodies' variable regions, giving them the ability to recognize nearly all pathogens. Throughout his career, Frederick Alt, PhD, has revealed multiple aspects of this process, known as V(D)J recombination. The newest work from his lab, in *Nature*, solves a long-standing question, revealing that distinct mechanisms generate heavy chain versus light chain gene rearrangements during V(D)J recombination. *Yiwen Zhang, Xiang Li, and Hongli Hu were co-first authors of the paper and Frederick Alt and Hongli Hu were co-corresponding authors.* *(continued on page 2)*

(continued from page 1)

Frederick Alt and instructor Xiang Li.

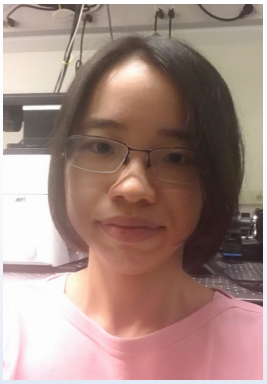
Former PhD student Yiwen Zhang, instructor Hongli Hu, and Frederick Alt.

A New Tool Could Exponentially Expand Our Understanding of Bacteria

Combining powerful genomic-scale microscopy with a technical innovation, the lab of Jeffrey Moffitt, PhD, is capturing what genes bacteria turn on in different situations and different spatial environments. Their work may give clues to how bacteria become more virulent, resist antibiotics, and more. Ari Sarfatis, Yuanyou Wang, PhD, and Nana Twumasi-Ankrah in the Moffitt Lab were coauthors on the paper, published in *Science*. BCH summary article can be found here.

Jeff Moffitt

Ari Sarfatis



Yuanyou Wang

Nana Twumasi-Ankrah

Jhullian Alston (Photo: Michael Goderre/BCH)

Bringing Order to Disorder: Jhullian Alston, PhD

Jhullian Alston, PhD, in the PCMM lab of Taekjip Ha, PhD, uses biophysics techniques to study disordered proteins, which don't fold into orderly structures and currently can't be targeted with drugs — something he hopes to change. He is currently focused on fusion proteins, which drive some childhood cancers. BCH summary article can be found here.

Postdoctoral Fellow and Graduate Student Awards

Jhullian Alston, PhD, a postdoctoral fellow in Taekjip Ha's laboratory, has been named an HHMI Hanna Gray Fellow. His research focuses on the role of intrinsically disordered protein regions, which lack fixed structure, in transcription factor function. Specifically, he studies disordered transcriptional activation domains in fusion oncoproteins such as PAX3-FOXO1, a driver of the pediatric cancer alveolar rhabdomyosarcoma. By combining single-molecule FRET and computational biophysics, Jhullian investigates how disordered regions and folded domains within PAX3-FOXO1 cooperate to regulate DNA binding and transcriptional activation, with the goal of developing innovative methods to target disordered regions for therapeutic benefit.

Camille Le Gall, PhD, a postdoctoral fellow in Hidde Ploegh's laboratory, has received the American Heart Association Postdoctoral Fellowship. Malaria is a mosquito-borne disease caused by apicomplexan *Plasmodium falciparum* (*P. falciparum*) parasites. Half of the world's population in developing (sub) tropical regions remains at risk. Infection can lead to stroke and severe cardiovascular complications, either directly or via hematological disorders. In the context of this work, Camille proposes an inexpensive, dual-function therapeutic strategy to provide immediate therapeutic benefit while also inducing long-term immunity to malaria in case of an infection, by using single domain antibodies targeting *P. falciparum*. Successful outcomes from this work will identify novel strategies for treating malaria that induce long-lasting immune responses.

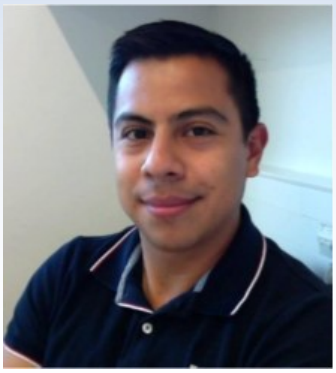
Molly Parsons, PhD, a postdoctoral fellow in Sun Hur's laboratory, was awarded an NIH Ruth L. Kirschstein NRSA postdoctoral fellowship. The innate immune effector TRIM56 restricts a surprisingly broad range of viruses, including influenza A, hepatitis B, and HIV-1 viruses, but its antiviral mechanisms are varied and poorly understood. After establishing an antiretroviral functional assay for

TRIM56, Molly recently found that the E3 ubiquitin ligase domain significantly contributes to its anti-HIV-1 activity. Additionally, the C-terminal domain of TRIM56 is a putative nucleic acid binding domain. Molly's initial crosslinking and pulldown experiments suggest that TRIM56 associates with regulatory domains of the HIV-1 RNA genome that are important for translation and for packaging, which suggests possible modes of action and targets for TRIM56-mediated ubiquitination. In this project, Molly will use cellular, biochemical, and structural assays to elucidate a detailed mechanism for TRIM56 antiretroviral activity, and thus provide insight into the innate immune roles of this emerging class of antiviral effectors.

