UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

Poster Session 1

Balcony, Easel 118

11:00 AM to 1:00 PM

Factors Affecting the Geographic Distribution of *Drosera* (Droseraceae) Species in the Cape Floristic Region of South Africa and Implications of Global Climate Change

Alexander Dietrick, Sophomore, Ecology, Evolution, & Conservation Biology, Seattle Central College
Mentor: Joshua Whorley, Science Technology Engineering Math, Seattle Central College

The Cape Floristic Region of South Africa (CFR) is the smallest of the world's six floristic regions, and is known for both its high biodiversity and high number of endemic species, or species that exist nowhere else. Among such species are sundews in the genus *Drosera*, which are small, herbaceous, and carnivorous flowering plants. Of the 34 species of *Drosera* in Africa and Madagascar, 21 of them are endemic to South Africa. *Drosera* in South Africa can be grouped into two categories: generalist species that are widespread in their distribution, and specialist species with highly restricted ranges, often known from only a few sites. The high diversity and variety of life history strategies of *Drosera* in South Africa make the genus an excellent model system for the study of species distribution through the lens of global change. For this project, occurrence data was sourced from citizen science platforms iNaturalist and iSpot, as well as from publicly accessible herbarium and biodiversity databases. Using MaxEnt niche modeling software, occurrence data for *Drosera* in South Africa were used in combination with geographic and climatic data from WorldClim to create a niche occupancy model, which predicted the distribution of these species. This model was used in combination with future climate estimates from WorldClim to predict changes in the distribution of *Drosera* species in South Africa in response to climate change. Results from this study suggest that generalist species are less susceptible to climate change than specialist species with already highly restricted ranges. Understanding how species respond to global climate change is crucial for the success of long-term conservation initiatives.

Poster Session 1

MGH 241, Easel 124

11:00 AM to 1:00 PM

Automated Device for Sensitive Detection of an Influenza Virus Surface Glycoprotein

Alexis Fleming, Junior, Bioengineering
UW Honors Program
Mentor: Joshua Buser, Bioengineering
Mentor: Caitlin Anderson, Bioengineering
Mentor: Paul Yager, Bioengineering

Influenza is a viral respiratory tract infection responsible for 15-60 million infections and more than 200,000 flu related hospitalizations in the United States annually. Diagnostics can help stop outbreaks and treat infections, however the current flu detection methods lack high sensitivity. The Yager Lab at the University of Washington is developing a simple diagnostic test to identify subtypes of the influenza virus. The assay targets the viral surface protein hemagglutinin (HA) and can specifically detect two strains of the influenza virus, the Solomon Islands strain of H1N1 and the Switzerland strain of H3N2. We have developed an automated device for this assay, supplying the reagents sequentially using a pinched valve and water soluble paper. I have tested and modified this device in order to enable hands free operation once the sample (a nasal swab) is inserted in the system. The paper based assay is fast, specific, and easy to use, allowing rapid diagnosis of influenza in any setting.

Poster Session 2

Balcony, Easel 104

1:00 PM to 2:30 PM

Implementation of Fluorescence-Based Detection Methods for Semi-Quantitative Pathogen Evaluation in Point-of-Care Diagnostics

Louise Hansen, Senior, Bioengineering
Mary Gates Scholar
Mentor: Paul Yager, Bioengineering
Mentor: Josh Bishop, Bioengineering

The leading cause of mortality in the developing world is infectious disease. Because such diseases are often treatable, the expanded use of accurate and rapid diagnostic devices plays an important role in reducing the burden of these illnesses. Point-of-care (POC) diagnostic devices can be inexpensive, which expands use in low resource settings, and rapid, which expands options for immediate patient care and treatment. The Yager lab designs low-cost, rapid and easy-

to-use POC diagnostic devices based on paper microfluidics. These devices perform sample preparation, nucleic acid amplification and visual result detection in sequential, automated steps. As with lateral flow tests (LFTs), these devices have generally employed colorimetric detection, but this method increases test duration compared to fluorescence detection, which can give results in real time. Additionally, we hypothesize that fluorescence detection could provide a semi-quantitative evaluation of pathogen load *via* competitive thresholding. When a second DNA species is introduced, competition for reagents can generate different chemical sensitivities by varying the concentration of the secondary species. The competitive threshold, which we aim to calibrate by this method, is the point at which a known concentration of competitive targets amplifies in preference to the pathogenic target. We first performed amplification reactions in aqueous solutions with the number of copies of the pathogenic target and the competitor varied across five orders of magnitude. We visualized this calibration matrix as a surface plot, which we used to establish an accurate, order-of-magnitude competitive threshold that did not hinder pathogenic target amplification. Implementation of this method in paper-based amplification reactions will lead to shorter test times and, possibly, semi-quantitative detection in a new family of optics-enabled POC diagnostic devices.

