Undergraduate Research Symposium May 18, 2018 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

MGH 258, Easel 186

11:00 AM to 1:00 PM

Integrated Local Coherence in Parkinson's Disease Patients Off and On Levadopa: A Resting-State fMRI Study

Kimia Preston, Freshman, Pre Engineering
UW Honors Program
Mentor: Thomas Grabowski
Mentor: Swati Rane, Radiology

Parkinson's disease (PD) is degenerative disorder of the nervous system that primarily impairs motor function along with other cognitive functions due to a specific loss of striatal dopamine neurons. The most challenging aspect of treating PD is that there is no explicit cause or any definitive long-term treatment. Current therapies restore dopamine levels in brain to alleviate the symptoms of PD. In this study, our goal is to address and identify possible markers of PD by studying the action of dopamine using functional MRI (fMRI) data. Resting-state fMRI allows us to examine the patterns of neuronal firing in vivo. We performed fMRI in 26 PD patients (age = 68 +/- 8 years) before (OFF group) and after (ON group) administration of dopaminergic medication to understand which brain regions showed altered neuronal firing with dopamine therapy. We applied the Integrated Local Coherence (ILC) approach to study temporal coherence of neuronal fluctuations within regions. High ILC corresponds to coherent neuronal fluctuations or recruitment of larger number of neurons as baseline. Low ILC corresponds to reduced temporal coherence in neuronal fluctuations due to neurodegeneration. ILC was computed in the cortex and compared between the ON and OFF groups using a paired t-test. Significance was considered for a voxel-wise threshold of p=0.001 and a Family-Wise Error correction for multiple comparisons at p=0.05. ILC measures were higher bilaterally in the superior parietal lobe, angular gyrus, and precuneal regions in the OFF compared to ON group. These results support another study where increased temporal coherence was observed in the same regions in PD patients compared to healthy controls. These regions are also part of the PD-related disease pattern network involved in motor dysfunction. With this work we believe that administration of levodopa reduces the excessive recruitment of neuronal regions during rest in PD patients.

POSTER SESSION 1

MGH 241, Easel 150

11:00 AM to 1:00 PM

Designing Humanized Model of Infective Endocarditis to Understand the Initial Stages of the Disease

Solomon T. Muche, Senior, Bioengineering
McNair Scholar
Mentor: Wendy Thomas, Bioengineering
Mentor: Olga Yakovenko, Bioengineering

Infective endocarditis (IE) is a life-threatening bacterial infection of heart valves. In comparison to treating other diseases, IE treatment is especially difficult to treat due to an increased resistance to conventional antimicrobial agents as well as a continuously high shear environment of the endocardium wall. Even after receiving prompt therapy, patients with streptococcal endocarditis often develop complications-including heart failure, progressive valve destruction, and stokes. Though the interactions between adhesive bacteria such as streptococci group of viridians (the major cause of IE) and platelets are known to facilitate bacterial vegetation in damaged heart valve, it is not well known how the bacteria bind through hitchhiker binding events (a specific interaction of platelets with streptococcus group viridans). Mainly, it is unclear whether bacteria bind to platelets and then to heart valves, or if the bacteria bind to platelets which are already found on inflamed heart valves. To successfully characterize these spatial and temporal events, we used a parallel microfluidic device to develop a model. Specifically, we used our humanized in vitro model of infective endocarditis to understand the initial stages of the disease. We are demonstrating that the number of adherent bacteria can be measured after bacteria mixed with various blood components is washed through to the device, with sufficient accuracy to test hypotheses regarding the adhesion phase. Finally, we quantified the number of bacteria in vegetation after a growth phase following the adhesion phase. The findings from this design can potentially lead for the development of novel molecular therapeutic mechanisms. These will have implications not only for infective endocarditis, but also all other endovascular infections with a shear environment.

