2025 ABRCMS Career and Research Summit Graduate Student Speakers: Scientific Abstracts

Tuesday, February 25

- **Arianna D. Daniel,** Doctoral Candidate, Quantitative and Systems Biology, *University of California Merced*
- **Beatrice Milnes**, Doctoral Candidate, Molecular and Cellular Biology Program, Department of Biochemistry, *University of Washington*
- **Ashley Greenlee**, Doctoral Candidate, Biomedical Sciences Graduate Program, *The Ohio State University*
- **Sabina Kubayeva**, Medical Student, *SUNY Downstate College of Medicine*
- **Diego Torres Martinez**, Doctoral Candidate, Department of Cell and Developmental Biology, *Vanderbilt University*
- **Bahati Nkera**, Doctoral Candidate, Department of Neuroscience, *Brown University*
- **Brittany Gomez**, Doctoral Student, Department of Biomedical Informatics, *University of Pittsburgh*

Thursday, February 27

- **Emmanuel Chijindu Allwell**, Department of Microbiology, Doctoral Candidate, *The University of Tennessee, Knoxville*
- **Shima Moradpour,** Ph.D. Candidate, Department of Microbiology and Immunology, *SUNY Upstate Medical University*
- **Darnell Campbell**, Doctoral Candidate, Department of Biomedical Engineering, *Wake Forest University School of Medicine*
- **Caitlin Therien**, Doctoral Candidate, Department of Biomedical Engineering, *Columbia University*
- **Aleandra Ortiz Santiago**, Doctoral Student, Department of Anatomy and Neurobiology, *University of Puerto Rico Medical Sciences Campus*
- **Jeremy Boutin**, Doctoral Candidate, Molecular Plant Sciences, *Washington State University* #GoCougs
- **Isabella Sirit**, PhD Candidate, Department of Molecular Pathology and Immunology, *Vanderbilt University*
- **Maria Mercedes**, Graduate Student, *Icahn School of Medicine at Mount Sinai (ISMMS)*

Arianna D. Daniel

Doctoral Candidate, Quantitative and Systems Biology, University of California Merced

Functional Insights into Coccidioides Immunoevasive Proteins Using Computational Tools

Coccidioides species are soil-dwelling fungi endemic to arid regions of the Southwest US, Mexico, and parts of Central and South America. These fungi cause coccidioidomycosis, a severe respiratory disease ranked as a significant fungal pathogen by the World Health Organization. With infection cases expected to rise, there is an urgent need to understand the biology of these organisms. A substantial portion of the fungal genome contains uncharacterized hypothetical proteins, which may play pertinent roles in pathogenicity, immune evasion, and host modulation. In this work, we characterize and functionally annotate Coccidioides immitis hypothetical protein sequences using bioinformatics tools, revealing conserved functional domains, gene ontology classification, and enzyme commission values. We evaluated ~9k high-quality proteins based on homology scoring, conserved domains, subcellular localization, and physicochemical properties, providing insights into their potential roles in disease and pathogenicity. Our research contributes to the identification and characterization of novel Coccidioides proteins, facilitating drug design and therapeutic development. These findings advance our understanding of this important pathogen and may lead to improved strategies for managing coccidioidomycosis, a disease of increasing concern in endemic regions.

Beatrice Milnes

Doctoral Candidate, Molecular and Cellular Biology Program, Department of Biochemistry, University of Washington

Characterizing the Contribution of Metabolic Reprograming to Myeloid Populations during X. tropicalis Tail Regeneration

