UNIVERSITY OF MINNESOTA

2014 SUMMER UNDERGRADUATE RESEARCH SYMPOSIUM

Life Sciences Summer Undergraduate Research Program (LSSURP)

Faculty Director: Dr. Janet Schottel

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Presenter: **Carmen Aguirre** **Poster Number:** 1
Home Institution: College of Saint Scholastica
Program: LSSURP
Faculty Mentor: Dr. Bryce Binstadt
Poster Title: **Engineering Of A Snorkel Tagged TCR To Aid In Detection Of Dual Tcrα T-Cells**

Abstract: One of the central tenets of clonal selection theory posits that a lymphocyte must have only one receptor specificity. However, it is known that dual TCR-expressing T cells actually account for 10% of all T cells. The goal of our project was to understand how dual TCR expression contributes to T cell development and to T cell-mediated immune responses. Dual TCR T cells have been suspected to play roles in several immune contexts including autoimmunity, alloreactivity, and protective immunity. However, a major limitation to studying dual TCR T cells has been the lack of reagents to study them. Currently, antibodies exist that recognize only 4 of the 25 different TCRα chain variable gene products. My project sought to create a dual TCR T cell reporter mouse by engineering an epitope tagged TCR α chain permitting easy detection of dual TCRα T cells. To accomplish this we use a snorkel tag consisting of an epitope tag connected to a trans membrane domain linked to the cytosolic end of the integral membrane protein that makes up the TCRαchain. This mouse would allow dual TCR T cells to be tracked in a wide variety of immune contexts.

Presenter: **Basem Al-Shayeb** **Poster Number:** 2
Home Institution: University of Minnesota - Twin Cites
Program: LSSURP
Faculty Mentor: Dr. Jeffrey Gralnick
Poster Title: **Design Of Silica Gel- Encapsulated Recombinant *E. Coli* For Mercury Bioremediation**

Abstract: Mercury is a heavy metal with the ability to biomagnify, therefore it is a significant issue in public health and environmental studies worldwide. Its levels are continually on the rise due to Cu-Ni mining activities, the industrial use of mercury catalysts, mercurial fungicides in agriculture, and the burning of fossil fuels. This has resulted in the pollution of many marine ecosystems and water reservoirs worldwide, the cleanup of which using current technology, is either not feasible or incredibly costly. This study describes the use of the standardized, modular design of the BioBrick™ systems to engineer recombinant *Escherichia coli* with the mercury resistance (mer) operon that can facilitate the biological remediation of the neurotoxin methylmercury and hazardous mercury ions from an aquatic target site into less toxic form. This synthetic microbe was tested on LB media containing 5 X 10-5 to 10-4 M mercury chloride, and incorporated in novel silica/polymer porous gel encapsulation technology within a cost-effective, scalable water filtering column that sequesters elemental mercury resulting from the bioremediation process. The employment of this device could rigorously change the practices used in mercury decontamination efforts as well as pave the way for the switch to biological rather than chemical processes. Furthermore, this technology can be applied towards bioremediation and biosensing of various other heavy metals and organic toxins in the environment.

Presenter: **Margaret Antonio** **Poster Number:** 3
Home Institution: Boston College
Program: LSSURP
Faculty Mentor: Dr. Michael Kyba
Research Advisor: Abhijit Dandapat, Lynn Hartweck
Poster Title: **Genetic Correction Of Facioscapulohumeral Muscular Dystrophy Using CRISPR-Cas9 Genome Editing**

Abstract: Facioscapulohumeral Muscular Dystrophy (FSHD) is a heritable, genetic disease without a cure or treatment. Mapping of sequence polymorphisms among FSHD patients identified a putative polyadenylation sequence that follows the macrosatellite D4Z4 repeat array on chromosome 4 as one of two necessary genetic causes of the disease (the other being deregulation of chromatin at the D4Z4 macrosatellite). The polyA sequence stabilizes expression of DUX4, a myopathic protein. The purpose of this project is to mutate the polyA sequence by homologous recombination using the CRISPR-Cas9 genome editing system as a first step in gene therapy for this disease. We determined the optimal CRISPR target sequence and transfection method, and devised a PCR assay to verify genetic correction. Small guide RNAs located at three different locations were tested and used with nuclease and nickase versions of Cas9. FSHD patient iPS cells were transfected with CRISPR-Cas9 using different ratios of GeneIn reagent. To detect induced mutations, we amplified the targeted region using flanking primers and performed a nested PCR using a primer specific to the mutant sequence. We observed genome editing only in cells transfected with Cas9-nickase and one combination of guide RNAs. This project presents an optimized protocol for targeting the polyA sequence in FSHD patient cells using CRISPR–Cas9 genome editing. Such a strategy introduces the possibility of gene therapy for FSHD.