POSTER SESSION 2

Balcony, Easel 105

1:00 PM to 2:30 PM

Point-of-Care Blood Sample Preparation for Zika Virus Diagnostics

Arielle Howell, Junior, Pre Engineering
Mentor: Paul Yager, Bioengineering
Mentor: Joshua Buser, Bioengineering

Point-of-care diagnostics are needed to improve the detection of pathogens like the Zika virus. The Zika virus can be transmitted by mosquitoes, or by the exchange of blood or other bodily fluids. The implications of the virus in pregnant women and their children are still largely unknown. So monitoring, and if possible, preventing the spread of the disease is critical. Paper-based diagnostic assays enable complex assays in settings without laboratory assets. Today, sample preparation for molecular testing requires training and expensive machinery. The Yager lab recently created a fully automated multi-material paper-based system that can extract DNA from urine, but had not yet developed an automated system for blood targets. My initial work centered on adapting the paper-based system to enable diluted blood processing. Our updated design successfully flowed ˜1.6 mL diluted blood sample through the paper in ˜20 minutes. By passing large volumes through the system, low-concentration DNA can be extracted from the sample, removing assay inhibitors at the same time. This work continues the study of fundamentals of fluid movement through porous networks in the Yager lab, enabling a foundation for DNA sample preparation necessary for blood diagnostics. These advances will help detect the Zika virus in blood samples outside the laboratory, contributing to the monitoring and prevention of the disease.

POSTER SESSION 2

MGH 241, Easel 141

1:00 PM to 2:30 PM

Finding Genes and Markers for Extracellular Vesicle Signaling in *C. elegans*

Shree Mehta, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Josh Russell, Pathology

Extracellular vesicles (ECVs) are small vesicles secreted into the extracellular space to transfer proteins, lipids, and genetic material throughout the organism. Although ECV signaling is thought to be a conserved cell-signaling modality, seen by all cells from bacteria to human, very little is known how this signaling occurs in a living multicellular organism. I used the simple genetic worm model organism C. elegans to conduct genetic screens to understand the mechanisms by which ECVs are formed and secreted in a living animal. In addition, I generated worm lines with fluorescent ECV markers allowing myself and others to monitor ECV signaling in an intact organism. C. elegans uses the ECV machinery to secrete extracellular vesicles that are needed to form alae – two rows of three "racing stripes" that run down the lateral sides of the worm. This clear phenotype allows me to perform a reverse genetics screen using RNAi (RNA interference) to identify the key molecular components for proper signaling. In my first screen, I selected 20 genes that my mass-spectrometry experiments indicated were on ECVs. I found that several of them also prevented proper alae formation, indicating they are key for proper ECV signaling. I then screened all 21 members of the C. elegans tetraspanin family, an ECV-associated transmembrane protein family that is implicated in loading, secretion and cell targeting of the vesicles. Then I generated transgenic lines with artificially introduced DNA that produce fluorescent versions of the genes that are key for ECV secretion. This research is significant because it will uncover new genes necessary for ECV secretion in C. elegans and will find markers that will monitor ECV trafficking in vivo. This results of this will open up the study of ECV signaling in a powerful model system, allowing us to identify physiologically relevant signaling pathways that utilize this communication modality.

POSTER SESSION 2

MGH 241, Easel 135

1:00 PM to 2:30 PM

Internal Deletion Induced Interferon Response to Influenza A

Jacob Kowalsky, Senior, Microbiology
Mary Gates Scholar
Mentor: Jesse Bloom, Division of Basic Sciences
Mentor: Alistair Russell, Basic Sciences