POSTER SESSION 1

MGH 258, Easel 187

11:00 AM to 1:00 PM

Host-Directed Macrophage Therapy with Kinase Inhibitors that Limit Mycobacterium Tuberculosis Replication and Modulate Cytokine Signaling

Natasha Bourgeois, Recent Graduate, Biological Sciences, University of Washington

Amgen Scholar, Howard Hughes Scholar, UW Post-Baccalaureate Research Education Program

Mentor: Thomas Hawn, Medicine

Antibiotic resistance, drug cytotoxicity, and lengthy treatment regimens are major barriers to ending the tuberculosis epidemic. Host-directed therapies (HDTs) that avoid direct targeting of Mycobacterium tuberculosis (Mtb) while enhancing macrophage clearance of the bacteria may overcome such challenges. Because host protein kinases (PKs) regulate multiple innate immune signaling pathways involved with Mtb clearance, we hypothesized that host PKs are HDT candidates. We investigated a group of ATP analogues with specific activity against parasite serine/threonine protein kinases called bumped kinase inhibitors (BKIs). BKIs inhibit few mammalian PKs, so they offer HDT potential while avoiding broad activity that could result in toxicity. To assess the effect of BKIs on Mtb growth within monocyte-derived macrophages (MDMs), MDMs from healthy donors were infected with a luminescent Mtb reporter strain (Mtb-lux) in the presence or absence of BKIs and daily luminescence readings were recorded. We found three BKIs that limit Mtb intracellular replication in MDMs at a final reaction concentration of 10-20 μM. Interestingly, none of these compounds inhibited Mtb-lux growth in 7H9 broth culture. To examine possible mechanisms, we measured the effect of the BKIs on pro-inflammatory cytokine release in MDMs in response to various stimuli including Toll-like receptor agonists, virulent Mtb, and the attenuated vaccine strain Mycobacterium bovis BCG. In response to all of these stimuli, the BKIs potently inhibited TNF secretion and moderately inhibited IL6 secretion by ELISA. In contrast, the mRNA expression level of antimicrobial peptide cathelicidin was maintained in the presence of the BKI tested. Since pro-inflammatory cytokine induction and cathelicidin expression are generally host-protective in early Mtb infection, it is unlikely that the effect of the BKIs on these pathways are responsible for inhibiting intracellular Mtb growth. We are currently evaluating the effect of BKI treatment on other antimicrobial pathways that restrict Mtb infection, including phagosomal uptake and autophagy.

SESSION 1E

FROM VIRAL PATHOGENESIS TO GENETIC DISEASES TO BUILDING A BETTER KIDNEY

Session Moderator: Michael Lagunoff, Microbiology

MGH 231

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

The NLRP3 Inflammasome in the Cystic Fibrosis Lung

Nihar Mahajan, Senior, Biology (Molecular, Cellular & Developmental), Biochemistry

Mentor: Thomas Hawn, Medicine

Cystic Fibrosis (CF) is a genetic disorder that is characterized by recurrent pulmonary infections and a progressive decline in lung function caused by increased inflammation. While the host immune response can help fight these infections, an overzealous inflammatory response in the lungs may be harmful. Inflammation can be induced by multimeric proteins called inflammasomes, which catalyze the maturation of pro-inflammatory cytokines and pyroptosis. Our laboratory is interested in investigating genetic variants that alter the response of the NLRP3 inflammasome in human CF patients as a means of identifying potential targets for host-directed therapies. Preliminary data from the EPIC study has identified two genetic variants in CF children that is associated with colonization by P. aeruginosa, a common bacteria in the lung that can induce deleterious inflammation. We hypothesize that genetic variants in the NLRP3 inflammasome will increase the inflammatory response to bacterial infection and will lead to worsened pulmonary outcomes in patients with CF. First, we used CRISPR/Cas9 and gene editing techniques to knock out the NLRP3 gene in human cell lines. We then inserted NLRP3 genetic variants and stimulate the NLRP3 inflammasome using Pathogen associated molecular patterns such as Nigericin and ATP. The extent of inflammation was measured using ELISA (Enzyme Linked Immunosorbent Assay), and results were compared among NLRP3 genetic variants and wild type controls. The ultimate goal of this investigation is to learn how genetic variants are associated with the clinical outcomes of CF patients. Future studies can involve harnessing host-directed therapies to target specific inflammatory pathways in order to reduce harmful inflammation in CF. Finding the relationship between the NLRP3 inflammasome and CF is critical to generating an effective treatment for CF.