Presenter: **Amanda Basham** **Poster Number:** 4
Home Institution: Manchester University
Program: LSSURP
Faculty Mentor: Dr. Nathan Springer
Poster Title: **Allele Specific Cis-Regulatory Responses to Abiotic Stress in Maize Genes**

Abstract: The United States account for 32% of the world's maize (Zea Mays) production. Previous breeding efforts have successfully increased grain yield in order to continue to feed the exponentially growing human population and better handle potential environmental conditions consistent with climate change. Abiotic stresses, such as drought or temperature extremes, continue to have negative effects on maize yield. This study aims to assess the variation in regulatory mechanisms for abiotic stress response in maize. Gene expression levels were surveyed in control and abiotic stresses (cold, heat, UV and salt) for three genetically distinct inbreds (B73, Mo17, and Oh43) and three F1 genotypes(B73xMo17, B73xOh43, and Mo17xOh43) under different abiotic stresses. We identified genes that exhibit differences in the response to abiotic stress in the different genotypes and focused on a subset of these genes that show evidence of allele-specific responses in the F1 generation. These genes are candidates for cis-regulatory variation that results from promoter variation. Detailed characterization of the promoter sequence variation and transcriptional responses in a panel of additional inbred genotypes revealed several candidate promoter differences that may cause the allelic variation for stress responsive expression. This study will contribute to our understanding of how complex regulatory responses might arise for genes and also has the potential to improve our ability to select stress-tolerant varieties of maize.

Presenter: **Christopher Bays** **Poster Number:** 5
Home Institution: Indiana University - Bloomington
Program: LSSURP
Faculty Mentor: Dr. Jakub Tolar
Poster Title: **Quantification of Secreted Type VII Collagen from Dermal Fibroblast Exosomes**

Abstract: Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic skin disease classified by severe skin fragility. RDEB is caused by mutations in the COL7A1 gene that impair function of type VII collagen (C7). Recent evidence suggests mesenchymal stem cell exosomes contain C7 and contribute to therapeutic tissue repair in skin. We hypothesize exosomes may provide a therapy for RDEB. The goal of this investigation is to isolate exosomes from dermal fibroblasts, measure C7 content, and identify the mechanism of action for C7 release from exosomes. Conditioned media were collected from dermal fibroblasts and enriched for exosomes. A CD63 marker was used to isolate and quantify exosome content by flow cytometry. We identified >80% of total particles as exosomes from exosome solutions by FACS. We expect to detect C7 in exosome solutions and lysates by western blot, and measure exosomal C7 secretion by ELISA. We predict extracellular C7 will be present from RDEB dermal fibroblasts treated with exosomes. Intravenous and local application of exosomes derived from dermal fibroblasts could represent a novel platform for skin tissue repair. Studies are ongoing to define the amount, the mechanism of action, and robustness of exosome-mediated wound therapy in preclinical murine model of RDEB.

Presenter: **Liam Beckman** **Poster Number:** 6
Home Institution: University of Oregon
Program: LSSURP
Faculty Mentor: Dr. George Weiblen
Research Advisor: Erin Treiber
Poster Title: **The Effects of EPIC Genetic Marker Addition on the Phylogenetic Analysis of the Tribe Cecropieae**

Abstract: Mutualisms remain an integral field of biological systematics as they may strongly affect the evolutions of the organisms involved. Similarly, mutualistic relationships between ants and members of the botanical genus Cecropia offer promising insights into their respective coevolutionary pathways. In order to obtain a greater understanding of the phylogenetic changes involved, we aimed to investigate the higher order relationships on the tribal level (i.e. within the Cecropieae tribe). Previous work has studied the effect of two loci—26S (ribosomal DNA) and ndhF (chloroplast DNA)—in phylogenetic relationships. However, suboptimal levels of support resulted, and further study was prompted. Our line of question examined the effect that inclusion of exon-primed intron-crossing (EPIC) markers had on the resolution of Cecropiaea's phylogenetic tree. To manifest this aim, we ran a wide set of samples through sequencing and Bayesian Analysis with the EPIC regions. By comparing the level of support for both non-EPIC and EPIC phylogenies, we were able to determine how such a change affects resolution with our studied organisms. While some of the Cecropieaen clades lost support with the addition of EPIC markers, we observed greater resolution in two particular clades and a sister group relationship bridging neotropical and afrotropical genera.