Influenza is a prolific and hazardous virus, affecting even those in the population who have been previously infected or vaccinated. The innate immune system serves as a key first line of defense against this pathogen, with the signaling components, called interferons, driving the production of a potent cellular antiviral response. Studies have indicated that viral populations replete in defective virus particles, virions with a deletion in a portion of their genome, are less efficient at blocking the antiviral response, as shown by increased interferon in the host. Our project seeks to explore this phenomenon of RNA deletions leading to increased interferon expression in host cells by testing the hypothesis that deletions in the three polymerase genes of influenza alone are sufficient to cause an increase in the interferon response. To begin answering this question, an interferon reporter system was used to analyze the viral genome of interferon positive cells, and deletions within various lengths of the PB1, PB2, and PA polymerase genes were found to be enriched. I then created pure populations of influenza with these empirically derived gene deletions in PB1 while my mentor, Dr. Alistair Russell, created pure PB2 deletion populations, which were grown on cell lines expressing functional PB1 and PB2 proteins respectively. When used to infect unmodified cell lines, it was found that pure populations of both PB1 and PB2 defective influenza were sufficient to induce the host interferon response. In the future, I will create PA expressing cell lines and influenza with deletions in PA to analyze the effect of PA deletions within the influenza genome on the host interferon response, and it is hoped that these results can be used to explore a mechanism for how influenza occasionally fails to escape the innate immune response.

POSTER SESSION 2

MGH 241, Easel 140

1:00 PM to 2:30 PM

Developing New Transgenic Models and Phenotypes for Modeling Alzheimer's Disease in *C. elegans*

Jennifer Nianing (Jennifer) Lee, Senior, Biology (Physiology)
UW Honors Program
Kevin La, Senior, Biology (General)
Mentor: Josh Russell, Pathology

With dramatic increases in life expectancy over the past century, millions of people are living long enough to develop age-related neurodegenerative diseases often associated with cognitive decline and dementia. These neurodegenerative diseases are all proteinopathies, or diseases associated with misfolded proteins. Abeta and Tau protein aggregation is a key hallmark of Alzheimer's disease (AD) while aggregated alpha-synuclein is a strong indicator of Parkinson's disease (PD). This categorization is not definitive by any means because many patients with dementia exhibit multiple neuropathologies with combined Abeta, Tau, and alpha-synuclein aggregation. However, there has been almost no research studying the effects of the combined expression of multiple neurodegeneration-associated protein species. Therefore, we have developed the nematode *C. elegans* as a powerful genetic model for quantifying the functional consequences and mechanisms of action of interacting neurodegenerative-related proteins in vivo. To do this we have generated new transgenic worm lines with neuronal expression of different AD-related toxic proteins (both singularly and in combination) and developed new ways for assessing neuropathologies in these animals. We have generated worm lines with neuronal expression of the individual proteins as well as combined expression and seen that we can discriminate between the physiological effects of neuronal Abeta, Tau, and alpha-synuclein through our touch response and pharynx pumping assays we developed. Our escape response assay measures the number of reversals a worm completes upon stimulation by bending a piece of surgical thread over its pharynx. Our pharynx pumping assay measures the electrical activity as the worm feeds and allows us to extract precise measurements for pump frequency, amplitude, and duration. This proposed research is significant because it develops new neurodegenerative worm lines and uncovers new phenotypes that can be used to determine the physiological impact of individual and combined neurodegenerative toxic peptides.

SESSION 2M

FUNDAMENTAL IMMUNE MECHANISMS

Session Moderator: Alanna Ruddell, Comparative Medicine

MGH 287

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

The New Pattern Recognition Receptor RECON Acts as Part of the Intestinal Immune System
Alexie Carletti, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, Innovations in Pain Research Scholar
Mentor: Joshua Woodward, Microbiology
Mentor: Adelle McFarland, Molecular and Cellular Biology/Microbiology

We have recently identified a new cytosolic sensor for bacterial cyclic dinucleotides, the aldo-keto reductase RECON. RECON plays a critical role in surveying the host cytosol for intracellular bacterial pathogens, orchestrating the innate immune response to those pathogens and aiding in bacterial clearance. In the host, RECON is most highly expressed in the small intestinal epithelium, enterocytes and M cells. Its high expression at these important mucosal immune sites raises the question as to whether RECON is part of the intestinal immune system. We have recently made a RECON knockout (KO) mouse and found significant alterations in intestinal inflammation and commensal abundance, particularly an increase in segmented filamentous bacteria (SFB). Using quantitative real-time PCR copy number analyses of SFB 16S gRNA, we detected high SFB burden in the RECON KO mice while SFB was undetectable in wild-type mice. SFB have been shown to impact the development of the immune system, particularly T cell populations, in rodents by colonizing and attaching to the follicle-associated epithelium of the small intestines. We predict that the high abundance of SFB in RECON KO mice may either be the result of immunosuppression due to the presence of too many T regulatory cells or will associate with the increased presence of proinflammatory T helper type 17 cells. Current work is aimed at establishing whether there are changes in these T helper cell types in the intestine, as well as looking at the broader dysbiosis by 16S deep sequencing. This project will establish which immune axes are dysregulated in the absence of RECON and will direct future mechanistic investigations into how loss of RECON enzymatic activity is driving immune dysregulation.