The capacity for tissue regeneration is highly variable across species. Successful cases require a coordinated cellular effort to close the injury site, generate requisite biomass, and pattern the newly formed tissue. This permissive environment is created in part by intracellular responses to lost or damaged body parts, including changes in the metabolic regulation and metabolite profile of the regenerate tissue. After tail amputation in Xenopus tropicalis tadpoles, an increased uptake of glucose is observed in the forming regenerative bud, and a switch to the pentose phosphate pathway (PPP) as its primary fate is required for successful regenerative outcomes. scRNA-seq data suggests that PPP enzyme expression is partitioned into distinct cell populations, specifically that of proliferating cells and the phagocytic myeloid lineage. Infiltrating immune cells have long been known to play a major role in mammalian wound repair and are critical to regenerative outcomes across species. In human cell lines, metabolic regulation has been linked to the repolarization of specific phagocyte populations. This work aims to test the central hypothesis that the metabolic program of myeloid cells is crucial for phagocytic innate immune driven regenerative outcomes in X. tropicalis. To define the primitive myeloid cells present in tadpole life stages, we performed a transcriptomics analysis which suggests distinct gene expression profiles of myeloid cell populations during both developmental and post-injury time points. Live imaging was used to identify the onset of phagocytic cell recruitment to the wound site, revealing a temporally defined requirement of myeloid cell infiltration after amputation. Phagocytes were shown to be required for tail regeneration after depletion by intravenous clodronate liposome injection. Systemic phagocytic cell depletion showed a direct correlation to regenerative ability by inhibiting tail elongation and reducing myeloid marker expression. Our next goal is to clarify the contribution of immune cells to the previously seen regenerative defects in PPP knockdown individuals. These ongoing experiments will quantify outputs of innate immune infiltration and activity, such as a matrix metalloprotease remodeling and scar tissue deposition, during regeneration in the presence of small-molecule antagonists for key PPP enzymes. This data leads us to conclude

that there is cellular heterogeneity within the metabolic reprograming initiated during X. tropicalis tail regeneration as the PPP is required for both the previously defined proliferative role and localized immune responses. Future work will determine the mechanism by which PPP activity directs specific myeloid cell activity during tail regeneration and identify potential roles by which metabolic sub-populations contribute to the regenerative milieu.

Ashley Greenlee

Doctoral Candidate, Biomedical Sciences Graduate Program, The Ohio State University

Investigating the Cardiotoxic Mechanisms of Newer Generation Tyrosine Kinase Inhibitors

Background: Tyrosine kinase inhibitors (TKIs) have been monumental in improving cancer survivorship but have also led to serious side-effects such as arrhythmias and heart failure. Studies elucidating TKI-cardiotoxicity mostly use older drugs such as sunitinib or sorafenib. However, investigating cardiotoxicities using TKIs that are currently used in the clinic such as cabozantinib have been rarely studied. Our goal is to explicate the cardiotoxic profile of cabozantinib in vitro.
Methods: H9c2 ventricular rat myoblasts were treated with cabozantinib (0.8 nM - 4 uM) for up to 72-hours. To understand the cellular changes, multiple activity assays were used to assess cell viability, cell death, apoptosis, and reactive oxygen species (ROS).
Results: In H9c2 myoblasts, cabozantinib led to significant decreases in viability and increases in cell death after 24 hours. Additionally, cabozantinib led to significant increases in capsase activation, decreases in the anti-apoptotic protein Bcl-2, and increases in mitochondrial ROS and cytosolic ROS at different timepoints.
Conclusions: Cabozantinib led to significant decreases in viability in H9c2 myoblasts. Cabozantinib additionally showed dysregulation in apoptosis and increased ROS activity. Future research will expand on transcriptional and morphological changes after cabozantinib treatment through the use of RNA-sequencing and cell painting. Additionally, inhibitors of ROS and apoptosis will be incorporated to determine if cell viability is increased and cell death is decreased. Finally, confirmation of these finding in H9c2 cells will be performed in human-induced pluripotent stem cell cardiomyocytes (hiPSC-CMs). Overall, this study will allow us to establish effective cardioprotective treatments for cancer patients.