Presenter: **James Brown** **Poster Number:** 7
Home Institution: Morehouse College
Program: LSSURP
Faculty Mentor: Dr. Colin DeYoung
Poster Title: **Agreeableness in Association to the Neuro-Cognitive Correlates of Social-Cognitive Theory of Mind**

Abstract: The personality trait of Agreeableness is linked to a wide-range of human social capacities, including the ability to empathize with others by adopting their perspective. Recent evidence has demonstrated that Agreeableness is related to the social-cognitive aspect of Theory of Mind (ToM),which concerns the ability to reason about others' mental states. In the present study, we attempted to first replicate this finding, and then later extend it to lower-level personality traits, as well as more pathological variants of Agreeableness. Subjects (N=243) completed a task designed to test memory and Social Cognitive ToM at five different levels of embedding. Personality was assessed via the Big Five Aspect Scales (BFAS), the Personality Inventory for the DSM-V (PID-5),and the Externalizing Spectrum Inventory Measure (ESI). Results indicated that there Agreeableness and Compassion are correlated to ToM. Manipulativeness and Empathy are correlated to ToM as well. Intelligence is also strongly correlated to ToM. Findings suggest that more compassionate aspects of Agreeableness, including those reflecting interests in the emotions of others, seem to drive the association between Agreeableness and ToM.

Presenter: **Nicholas Cook-Rostie** **Poster Number:** 8
Home Institution: Normandale Community College
Program: LSSURP
Faculty Mentor: Dr. David Bernlohr
Poster Title: **Detection Of Protein Carbonylation In Models Of Insulin Resistance And Oxidative Stress**

Abstract: Over one third of the United States population is obese. This results in many medical complications including type II diabetes mellitus, making it one of our nation's top health priorities. Protein carbonylation is a post-translation modification of proteins that occurs under conditions of oxidative stress and is positively correlated with obesity and insulin resistance. Despite this known correlation, difficulties in detecting protein carbonylation have prevented the study of this modification in vivo. Using a new method in which free carbonyls are labeled with a carbonyl-reactive tag and then detected with an antibody directed against the tag, I profiled protein carbonylation in several models of obesity and insulin resistance. In cultured 3T3-L1 adipocytes, treatment with inflammatory cytokine TNF-α resulted in increased protein carbonylation. Additionally, protein carbonylation was significantly increased in visceral adipose depots of high fat fed mice compared with lean controls. These results substantiate the positive correlation between carbonylation and insulin resistance and highlight the utility of carbonyl-reactive tags for the detection of carbyonlated proteins.

Presenter: **Wilfredo Cruz - Velez** **Poster Number:** 9
Home Institution: Metropolitan University of Puerto Rico
Program: LSSURP
Faculty Mentor: Dr. Kevin Wickman
Poster Title: **GIRK3 AAV: A Tool for Understanding the Role of GIRK3 in Addiction**

Abstract: Addiction can be defined as the continued repetition of a behavior despite adverse, sometimes serious, consequences. Even though drug abuse might be the most publicized, there are many others such as food, exercise or gambling. A common pathway that all these addictions share is an increase in the extracellular dopamine levels in the mesocorticolimbic system, also known as the reward system. Some drugs of abuse such as opioids and cannabinoids bind to specific receptors located in the membrane of neurons, called GPCRs, and its activation leads to a modulation of ion channels, also present in the cell membrane, which modify the electrical properties of the cell. Other drugs, such as ethanol, can directly activate some particular types of ion channels and in the case of cocaine, for example, it blocks a dopamine transporter producing the known increase in extracellular dopamine. A family of ion channels deeply involved in the effects of all these drugs of abuse is the GIRK family or G-protein inwardly rectifying potassium channels family. There are 4 members in the family GIRK1-4, with 1-3 being widely expressed in the nervous system including those areas involved in reward and addiction. To date not much is known about the specific role of one of these channels, GIRK3, so the main goal of this project was to develop, produce and test a novel AAV virus, containing the ORF of GIRK3, that could be used as a tool for a better understanding of the role of GIRK3 in addiction.