POSTER SESSION 3

Balcony, Easel 90
2:30 PM to 4:00 PM

Using Thermal Infrared Remote Sensing to Identify Effects of Beaver Dams on Stream Temperature
Alishia Orloff, Sophomore, Environmental Science & Resource Management
Mentor: Benjamin Dittbrenner
Mentor: Joshua Lawler, School of Environmental and Forest Sciences

Beavers create complex modifications to the physical and biological components of stream ecosystems through their creation of dams and wetland complexes. Beaver impoundments alter the movement of surface and subsurface waters through riparian systems, which substantially affects biotic and abiotic ecosystem processes that together make up habitats for different species. Although there is general agreement that beaver impoundments modify stream temperature, there has been very little focus on their effect at larger spatial scales. In prior research, traditional temperature assessment methods have failed to capture the full spatial breadth of riverine systems. The use of thermal infrared remote sensing (TIR) is an effective tool for measuring surface stream temperature variability at landscape scales. To better understand the role of beavers in the regulation of stream temperature, we evaluated TIR imagery collected within the Snoqualmie and Stillaguamish River basins in Washington State. Our objective was to identify whether TIR imagery could be used to identify areas where beaver dam complexes have measurable effects in decreasing downstream water temperature due to the upwelling of groundwater. We used an intrinsic potential beaver habitat model to identify areas within the TIR flight paths where beavers were likely present and surveyed these areas to confirm presence of beaver wetland complexes. We compared upstream and downstream surface temperature, thermal complexity, and heterogeneity within these areas using a suite of spatial statistics. Our results have the potential to demonstrate that the use of TIR is a novel approach for monitoring the effects of beaver on riparian systems. Its use provides for assessments at scales and breadth not previously possible using traditional approaches.

POSTER SESSION 4

Commons East, Easel 69
4:00 PM to 6:00 PM

Super-Resolution Imaging of *Drosophila* Tissues Using Expansion Microscopy
Hyeon-Jin Kim, Senior, Biochemistry, Chemistry
Mary Gates Scholar, UW Honors Program, Washington Research Foundation Fellow
Mentor: Joshua Vaughan, Chemsitry

Expansion Microscopy (ExM) is a revolutionary fluorescence microscopy technique that enables super-resolution imaging of fixed biological specimens on ordinary microscopes. By chemically incorporating the specimens into a hydrogel polymer matrix, enzymatically digesting the proteins that hold the structures together, and physically expanding the hydrogel complex, this technique yields expansion of fourfold along each x/y/z axis and a spatial resolution of ~70nm. The original ExM approach utilized special tri-functional antibodies containing a DNA-dye-linker complex to attach the fluorescent dyes to the expandable polymer. Previously, our group has

developed an improved and simplified ExM protocol, which is compatible with conventional antibodies and fluorescent proteins, by using only commonly available reagents, yielding increased fluorophore retention for cultured cells and tissue specimens. In this project, we extend this new approach of ExM to enable ultrahigh resolution imaging of *Drosophila melanogaster* tissues which had previously been incompatible with expansion. To do so, we adapted our existing ExM protocol to *Drosophila* embryos, larval brains, and larval and adult body walls by utilizing an enzyme system that can efficiently digest the tough outer cuticle of the organism. We assessed the uniformity of expansion achieved by our new procedure using correlative pre-expansion and post-expansion imaging and found the distortions to be minimal (<2%). With our new ExM approach, we were able to resolve features of presynaptic active zone structure and study the interactions between the epidermis and dendrites of somatosensory neurons in *Drosophila*. This work should open up this powerful super-resolution microscopy method to a large community of researchers who utilize *Drosophila* as an important model system in biology.