Aritra Bhattacherjee, PhD, a Research Fellow in Yi Zhang's laboratory, was awarded a Harvard Brain Science Initiative Postdoc Pioneers Grant. Emerging evidence indicates that chronic pain can worsen opioid addiction. Mechanistically, the prefrontal cortex (PFC) is thought to be instructive in the pain/addiction interaction, but its cellular heterogeneity and functional complexity presented a challenge in understanding the mechanisms of such interaction. Using single cell genomics techniques, Aritra recently identified a distinct spatially and molecularly-defined neuron subtype, the activation of which can aggravate pain and addiction. In his project, he will use a genetic mouse model that he created to study how chronic pain facilitates addiction liability through these neurons, which could reveal molecular mechanisms that can promote better understanding for potential therapeutic interventions.

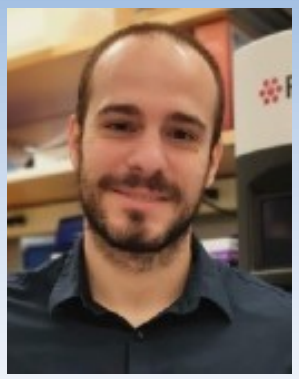
Paolo Cadinu, PhD, a Research Fellow in Jeffrey Moffitt's laboratory, was awarded a Charles A. King Trust Postdoctoral Research Fellowship. In addition to providing tissue structure, fibroblasts have been recently shown to affect the immune response and become inflammation-associated fibroblast (IAFs). By using spatial transcriptomics, Paolo has recently shown an unprecedented IAF diver

sity within the gastrointestinal (GI) tract of a colitis mouse model. However, it still remains unknown how these IAFs contribute to tissue repair, interact with other cell types, and the extent to which they retain memory of the insult. To address this question, he will create a comprehensive atlas characterizing these heterogenous fibroblast populations further, to potentially uncover mechanisms of tissue repair that can be targeted in treatment of gut-related diseases.



Uriel López-Sánchez, PhD, a Research Fellow in Timothy Springer's laboratory, was awarded a Charles A. King Trust Postdoctoral Research Fellowship. Integrins α4β1 and α4β7 are cell surface receptors that play essential roles in cell adhesion and migration and are involved in processes such as circulation of immune cells during inflammation. However, the mechanism of how these integrins affect cell migration remains not fully understood. Using biochemical, structural, and cellular approaches, Uriel's research aims to elucidate the principles underlying the conformational changes in α4β1 and α4β7 and to clarify how these changes influence their ability to control immune cell migration. Insights gained from this study may contribute to accelerating the development of new drugs and improvement of therapeutics for conditions such as multiple myeloma and inflammatory bowel diseases.

Yingying Zhang, PhD, a Postdoctoral Fellow in Michael Carroll's laboratory, was awarded a Brain and Behavior Research Foundation Young Investigator Grant. Genetic studies have implicated an increase of an innate immune system protein C4A as a major risk factor in schizophrenia. Yingying has established a mouse model of C4A overexpression, in which complement-mediated microglia excessively prune synapses. Additionally, other schizophrenia-like phenotypes were observed: increased monocyte infiltration into the choroid plexus (ChP), hydrocephalus, dramatically enlarged cerebral ventricles (ventriculomegaly), and subsequent behavioral anomalies. In this study, Yingying will probe the complement overactivation-mediated mechanisms underlying the observed pathology and test for a causal relationship between ChP inflammation and schizophrenia-like neuropsychiatric manifestations.