Presenter: **Cody Dail** **Poster Number:** 10
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Dan Kaufman
Research Advisor: Mat Angelos
Poster Title: **Effects of Extracellular Matrix Substrates on Osteogenic Differentiation from Human Pluripotent Stem Cells**

Abstract: Human pluripotent stem cells (hPSCs) represent an ideal platform to understand the developmental biology of all cell and tissue lineages. While hPSCs are capable of differentiating into osteogenic cells in vitro, many systems neglect the role of the bone marrow microenvironment where osteogenic cells develop. We hypothesized that defined, extracellular matrix (ECM) substrates representative of the endosteal stem cell niche would enhance osteogenic differentiation from hPSC and hPSC-derived mesenchymal stem cells (hPSC-MSCs). Using qRT-PCR, we first determined that hPSC-MSCs significantly expressed less decorin, a proteoglycan found within the endosteal niche, relative to endogenous human bone marrow derived MSCs (hBM-MSCs). Experiments are ongoing to validate this effect using immunofluorescent imaging. We next questioned whether exogenously seeded biglycan, decorin, fibronectin, or type I collagen would affect the differentiation kinetics of hPSC-MSCs into osteogenic cells. Using flow cytometry, we determined culture on at least fibronectin resulted in a more homogeneous immunophenotype for osteogenic cell surface antigens relative to a gelatin control. Results with other substrates and a qRT-PCR time course kinetic analysis for osteogenic specific genes are ongoing. Collectively, our preliminary data suggest ECM can modify osteogenic potential from hPSCs and may be critical in supporting osteogenic differentiation for future experiments.

Presenter: **Sarah Dremel** **Poster Number:** 11
Home Institution: University of Minnesota - Twin Cites
Program: LSSURP
Faculty Mentor: Dr. Burckhard Seelig
Research Advisor: Ravi Patel, Misha Golynskiy
Poster Title: **Enzymatic Origins: Using Synthetic Libraries to Rescue Essential Biological Functions in *E. coli***

Abstract: Billions of years of evolution have resulted in modern enzymes only sampling a portion of the vast amino acid sequences possible. We explore whether that limited sequence space is special—or merely an artifact of early selective pressures and environmental conditions—by testing if enzymes can emerge from libraries of unevolved random peptides and folding enriched $(\beta/\alpha)_8$ proteins. We established a selection method wherein a protein library is tested for numerous enzymatic reactions essential to *E. coli.* Variants of the unevolved peptide library contain 80 randomized amino acid positions bordered by constant sequences at both termini. Variants of the $(\beta/\alpha)_8$ library are randomized in seven of the eight loops present on the catalytic face. The utilized strains are selectively auxotrophic, capable of growth on nutrient rich media and incapable of growth on minimal media. Only if a library variant supplements the missing enzymatic function will the strain grow on minimal media. We have performed *in vivo* library selections in 67 strains. Three different strains—ΔmetC, ΔserB, and ΔlipA— were rescued by different library variants. The essential enzymes potentially being replaced are involved in amino acid or cofactor biosynthesis, and function in three different enzymatic classes—lyase, hydrolase, and transferase. Further testing is in progress to determine if the library variants are actually catalyzing an essential metabolic reaction. Demonstrating how catalytic function can emerge from primordial molecules will provide valuable insight into origin of life theories, and the limited sequence space found in nature.

Presenter: **Thu Duong** **Poster Number:** 12
Home Institution: California State University - Northridge
Program: LSSURP
Faculty Mentor: Dr. Kalpna Gupta
Poster Title: **Morphine and Mast Cells Stimulate Endothelial Dysfunction**

Abstract: Although morphine is currently a predominant treatment for cancer-induced chronic pain, its pharmacological effects accelerate tumor progression. Morphine stimulates mast cells in the skin and tumors of mice, leading to the release of cytokines and neuropeptides. Additionally, morphine-induced activation of mast cells stimulates angiogenesis, tumor progression, and contributes to vascular leakage leading to neurogenic inflammation and pain. Therefore, we examined if mast cell activation, particularly the release of cytokines and neuropeptides, has direct effects on tumor endothelium. To demonstrate the developmental spectrum of human breast carcinoma, transgenic mice with a rat C3(1) simian virus 40 large tumor antigen fusion gene were used. We also used primary mouse brain microvascular endothelial cells (MBMEC). We observed that morphine alters the tumor microenvironment by stimulating inflammation and neuroinflammation. IL-17, the master cytokine mediating the release of other neuropeptides and cytokines, was significantly increased in tumors of morphine treated mice vs PBS treatment ($p<0.01$). We also examined supernatant from morphine-activated mast cells and observed endoplasmic reticulum stress and mitochondria dysfunction, with generation of reactive oxygen species (ROS) in the MBMEC. Thus, morphine augments mast cell activation, which may lead to endothelial dysfunction and cancer progression.