Sabina Kubayeva

Medical Student, SUNY Downstate College of Medicine

Development of an Advanced Care Planning Pilot Module for New York City Community Members

Advanced care planning (ACP) is the process of planning for future medical decisions in the event that an individual is unable to do so independently. This process includes completing advanced directives (AD), legal documents that detail patients' medical and financial wishes. The lack of knowledge around ACP and AD was identified as a community-need by the Tisch Cancer Institute Community Outreach and Engagement (COE) Department at Mount Sinai. In response, we developed a community-based educational module to address this gap in knowledge. Curriculum development was guided by an in-depth literature review and collaboration with an interdisciplinary team of health educators, social workers, and palliative care experts. The workshop comprised three elements: a 30-minute slideshow presentation, a 2-page glossary and resource handout, and a healthcare proxy AD form. It was presented at five community organizations (n=50) selected based on existing relationships with the COE program. The presentation addressed the importance of ACP and ADs, debunked

myths, and included interactive questions and reflective exercises to engage participants. To evaluate the workshop's impact, acceptability, and gather feedback, we used a previously validated, standardized survey tool that included a 5-point Likert scale and two open-ended questions. Pilot results suggest that our novel ACP workshop is effective in enhancing participants' understanding and self-efficacy in ACP.

Diego Torres Martinez

Doctoral Candidate, Department of Cell and Developmental Biology, Vanderbilt University

Exosomes promote extracellular matrix assembly in breast cancer cells

Exosomes are small extracellular vesicles (SEVs) that are critical for intercellular communication and promote cancer progression and metastasis. Tumor cell-derived SEVs carry distinctive functional cargoes, including transmembrane adhesion receptors known to bind extracellular matrix (ECM) proteins. We previously found that the ECM protein fibronectin binds to cancer cell exosomes in an adhesive form and that the fibronectin-exosome complexes promote cell migration. Those data suggested that exosomes may, in fact, assemble soluble ECM into an insoluble adhesive form. To test whether cancer exosomes can indeed promote ECM assembly, the exosome secretion regulator, Rab27a, was stably knocked down (KD) in 4T1 breast cancer cells. Rab27a KD cells were grown for 3 or 6 days and cell-derived ECM samples were then collected for biochemical characterization and immunofluorescence analysis. Western Blot analysis of the cell-derived ECM deposited by Rab27a KD cells revealed lower levels of perlecan and nidogen compared to control cells. Moreover, immunofluorescence analysis of collagen IV content of the cell-derived ECM demonstrated that Rab27a KD cells deposit less ECM compared to the control. Taken together, these studies suggest that exosome secretion may contribute to ECM assembly. Future studies will determine if SEVs isolated from parental 4T1 cells can rescue the ECM assembly defects of Rab27a KD cells and identify the EV cargoes that mediate exosomal ECM assembly.

Bahati Nkera

Doctoral Candidate, Department of Neuroscience, Brown University

From Receptor to Behavior: Introducing Vmn2rC mice as a model for studying pheromone-mediated social behaviors

Pheromones are chemicals emitted from one member of a species and detected by others where they elicit stereotyped physiological and behavioral responses. Pheromone signaling is initiated as they bind to specific receptors on sensory neurons in the vomeronasal organ (VNO). These receptors belong to two families: Vmn1r or Vmn2r. Like sensory neurons in other olfactory subsystems, apical vomeronasal sensory neurons (VSNs) adhere to the one-receptor-per-neuron rule and express only one Vmn1r per neuron. Conversely, basal VSNs co-express at least one receptor from the Vmn2rC class and another from the Vmn2rABD classes in a non-random fashion. However, the role of Vmn2rCs and the functional significance of Vmn2r co-expression has yet to be elucidated. We hypothesize that Vmn2rCs are required for the cellular and behavioral responses mediated by Vmn2rABD ligands. To test our hypothesis, my lab has used recent advances in gene-editing technology to establish Vmn2rC-/-, a mouse line where the gene cluster comprising the entire class C family of Vmn2rs is deleted. My project utilizes this Vmn2rC-/- line and examines the effect of deletion of Vmn2rCs on the display of stereotyped parental and aggressive behaviors in adult mice. Specifically, I have assessed pup retrieval and territorial aggression, as these are two sexually dimorphic, innate behaviors that are mediated by parental