Pietro Fontana, PhD, an instructor in Hao Wu's laboratory, was awarded a 2024 Boston Children's Hospital OFD/BTREC/CTREC Faculty Career Development Fellowship. He will continue his research on the mechanistic study of innate immune pathways, with a particular focus on immunogenic cell death and the role of gasdermin D (GSDMD) in this process. While GSDMD has been recognized for its involvement in pyroptosis, its broader functions and potential roles in immune regulation have not been fully explored. He will use small molecule agonists to induce cancer cell death in a GSDMD-dependent manner and investigate combinatorial therapy with existing cancer therapies. Overall, the project contributes to filling gaps in knowledge about immunogenic cell death, the potential of agonizing GSDMD for anti-tumor immunity, the development of targeted immunotherapies, and mechanistic understanding of GSDMD regulation.

Ibraheem Alshareedah, PhD, a postdoctoral fellow in TJ Ha's laboratory, has been named a Jane Coffin Childs-HHMI Fellow. In the area of DNA break repair, it is known that BRCA2 loads RAD51 onto single stranded DNA (ssDNA), yet homologous recombination still occurs in BRCA2-mutant cancers, suggesting that there is redundancy in this pathway. Hypothesizing that RAD52 nanoclusters in cells recruit RAD51 and load it onto ssDNA, even in the absence of functional BRCA2, he will examine if RAD51, ssDNA, and other DNA-break repair proteins are recruited to RAD52 nanoclusters in cells, and will then determine which RAD52 protein features are required for cluster formation. By understanding the partial redundancies in the DNA repair pathway, his research may reveal novel targets for treating BRCA2-mutant cancers.

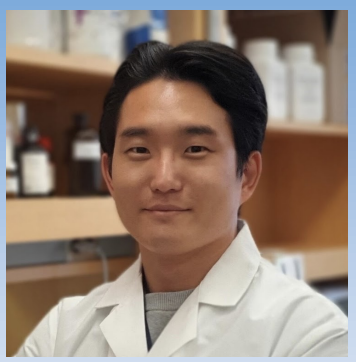
Man Wu, PhD, a postdoctoral fellow in Hao Wu's laboratory, has received an Irvington Postdoctoral Fellowship from the Cancer Research Institute. She will focus on the mechanism for LPS-rendered NEK7-independent NLRP3 inflammasome activation in human macrophages. NEK7 was previously found as a scaffolding protein mediating NLRP3 cage structure opening to further assemble into supracomplexes together with ASC and caspase-1, which is the hallmark of NLRP3 inflammasome activation. Using biochemical, structural, and cellular imaging approaches, she aims to interrogate whether and what condition of NLRP3 activation requires NEK7 and the mechanism of NEK7-indenpendent NLRP3 activation, which will provide a comprehensive understanding of NLRP3 activity to guide future therapeutic intervention.

Saket Rahul Bagde, PhD, a Research Fellow in Timothy Springer's Laboratory, was awarded a Damon Runyon Fellowship Award. About 80–90% of all cancer cases develop in the epithelial tissue including the skin and organ linings. Saket is investigating how the disruption of the epithelial tissue integrity contributes to cancer metastasis. Hemidesmosomes (HDs) are velcro-like adhesive structures that anchor the epithelial cells to the underlying base layer, maintaining the integrity of the tissue. While disassembly of HDs is a normal process in wound healing, it can be exploited by tumor cells to detach and spread to other parts of the body. Saket will study how multiple components of HDs interlock like Lego blocks to make stable HDs in normal tissues, and how HDs are disassembled in cancer tissues.
Additionally, Saket aims to develop tools to support the growth of organoids, self-organizing mini-organs grown in a petri dish, used for studying disease progression. He will develop simple base layers that simulate supportive properties of the native base layer in organs to promote the growth of normal and cancerous organoids. This work will be valuable for growing patient-derived tumor organoids with potential applications in creating personalized cancer therapies.

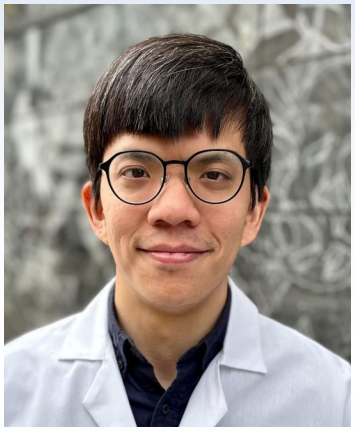
Liam Healy, a graduate student in Hao Wu's Laboratory, won an NIH F31 Ruth L. Kirschstein Predoctoral Individual National Research Service Award. Liam's research investigates the molecular mechanisms of pyroptosis, a form of programmed cell death crucial for innate immune defense. Specifically, he studies Gasdermin D (GSDMD), a key protein in this process, which forms pores in cell membranes upon cleavage by inflammatory caspases. His work focuses on understanding how GSDMD activation is influenced by post-translational S-palmitoylation and how reactive oxygen species (ROS) enhance this modification during inflammasome activation. Through biochemical and structural studies, he aims to resolve the mechanisms by which palmitoylation regulates intact and cleaved GSDMD pore formation and membrane damage. Ultimately, his work seeks to uncover therapeutic strategies for diseases involving dysregulated pyroptosis, such as sepsis, cardiovascular diseases, and neurodegenerative disorders.