SESSION 1J

MECHANISMS OF CELLULAR REGULATION

Session Moderator: Hannele Ruohola-Baker, Biochemistry
MGH 251
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

The Impact of miRNAs on Postnatal Retinal Progenitor Cell Development
Ellen Riddle, Senior, Biochemistry
Mentor: Thomas Reh, Biological Structure
Mentor: Stefanie Wohl, Biological Structure

Small RNA molecules called microRNAs (miRNAs) are translational repressors and are involved in a variety of processes important for normal cell development and function. Previous studies in our lab showed that miRNAs are required in early (embryonic) stages of retinal development. After depletion of miRNAs in early retinal progenitor cells (RPCs), late RPCs and their progeny, such as Müller glia (MG) which are the primary glial in the retina, are not generated. Instead, only early born neurons are produced. The depletion of miRNAs can be obtained through deletion of an enzyme that generates mature miRNAs called Dicer. Since we know that miRNAs are crucial for early retinal development, this study investigated if miRNAs are also important for proper late (postnatal) retinal development. We used a Sox2 reporter mouse which allows visualizing late retinal progenitors (Sox2 is a gene expressed in RPCs) and selectively deleted Dicer in RPCs at postnatal day 2-6. The tissue was analyzed 2, 4 and 7.5 weeks after Dicer deletion and compared to normal retinas using immunofluorescent staining of retinal cross sections and confocal microscopy. We found massive disruptions in the retinal architecture such as the formation of rosette/ bubble-like structures, indicating that miRNAs are required for proper formation of the retinal layers. Since MG play a role in maintaining the retinal structure, in a second series of experiments we induced Dicer deletion specifically in MG at postnatal day 11 –14, when the retina is almost completely developed. The tissue was analyzed 8 and 20 weeks after Dicer-deletion and compared to normal tissue. We found an atypical location of MG and disruptions in the layered retinal structure, indicating that miRNAs are also required for accurate MG location in the retina, which is essential for normal retinal architecture.

SESSION 1J

MECHANISMS OF CELLULAR REGULATION

Session Moderator: Hannele Ruohola-Baker, Biochemistry
MGH 251
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

DNA Origami for Single Molecule Force Measurements
Amy Stegmann, Senior, Materials Science & Engineering
Levinson Emerging Scholar, Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program
Mentor: Wendy Thomas, Bioengineering
Mentor: Molly Mollica, Bioengineering

Characterizing biological functions on a single molecule scale increases understanding of biological functions by providing information about the indiviual contributions which combine to create larger scale functions. Single molecule measurements are a crucial part of characterizing molecular interactions. Although atomic force microscopy (AFM) and magnetic tweezers are able to measure the response of single molecules to mechanical force, it is challenging to ensure single molecules are being measured. In this project, a precise DNA Origami structure was used to space molecules for single molecule force measurements. Base pair association between DNA nucleotides allowed specific nanostructures to be designed and fabricated. Molecules of interest self-assemble to specific sites of the structure. AFM was used for imaging and obtaining force measurements. This research investigates the strength of adhesion for double stranded DNA when subjected to different loading rates as a proof of concept. In the future, this structure will be used to determine force properties of diverse molecular interactions like platelet and bacterial adhesions.