Presenter: **Nimasha Fernando** **Poster Number:** 13
Home Institution: University of Maryland - Baltimore County
Program: LSSURP
Faculty Mentor: Dr. Walter Low
Research Advisor: Holly Hewitt
Poster Title: **Optimization of an Immunohistochemical Fluorescent Antibody Staining Protocol for Use on Chimeric Tissues**

Abstract: Biomedical research applications and disease treatments generate a significant demand for human organs and tissues. This need can be potentially satisfied by developing human organs in chimeric porcine that contain human and pig genetic material. To obtain this goal, firstly it is necessary to optimize a method for the precise identification of human cells versus other species, especially in chimeric models. We have optimized an indirect immunohistochemical fluorescent staining protocol for the differentiation of human cells from those of other model species. Primary (1°) antibodies including human nuclear MAB (monoclonal antibody) 1281 and human mitochondrial MAB 1273 were tested in coordination with secondary (2°) antibodies such as the goat – anti – mouse antibody tagged with the Alexa Fluor 555 fluorescent red signal. Various concentrations and types of blocking serum, washing agents, fixation times, tissue types and other variables were tested to determine the optimal experimental conditions for human cell identification. We concluded that tissues thoroughly fixed upon harvest which experienced minimal time between cryo-sectioning, staining, and fluorescent imaging displayed reduced nonspecific binding and best preserved the antibody-targeted antigen. Additionally, liver tissues demonstrated an excessive level of nonspecific binding while pancreatic specimens produced exceedingly clear staining. Overall, our results suggest that human cells can be accurately distinguished from those of other species. When our protocol is utilized for chimeric tissue, the extent of human cell incorporation may be quantitatively analyzed to determine the progress achieved for developing human organs in other species.

Presenter: **Eleanor Fireside-Ostergaard** **Poster Number:** 14
Home Institution: Carleton College
Program: LSSURP
Faculty Mentor: Dr. Yoji Shimizu
Research Advisor: Brandon Burbach
Poster Title: **Generation of WT and ADAP-KO Resident Memory CD8+ T Cells Using An In Vitro Cytokine Programming Treatment**

Abstract: Antigen recognition by T cells drive the immune response. Different subsets of T cells exist throughout the body with varying functions. Resident memory (RM) T cells confer long term resistance to pathogens and are found in peripheral, non-lymphoid tissues. The Adhesion and Degranulation Adapter Protein (ADAP) regulates signaling pathways in adhesion events between T cells and antigen-presenting cells (APC). Previous research has shown that ADAP-knockout mice have less RM T cells than WT mice when compared 60 days after infection. We investigated the differences in RM T cell generation between WT and aKO OT-1 T cells by stimulating activated T cells in vitro with TGF-B and IL-33. We used CD103 expression, measured by flow cytometry, to judge the success of the treatment. We also examined basal CD103 expression in naive WT and aKO T cells. We found that activated WT and aKO cells responded similarly to the cytokine treatment. Basal levels of CD103 expression were comparable in naive WT and aKO cells, but aKO cells tended to have lower CD103 expression. We concluded that activated aKO cells were able to respond to the cytokine treatment and express the resident memory phenotype.