experience. I have assessed these innate social behaviors by performing pup retrieval and resident-intruder assays on virgin male and female Vmn2rC-/- and their heterozygous and wild-type littermates. After assessing these mice as virgins, I paired them with a wild-type breeding mate, and upon birthing a litter, I reassessed pup retrieval and territorial aggression. Interestingly, I have seen that territorial aggression is decreased in Vmn2rC-/- males. Further, parental behavior in virgin Vmn2rC-/- males deviates from their wild-type littermates in that they retrieve—as opposed to ignore—foreign pups. However, upon sexual and parental experience, Vmn2rC-/- fathers show increased parental behavior when compared to their virgin state—an improvement that's also seen in their wild-type littermates. For females, I have not seen any differences in parental behavior when comparing genotype and sexual experience. My results have provided insight into the function of Vmn2r co-expression in pheromone detection and the initiation of pheromone-mediated social behaviors. Further, our newly established Vmn2rC-/- mouse line along with the antibodies we have generated against various Vmn2rs contribute innovative tools for future studies of pheromone signal processing.

Brittany Gomez

Doctoral Student, Department of Biomedical Informatics, University of Pittsburgh

Enhancing Artery Segmentation in Placental Analysis with Hyperspectral Imaging and Wavelet U-Net

Accurate segmentation remains a challenging task in biomedical image analysis due in part to small datasets and the limitations of traditional techniques. Hyperspectral imaging (HSI) offers a promising approach to segmentation by leveraging detailed spectral data of the tissues being segmented. It has the potential to enhance usual segmentation methods by responding to tissue chemical composition. The near-infrared spectrum's ability to penetrate tissue further aids in analyzing subsurface features, opens the door to more detailed physiological assessments and broader clinical applications. This project aims to leverage HSI to improve artery segmentation in placental analysis using a wavelet-enhanced U-Net model. The objective is to utilize the rich spectral information inherent in HSI to reduce the number of training samples needed for high performance segmentation. Merging the spatial hierarchies of U-Net with the signal processing power of Haar wavelets, the approach seeks to achieve finer and more detailed segmentation, especially when using small biomedical datasets. The study analyzed 101 hyperspectral images from four human placentas, covering veins, arteries, stroma, and the umbilical cord. Initially, each image contained 37 to 77 spectral slices, which were reduced to 78 images with 3 slices each after preprocessing. The wavelet U-Net was trained to perform the segmentation task using 70 of these preprocessed images. Results showed that the model effectively segmented arteries, often extending beyond the manually annotated ground truth labels. This result suggests that the model leverages the rich spectral data in HSI to identify features undetectable by the naked eye, highlighting its ability to capture nuanced arterial signals in mixed tissue samples. Combining HSI with a wavelet-enhanced U-Net represents a promising method for biomedical imaging. The preliminary results reported here provide support that the HSI is a promising tool for accurate and detailed tissue segmentation, even with limited training data. Additional investigation is warranted.

Emmanuel Chijindu Allwell

Department of Microbiology, Doctoral Candidate, The University of Tennessee, Knoxville

A Tale of Tailocins: Defining the role of the hypervariable region within tailocin-encoded locus for targeted antimicrobial activity