SESSION 1J

MECHANISMS OF CELLULAR REGULATION

Session Moderator: Hannele Ruohola-Baker, Biochemistry
MGH 251
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Development of a Robust DNA Origami Scaffold
Lesley Martinez, Sophomore, Bioengineering
NASA Space Grant Scholar
Mentor: Wendy Thomas, Bioengineering
Mentor: Molly Mollica, Bioengineering

DNA origami nanotechnology has evolved rapidly since its conception eleven years ago. Both two-dimensional and three-dimensional nanostructures have been created with potential applications in targeted drug delivery, "smart" diagnostic technology, and the study of cell behavior. By annealing "staple" oligonucleotide strands to a single-stranded DNA scaffold we can effectively fold the DNA onto itself to build the nanostructures of interest. One of the primary physical limitations to what one can build is the scaffold. The most commonly used scaffold is derived from the bacteriophage M13mp18 and has a length of 7,249 nucleotides. Its length has previously been varied; however, an overlooked limitation is the secondary structure DNA naturally exhibits. These are sites in which the scaffold binds to itself, thus creating competition for staples to bind during folding reactions. To predict the impact that a designed sequence with little secondary structure could have, we analyzed the first 6,000 bases of the M13mp18 DNA sequence using NUPACK, a nucleic acid sequence analyzer, for their minimum free energy (MFE) at storage, manipulation, and maximum folding reaction temperature. Preliminary data shows M13mp18 exhibits less secondary structure at a high temperature (65C) than at a low temperature (4C) and increasing the concentration of divalent salts linearly increases the amount of secondary structure. Additionally, alternative, shorter sequences have been engineered and their secondary structure is being analyzed at varying conditions. To further determine the effects on yield and stability, structures will be folded using the designed sequence and the standard sequence as a scaffold. These will be compared through agarose gel electrophoresis and transmission electron microscopy. The results from this preliminary data could help us move us toward using a scaffold with decreased secondary structure present at folding temperatures which could potentially result in higher yields, shorter folding reactions, and increased stability.

POSTER SESSION 2

MGH 241, Easel 149

1:00 PM to 2:30 PM

Application of Rationally Modified Self-Assembled Two-Dimensional Protein Array

Karl Benjamin Gilmore, Sophomore, Chemical Engineering
Mentor: Fran??ois Baneyx, Chemical Engineering
Mentor: Alexander Thomas

Although crystalline two-dimensional (2D) protein arrays are often found on the surface of archaea and bacteria where they form a protective S-layer, their potential in bionanotechnology applications remains unfulfilled. Progress in computation has recently allowed the (re)design of proteins for self-assembly into arbitrary structures. We are working with a rationally modified protein from S. typhimurium that can self-assemble into large (> 100 μm) and thin (˜5 nm) hexagonal 2D arrays pierced by ˜3 nm pores upon addition of divalent cations (e.g., Ca^2+). The goal of our research is to test the ability of these arrays to organize gold nanoparticles (AuNPs) with desirable plasmonic characteristics. To this end, we stain protein arrays with the lipophilic fluorescent dye Nile Red, and analyze fluorescence microscopy images to quantify how the decoration of arrays with various concentrations of AuNPs affects the rate of photobleaching of the Nile Red fluorophore. Understanding how AuNPs bind to protein arrays could lead to further applications, such as templated growth of inorganic materials or co-assembly of enzymes and inorganic catalysts.

POSTER SESSION 2

MGH 241, Easel 163

1:00 PM to 2:30 PM

Inhibition of Glycoprotein Horseradish Peroxidase from *E. coli* Receptor Protein, FimH

Chantalle Sasha Bell, Junior, Biochemistry
Mentor: Wendy Thomas, Bioengineering
Mentor: Laura Carlucci, bioengineering