Ph.D. Thesis Defenses

Dr. Jimin Kang of TJ Ha's Laboratory Successfully Defends his Ph.D. Thesis



On January 22, Dr. Kang successfully defended his thesis titled "SHARP Amplification of PCR-challenging Nucleic Acid Templates". His thesis seminar was held at Johns Hopkins University as part of the Jenkins Biophysics Program. Special thanks go to his review committee, in addition to TJ: Doug Barrick (JHU, Chair), Sua Myong, Wesley Wong, and Yaojun Zhang (JHU). We wish him continued success in his future endeavors!

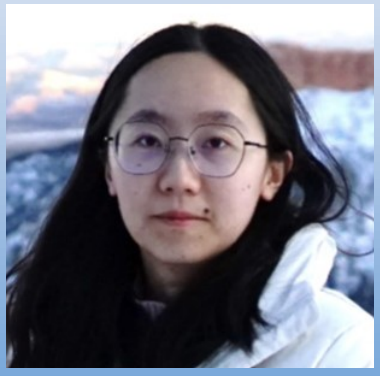


Dr. Ting-Wei Liao of TJ Ha's Laboratory Successfully Defends his Ph.D. Thesis

Titled "Exploring the Functional Kinetics of Biological Machines," his thesis seminar was held on December 9 at Johns Hopkins University as part of the Jenkins Biophysics Program. Special thanks go to his review committee, in addition to TJ: Sarah Woodson (Chair), James Berger, Carl Wu, and Stephen Fried. We wish him continued success in his future endeavors!

Dr. Rosalind Xu of Jeff Moffitt's Laboratory Successfully Defends her Ph.D. Thesis

Titled "Constructing a Spatially Resolved Single-Cell Reference Atlas of the Murine Gastrointestinal Tract with MERFISH," the successful defense took place at the end of August this year. We wish Rosalind continued success in her future endeavors!



Dr. Yiwen Zhang of Fred Alt's Laboratory Successfully Defends Her Ph.D. Thesis

Titled "Molecular Basis for Differential V(D)J Recombination Mechanisms in Immunoglobulin Loci," the thesis was successfully defended on November 12. Special thanks go to her review committee: Raul Mostoslavsky (Chair), TJ Ha, Shiv Pillai, and guest examiner Kefei Yu from Michigan State University. We wish Yiwen continued success in her future endeavors!

Faculty News

Julia Li joins PCMM

Julia Li, PhD, has joined the Boston Children's faculty as an Investigator in PCMM and the Harvard Medical School faculty as an Assistant Professor of Genetics. She joins us from University of California at San Diego, where she was a postdoctoral fellow in the laboratory of Don Cleveland and received the Damon Runyon-Dale F. Frey Award for Breakthrough Scientists. The mission of the Li Lab is to "uncover the missing link between repeat DNA, genome stability, and viral infection that underlies the etiology of virus-associated cancer and genetic diseases".

Judy Lieberman Elected to the American Academy of Microbiology

On behalf of the PCMM leadership, we are delighted to announce the election of Judy Lieberman, PhD, MD, to the American Academy of Microbiology! The Academy is the honorific leadership group within the American Society for Microbiology, and its official mission is "to recognize scientists for outstanding contributions to microbiology and provide microbiological expertise in the service of science and the public." Please join us in congratulating Judy for becoming a fellow of the Academy's Class of 2025!

Hao Wu elected to the National Academy of Medicine and named a 2025 fellow of the American Society for Biochemistry and Molecular Biology

Congratulations to Hao Wu, PhD, who was honored twice recently. First, she was elected to the National Academy of Medicine for her discovery of signalosomes, central organizing structures that have changed our understanding of innate immune signaling. Her work could lead to new therapeutic strategies for inflammation and cancer. Additionally, as a ASBMB Fellow, she was selected for "exceptional and sustained service to the society as well as accomplishments in research, education, mentorship, diversity and inclusion, advocacy and service to the scientific community." The society will recognize the 2025 class at its annual meeting on April 12–15 in Chicago. Congratulations, Hao!