Increasing resistance to antibiotics is a top global health issue. Headless phage-like structures known as tailocins are one potential alternative to antibiotics. Tailocins are encoded in bacterial genomes and resemble P2 phage-like contractile structures. They mediate inter-microbial competition by binding to target cell surfaces through a tail fiber protein, and eliminating target cells by causing membrane depolarization. Tailocin are composed of tail sheath, tube, fibers, and baseplate. The tail fibers comprise a DUF3751 domain mediating attachment to the baseplate and a C-terminal receptor-binding domain (RBD), crucial for binding to the target bacterial cell surface. Xenorhabdus nematophila are bacterial mutualists of entomopathogenic nematodes, and all known strains encode a tailocin, each with a main-tail-fiber protein-encoding gene that varies across strains in the RBD, with the predicted consequence of varying target strain specificity. Each X. nematophila strain also contains a variable region within the tailocin locus, which we have termed the intervening sequence (IVS), between the genes encoding the main tail fiber and the tail sheath. IVS genes include those predicted to encode RBD without the DUF3751 domain and those predicted to encode glycosyl transferases (GT). The role of genes within the IVS in tailocin activity and sensitivity is presently unknown. We hypothesize the IVS serves as a reservoir for alternate receptor binding domains that can expand tailocin target range. In addition, we hypothesize that free-standing RBDs could serve as defensive molecules by binding to the producer cell surface and blocking binding by tailocins with the same RBD. We hypothesize that GT within the IVS may confer tailocin immunity by modifying cell surface receptors. To test these hypotheses, IVS deletion mutants were created in two X. nematophila strains (ATCC19061 and F1). These mutants exhibited increased sensitivity to tailocins compared to their wild-type counterparts. This does not appear to be due to loss of tailocin production, since transmission electron microscopy of IVS mutant supernatants revealed visible, intact tailocins. Furthermore, deletion of the IVS did not prevent expression of transcripts, detected using reverse-transcriptase PCR, from the flanking genes encoding the main tail fiber or the tail sheath proteins. These findings suggest that hypervariable regions within tailocin-encoding loci contribute to both protection from and activity of tailocins. We will discuss our ongoing research to unravel the molecular mechanisms underlying these observations.

Shima Moradpour

Ph.D. Candidate, Department of Microbiology and Immunology, SUNY Upstate Medical University

Human cytomegalovirus spatially regulates cellular transcription factor Heat Shock Factor 1 to promote the establishment of a silent/quiescent infection in primary monocytes

Human cytomegalovirus (HCMV) is a member of the betaherpesviridea family that establishes lifelong infection in nearly 80% of the world's population. Following primary infection, HCMV infects circulating monocytes to spread throughout the infected host. Early infection of monocytes is characterized by the lack of viral replication, which allows the virus to evade the immune response. How HCMV establishes a quiescent infection remains unclear. Heat Shock Factor 1 (HSF1) is a cellular stress-responsive transcription factor that promotes the expression of viral genes following infection. In this study, we showed that HCMV infection induces a rapid and unique activation of HSF1 in monocytes. Surprisingly, HCMV-activated HSF1 remained cytoplasmic, which contrasts with the nuclear re-localization of HSF1 following heat shock (HS). Consistent with its role in promoting HCMV replication, forced re-localization of HSF1 prior to HCMV infection with HS treatment prevented the establishment of a quiescent infection and led to the early expression of viral proteins and progeny virus production. Accordingly, inhibition of HSF1 with a small-molecule inhibitor or genetic knockdown of HSF1 with siRNAs prevented HCMV replication in HS-treated, HCMV-infected monocytes. Moreover, we validated that forced nuclear HSF1 directly interacted with major immediate early promoter (MIEP) to induce IE protein abundance. Mechanistically, we found that inhibition of the chaperone HSP90 allowed for re-localization of HSF1 in HCMV-infected monocytes. Together this data suggests a critical role of HSF1 localization in the establishment of HCMV quiescent infection in primary monocytes.

Darnell Campbell

Doctoral Candidate, Department of Biomedical Engineering, Wake Forest University School of Medicine

Breast Biopsy Deployment System

In breast biopsies, accurate marker placement is crucial for imaging and potential surgeries. Current systems forcefully deploy markers, causing migration and increasing the risk of incomplete excisions. To address this, we developed a three-pronged approach: an elastic biodegradable scaffold to encapsulate the marker, a transparent breast tissue phantom for real-time visualization, and a non-forceful deployment device.