The majority of urinary tract infections are caused by *E. coli* bacteria. *E. coli* infections are strengthened due to *E. coli*'s ability to bind to mannosylated cells. A receptor protein on *E. coli*, FimH, has two domains: a regulatory pillin domain and a lectin domain (LD). In the presence of a force, the pillin domain detaches from the LD allowing the LD to go from its low to high affinity state. The naturally occuring mannose sugars on Horseradish Peroxidase (HRP) are known to bind the LD of FimH. The mannose binding pocket is suspected to open transiently, even when bound to mannose sugars. Based on the interaction of HRP with FimH, HRP may not regularly dissociate during these episodes, but we suspect that free mannose can induce the dissociation of HRP from FimH in these moments. To determine if mannose can improve HRP dissociation from LD, we are using an assay similar to a competitive Enzyme Linked Immunosorbent Assay. We expect to see a decrease in HRP bound to LD in the presence of free mannose compared to the absence. Ultimately this experiment provides an explanation of mannose monomers as an inhibitor for longer chains of mannose binding and a premise for a larger study on alternatives to *E. coli* antibodies that can competitively inhibit FimH from binding mannosylated cells.

POSTER SESSION 4

Commons East, Easel 75

4:00 PM to 6:00 PM

The Effects of RAM Pressure Stripping on Galactic Evolution in a Simulated Galaxy Cluster
Julia Bridget Jansen, Sophomore, Pre-Sciences
Mentor: Thomas Quinn, Astronomy
Mentor: Iryna Butsky, Astronomy

In our universe, galaxies tend to fall into one of two categories: those that inhabit a clustered environment, and those that lead more solitary lives in the field. Galaxies that reside within a cluster evolve differently from their solitary counterparts; namely, their ability to form stars seems to be stunted over time. However, understanding this phenomenon is hard since it's difficult to obtain detailed data about the evolution of distant galaxies through strictly observational techniques. Instead, we use a cosmological simulation of a galaxy cluster similar to the real Virgo cluster to discover the mechanisms of star suppression in such environments. Comparing our synthetic observations with existing observations of galaxies in the Virgo cluster will help constrain theories of galaxy evolution. Our preliminary results show signs of all the major forms of star suppression that are believed to exist, as well as a few outlying cases that don't fit any models we've seen so far. Now, we are continuing to look into one specific theory of galaxy evolution, called RAM pressure stripping, that removes the gas required to form stars from a galaxy via an external pressure. We are trying to discern what kinds of galaxies tend to undergo this process, and how exactly it impacts a galaxy over time. Understanding this fascinating phenomenon will result in a more accurate model of galactic evolution.

POSTER SESSION 4

Commons West, Easel 33
4:00 PM to 6:00 PM

Developing an Effective Microfluidic Instrument for Medical Diagnostics of HIV and HPV
Bob Weng, Junior, Pre-Health Sciences
Mentor: Daniel T. Chiu, Chemistry
Mentor: Thomas Schneider, Chemistry

Over 70 million people are infected by HIV and 79 million of Americans by HPV every year. Even in developed countries, these particular sexually transmitted diseases (STDs) grow at an unyieldingly steady rate. In the Chiu Group, we address this increase in infection by working on the development of a fully automated microfluidic instrument to help improve early diagnosis of HIV and HPV. While many diagnostic techniques already exist, our goal is to advance state-of-the art approaches in novel microfluidic technologies, primarily concerning the reduction of cost to run clinical samples, shortening the time required to analyze samples, and enhancing the reliability of results. We are developing our automated microfluidic instrument in two phases. The first is a preparative step in which we create high-quality microfluidic chips that help digitize thousands of nanoliter droplets in a static array of wells. In the following experimental step, we amplify target HIV/HPV sequences in these nanodroplets from patient samples through polymerase chain reaction (dPCR) and the static array format provides a direct readout through changes in fluorescence of the sample droplets.The successful implementation of the instrument together with the microfluidic chip will maintain a high level of accuracy and sensitivity as well as observe a large decrease in cost and time for diagnostics. Among the conventional diagnostic instruments available today, our development will offer a faster, cheaper, and more accessible way to diagnose HIV and HPV, and ultimately slow the rate of STD cases throughout the world.