POSTER SESSION 4

Commons East, Easel 68

4:00 PM to 6:00 PM

Design, Synthesis, and Caracterization of Cyanine Fluorophores for Super-Resolution Microscopy

Sarah West, Senior, Chemistry (ACS Certified)
Mary Gates Scholar, UW Honors Program
Mentor: Joshua Vaughan, Chemsitry

Optical microscopy is a cornerstone of biological research. It reveals to scientists phenomena that explain many of life's secrets. Despite its capabilities, traditional optical microscopy techniques are limited to a spatial resolution of ˜250 nm due to the diffraction limit of light. Consequently, features smaller than ˜250 nm cannot be distinguished. However, the development of super-resolution microscopy techniques such as the Stochastic Optical Reconstruction Microscopy (STORM) now enables resolution up to ˜60 nm. An essential criteria of STORM requires that the fluorophores turn on and off (photoswitch) stochastically. Up to date, the fluorophores used in these techniques are commercially available fluorophores with photophysical properties optimized for traditional optical microscopy methods. Since the photoswitching properties of these commercial dyes for use in STORM is not well understood, my project thus involves tuning the electronic properties of the cyanine scaffold by adding various functional groups to the dye in order to improve the photoswitching properties. I am currently working on synthesizing cyanine fluorophores in the red region that have electron-withdrawing groups on the indole molecule and a carboxylate linker attached for bioconjugation. Organic synthetic chemistry techniques will be used in the creation of these dyes. The structure of the dyes will then be characterized by proton NMR and mass spectrometry, while the photophysical properties will be characterized by Ultraviolet-Visual spectroscopy. The dyes will eventually be applied to fixed sample staining and STORM. If successful, these new dyes will improve multi-color STORM and researchers will be able to view cells in greater detail.

POSTER SESSION 4

Commons West, Easel 36

4:00 PM to 6:00 PM

Ice Belt Formation on High Obliquity Planets

Caitlyn Wilhelm, Freshman, Pre-Sciences
Mentor: Russell Deitrick, Astronomy
Mentor: Rory Barnes, Astronomy

At low obliquity, planets like Earth will tend to form permanent ice sheets at the poles, if anywhere. However, at high obliquity, the poles receive more sunlight on average than the tropics, and so ice sheets might be expected to form at the equator. We have used a simple energy balance model for investigating the orbital, atmospheric, and obliquity parameters that allow for the formation of permanent ice at the equator. We find there is a narrow range of semi-major axis and obliquity values that allow for such "ice-belts". First, we experimented with the range of stability of ice-belt formation by increasing the semi-major axis to the edge of the habitable zone, increasing CO2 levels to counteract the decrease in solar flux. This led to a very small range of conditions that allow for stable ice-belts. To further investigate the cause of this, we decreased the luminosity of the host star while increasing CO2 levels (to simulate the control temperatures). This stabilizes the otherwise unstable system, although the range of ice-belt formation is still narrow. Further, we find that this equatorial ice formation often leads to instability due to the ice-albedo feedback. This work suggests that it should not be common for Earth-like worlds at high obliquity to maintain large ice sheets.

POSTER SESSION 4

MGH 241, Easel 151

4:00 PM to 6:00 PM

Using *C. elegans* to Understand the Cellular Mechanisms that Connect Alzheimer's Disease and Cancer

Paula Pabustan, Junior, Biochemistry
Ashmi Chakraborty, Junior, Psychology
Ting I (Ting) Lee, Senior, Biochemistry
Mentor: Matt Kaeberlein, Pathology
Mentor: Josh Russell, Pathology

Alzheimer's disease (AD) and cancer are two of the most devastating public health challenges in modern society costing 361 billion dollars and 685,240 lives each year. As these two classes of pathologies progress they both generate protein stress inside cells. Tumor cells are often characterized by greatly increased protein production while most neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease are classified as a "proteinopathies"; characterized by the accumulation of the misfolded proteins. Some recent reports suggest that the Parkinson's disease related protein α- synuclein influences cancer growth in mice and cell culture models. The goal of our project is to uncover the cellular pathways that are shared between neurodegeneration and oncogenesis. We will study this using the *C. elegans* genetic model system. I have generated a transgenic line that ubiquitously expresses a human form of the AD-related protein, Amyloid-βeta, and observed that it develops similar tumors to cancer-model worm lines. We are also generating transgenic worm lines that ubiquitously express the other canonical neurodegenerative proteins α-synuclein and Tau to see if they also form tumors. These transgenic lines will allow me to knock down different cellular pathways using RNA interference (RNAi) which silences or inhibits the gene expression using double stranded RNA. First we will look chaperone functioning because this class of proteins is necessary for the cells to deal with protein stress. Research in our lab has already shown that RNAi of some chaperones decreases the occurrence of tumors in a worm cancer line. Next, we will generate worm lines with fluorescent reporters of the tumor altering genes and reporters of cancer-related cell-signaling pathways. Thus, we will begin to uncover the ways in which AD-related protein stress impacts cell processes that promote cancer. This could lead to new gene targets for future therapies.