We developed a biodegradable POC scaffold for fiducial markers, with properties fine-tuned by adjusting crosslinking. We used 400µm NaCl as porogens for elasticity and pore size. POC compression and expansion were optimized, revealing self-healing capabilities. An optically transparent breast tissue phantom was created using Dermasol for real-time demonstration of POC scaffold deployment. Lastly, a 3D-printed device was developed for passive marker deployment, reducing inaccurate placement. This device was used in in vivo testing on Sprague-Dawley rats, with marker retention and migration monitored via weekly x-ray imaging.

POC scaffolds, heated to 80 ^{o}C for various durations, showed decreased compressibility with longer treatment. Scaffolds exposed for 2, 4, and 6 hours were selected for degradation tests. Notably, 2-hour heated scaffolds were fully degraded after 60 days in SBF at 37 ^{o}C. These scaffolds, tested with 3T3 mice fibroblasts, showed no significant viability change, making them suitable for in vivo experiments. POC encapsulated markers were successfully deployed using both CDD and WFDD, limiting marker migration. The WFDD passively released POC, MM, and SM markers, further reducing migration compared to the CDD method. Data showed a 52% reduction in POC marker movement compared to SM, and a 41% reduction for WFDD compared to CDD.

We presented solutions for breast biopsy deployment challenges. Our POC scaffolds, especially those heated to 80 ^{o}C for 2 hours, showed promise in biocompatibility, degradation rate, cell viability, and reducing migration. They were deployed using both a CDD and WFDD, with the latter significantly reducing marker migration. The use of POC encapsulated markers and WFDD resulted in a substantial reduction in marker migration.

Caitlin Therien

Doctoral Candidate, Department of Biomedical Engineering, Columbia University

Advances in Single Molecule Oligopeptide Fingerprinting Based on Templated Self-Assembly of Oligonucleotide Structures

Introduction
Our objective is to enable massively parallel identification and quantification of single oligopeptide molecules in small samples. The development of such method will be complementary to the mainstay technologies for large-scale protein sequencing and quantitation, such as mass spectrometry, and would enable routine analysis of small amounts of protein as well as variations in posttranslational modification (PTMs).

Materials and Methods
Single molecule measurements were obtained via TIRF microscopy using fluorescently labeled strands of DNA in solution. Ensemble measurements were obtained via biolayer interferometry. Streptavidin coated biosensors

were first loaded with the biotinylated peptide-oligonucleotide conjugate and the subsequent binding and unbinding of the ketoboronate-oligonucleotide conjugate was observed by measuring changes in layer thickness at the sensor tip. Surface plasmon resonance was also used to observe the differences in binding affinity of our various oligonucleotide conjugate pairs. Gold sensor chips modified with biotin-PEG moieties were first loaded with avidin to immobilize the biotinylated peptide-oligonucleotide conjugate on the surface. The binding and unbinding of the ketoboronate-oligonucleotide conjugate was then observed through changes in the refractive index at the surface of the sensor chip. These changes in surface thickness reveal the contributions from various functional moieties.

Results, Conclusions, Discussions
The behavior of the oligonucleotide conjugate pairs was observed and cross verified across single-molecule and bulk measurements (biolayer interferometry via Octet and surface plasmon resonance via Bionavis). We found an enhanced affinity upon the addition of the organic ion receptor-peptide interaction across all platforms. However, the multiphasic nature of the unbinding curves complicates the interpretation of the results. Control experiments show that undesired interactions between probes and sensor chips may be present for both the biolayer interferometry and the SPR experiments. Due to the proprietary nature of the sensor chip designs, the origin of the cross reactivity is difficult to identify. Overall, the experiments highlighted the strengths of the single molecule approach, to which we plan to return. Additionally, we made progress in the isolation of aptamers for C-terminal, N-terminal and internal residues of amino acids in peptides.

Aleandra Ortiz Santiago

Doctoral Student, Department of Anatomy and Neurobiology, University of Puerto Rico Medical Sciences Campus

Modulation of Microglia Phenotype by Glioblastoma Derived Exosomes

Glioblastoma, specifically the IDH-wildtype (GBM) variant, represents the most common type of primary malignant brain tumor in adults, with over 300,000 new diagnoses annually. As a grade 4 diffuse glioma, GBM is highly aggressive, with a typical survival duration of only 12-15 months following diagnosis. Standard treatments, including surgery, chemotherapy, and radiation, encounter significant obstacles such as incomplete tumor removal, substantial genetic heterogeneity, the challenge of surpassing the blood-brain barrier (BBB), and an immunosuppressive tumor microenvironment. These challenges underscore the need for a deeper understanding of GBM mechanisms to improve early diagnostic and therapeutic approaches. Microglia, the central nervous system's primary immune cells, play a crucial role in the GBM tumor microenvironment. They can adopt either pro-inflammatory or anti-inflammatory states in response to the tumor environment, though the precise mechanisms and their impact on tumor progression are not well understood. We propose to study exosomes as key intercellular communication in microglial polarization and GBM progression. We hypothesized that glioblastoma-derived exosomes (GDE) would induce an anti-inflammatory polarization in microglia leading to increased levels of anti-inflammatory proteins and cytokines, thereby promoting GBM growth and survival. To test this, we cultured U-87MG glioblastoma cells, isolated exosomes from their culture medium using ExoQuick-TC ULTRA and confirmed their presence with Western Blot and ExoAB kit. We then exposed HMC3 microglia cultures to GDE for 24 hours, fixed the cells with 4% PFA, and assessed the expression of CD206 (an anti-inflammatory marker) and CD68 (a pro-inflammatory marker) using fluorescence microscopy. Preliminary results indicated that GDE exposure led to increased CD206 expression in a concentration-dependent manner compared to unexposed microglia, while CD68 showed no changes in expression. These results suggest that GDE may contain factors that drive microglia towards an anti-inflammatory phenotype, which could facilitate glioblastoma progression. Our next objective is to evaluate morphological changes in microglia associated with this polarization.

Jeremy Boutin

Doctoral Candidate, Molecular Plant Sciences, Washington State University #GoCougs

Unravelling the Mechanism of Cannabis sativa Acyclic Monoterpene Synthases

Monoterpene synthases (MTS) are highly versatile enzymes that catalyze the first committed step in pathways toward terpenes, the most structurally diverse class of plant products. Even though MTSs have been identified and characterized, the mechanism controlling product selectivity is not fully understood. While some MTSs are remarkably specific, others release a larger number of products from the same substrate. The following research represents a site-directed mutagenesis study that expresses select Cannabis sativa terpene synthases in E. coli with and without targeted mutations and assess their catalytic properties by quantifying the diversity of terpenes produced. Recent studies have demonstrated that different MTSs appear to stabilize carbocation intermediates differentially, thus leading to the formation of different monocyclic or bicyclic products, supporting the theory that increased carbocation stability leads to increased structural complexity of products. To investigate the mechanism of MTSs that form acyclic products in C. sativa, we compared the acyclic MTSs, β-myrcene synthase (CsTPS15CT; Cs-BMCS) and (E)-β-ocimene synthase (CsTPS38FN; Cs-EBOS) to the monocyclic MTS (-)-limonene synthase (CsTPS14CT; Cs-LMNS) from C. sativa. To test the hypothesis that carbocation intermediates are not sufficiently stabilized by acyclic MTSs experimentally, we've converted Cs-LMNS to a variant enzyme that produces β-myrcene or (E)-β-ocimene as a primary product, through active site targeted point mutations. In parallel, to further support our hypothesis, we've also converted Cs-BMCS and Cs-EBOS to variant enzymes that form (–)-limonene as a primary product, through active site targeted point mutations. Molecular dynamics (MD) simulations were then be employed to evaluate carbocation stabilization among our MTS mutants. Our structure-function analysis has allowed for the identification of active site residues that confer product specificity in acyclic MTSs. Additionally, our research has the implication of enhancing desired terpene yield within cannabis cultivars.

Isabella Sirit

PhD Candidate, Department of Molecular Pathology and Immunology, Vanderbilt University

Translocation of ADP-heptose, dependent on NF-kB activation, by Helicobacter pylori drives STING suppression

Helicobacter pylori infection and the ensuing gastric inflammatory response are the strongest known risk factors for gastric cancer, the 5th leading cause of cancer-related death in the world. One of the most intensively studied H. pylori strain-specific microbial virulence determinants is the cytotoxin-associated gene (cag) pathogenicity island (cag PAI), which encodes a type IV secretion system (T4SS) that can translocate the oncoprotein CagA, microbial DNA, and LPS precursors, such as ADP-heptose, into host epithelial cells. Microbial DNA classically activates the intracellular DNA sensor stimulator of interferon genes (STING); however, STING deficiency is associated with gastric carcinogenesis and our laboratory has previously demonstrated significant suppression of STING activation following infection with H. pylori in vitro, ex vivo, and in vivo. RNA-seq data derived from murine gastric tissue further revealed H. pylori-dependent upregulation of a known STING inhibitor, TRIM30a. Tripartite motif (TRIM) proteins are functionally diverse regulators of biological processes, shown to function as E3 ubiquitin ligases, capacity to inhibit STING function. We hypothesize that translocation of ADP-heptose via the cagT4SS of H. pylori leads to increased production of STING inhibitors, resulting in the suppression of STING following infection and increased inflammation and risk for gastric cancer development in the host. Multiple sequence alignment revealed that mouse-specific protein TRIM30a shares greatest homology to human-specific proteins TRIM5, TRIM6, and TRIM22. Performing a tissue micro array (TMA) on human gastric tissue revealed that among the human-specific TRIM proteins identified, TRIM5 and TRIM22 were shown to be

upregulated in gastric epithelial cells (GECs) in intestinal-type tumor samples, compared to GEC in non-tumor samples. Another cag T4SS substrate, ADP-heptose triggers various signaling pathways, such as NF-kB following translocation into host cells, in an ALPK1/TIFA-dependent manner. Since TRIM proteins are largely induced by NF-kB, our aim is to define the role that H. pylori ADP-heptose exerts on induction of STING-inhibiting TRIM proteins. Directly relevant to this aim, AGS and HEK293 cells transfected with NF-kB-specific and STING-specific reporters, respectively, displayed increased NF-kB expression in an ADP-heptose dose-dependent manner and decreased expression of STING. Further, use of a specific NF-kB inhibitor, BAY-11-7082, markedly decreased expression of these targets, implicating NF-kB in the production of potential STING inhibitors in response to H. pylori. These data further implicate the role of ADP-heptose inducing an inhibitory effect on STING, that is dependent on NF-kB.

Maria Mercedes

Graduate Student, Icahn School of Medicine at Mount Sinai (ISMMS)

Bap1 regulates stress-associated factors during skin development

The skin is the body's largest organ, serving as a shield against external insults while protecting internal organs. The basal layer of the skin contains epidermal stem cells (EpSCs) that not only maintain this layer but also replenish the terminally differentiated suprabasal layer. Increasing evidence indicates that chromatin-modified proteins regulate the cell renewal and differentiation processes in the basal layer during development; however, the role of BRCA1-Associated Protein 1 (BAP1), a chromatin modifying deubiquitinase, remains unclear. Published bulk RNA sequencing data show that Bap1 is primarily expressed in the basal layer, with reduced expression in the suprabasal layer. To investigate Bap1's role in skin development, I generated conditional ablation, Bap1 Flox/Flox; K14-Cre+. So far, I have discovered that the loss of Bap1 does not lead to a thicker epidermis or hyperproliferation but instead results in aberrant expression of keratins Krt6, Krt16, Krt17, and Sox9; the expressions of these stress-associated factors are hallmarks of diseases like psoriasis, atopic dermatitis, and barrier defect disorders. The mechanism by which Bap1 regulates the expression of these genes is not well understood. Therefore, I will conduct genetic studies and transcriptomic analysis to uncover this important biological function of the skin.