Presenter: **Adrian Garcia** **Poster Number:** 15
Home Institution: New Jersey City University
Program: LSSURP
Faculty Mentor: Dr. Marija Cvetanovic
Poster Title: **The Effect of ATXN1 on Cell Cycle**
Abstract: Spinocerebellar ataxia type 1 (SCA1) is an inherited neurodegenerative disease caused by the expansion of CAG repeats in the *Atxn1* gene. SCA1 patients suffer from impaired motor control and cognition. While neuronal degeneration in the cerebellum accounts for motor problems, the cause of cognitive impairments in SCA1 patients is much less understood. Adult hippocampal neurogenesis, the formation of new neurons by proliferation of neural stem cells (NSCs), has been correlated with cognition and impairment in hippocampal neurogenesis, and could cause cognitive deficits in SCA1. Indeed we have detected decreased proliferation of neural stem cells (NSCs) /decreased neurogenesis in the mouse models of SCA1, SCA1 $^{154Q/2Q}$ mice. We are now interested in understanding the mechanism of decreased neurogenesis in SCA1 mice. All proliferating cells, including NSCs, go through the cell cycle whose various checkpoints determine whether cell proliferates and how fast. It is possible that mutant ATXN1 affects cell cycle of the neural stem cells in the hippocampus thus decreasing their proliferation and neurogenesis. To investigate the effects of ATXN1 on the cell cycle, we have used flow cytometry to examine the cell cycle in human neuronal cell line (DAOY cell) expressing normal or mutant *Atxn*1 gene. By gaining greater insight on the ATXN1 effect on the cell cycle and its role in adult neurogenesis, we hope to increase our understanding and promote development of new therapeutic approaches to treat cognitive problems in SCA1.

Presenter: **Kacey Guenther** **Poster Number:** 16
Home Institution: University of Minnesota - Twin Cites
Program: LSSURP
Faculty Mentor: Dr. Peter Bitterman
Poster Title: **The Role of Decellularized Matrix in Idiopathic Pulmonary Fibrosis**
Abstract: Idiopathic pulmonary fibrosis (IPF) is a progressive disease of the lung characterized by scarring of the lung tissue. This generally fatal disease results in stiffening of the extracellular matrix (ECM) in lung tissue due to increased deposition of collagen I (Col1) and other ECM proteins. Our lab has previously shown that mesenchymal progenitor cells are the cell of origin for fibrosis. Additionally, the lab has shown that microRNA29 (miR29) levels are suppressed in healthy lung fibroblasts seeded on decellularized ECM from the lung of a patient with IPF. miR29 is known to post-transcriptionally regulate the deposition of ECM proteins. The purpose of this study is to determine whether a similar phenomenon occurs in mesenchymal stromal cells (MSCs) isolated from human bone marrow. We want to see if a naïve cell seeded on extracellular matrix from the lung of an IPF patient will be corrupted and exhibit phenotypes characteristic of IPF fibroblasts. So far, we have shown that bone marrow MSCs form colonies when grown in methylcellulose and that cells from these colonies integrate into the embryos of zebra fish when grafted. This indicates that the cells we isolated are in fact MSCs.

Presenter: **Kierra Hayes** **Poster Number:** 17
Home Institution: Northwestern State University
Program: LSSURP
Faculty Mentor: Dr. Timothy Ebner
Poster Title: **Cerebral Cortical Activity Evoked By Optogenetic Modulation Of Cerebellar Purkinje Cells**

Abstract: Sensory feedback and motor commands are integrated in the cerebellum and provide the necessary information for producing coordinated motor output. Purkinje cells are the sole output neuron of the cerebellar cortex and are therefore central to cerebellar function. Optogenetics is a recently discovered technique that uses light to stimulate ion channels or pumps to modulate cellular excitability. This study examines the cerebellar projections to the cerebral cortex in transgenic mice using a combined approach of *in vivo* flavoprotein optical imaging and optogenetics. The strategy used Cre-loxP transgenic mouse lines to obtain Purkinje cell specific expression of the inhibitory, light activated Cl^- pump, halorhodopsin (eNpHR) or the excitatory, light activated cation channel, channelrhodopsin-2 (ChR2). The primary aim of this study is to determine if activity in the cerebral cortex, evoked by either excitation or inhibition of Purkinje cells, can be mapped with flavoprotein imaging. We have observed Purkinje cell specific expression of eNpHR and ChR2 in both transgenic mouse lines. We also demonstrate robust inhibition (eNpHR) or excitation (ChR2) of spontaneous Purkinje cell firing to photostimulation as measured with single unit extracellular recordings. We expect to observe cerebral cortical responses to photostimulation and electrical stimulation of the Purkinje cells using the flavoprotein imaging technique. The results of this experiment could provide a better understanding of the connecting neuronal pathways between the cerebellum and cerebral cortex as well as give insight to the circuitry that may underlie cerebellum associated cognitive dysfunction.

Presenter: **Gabriel Hernandez-Roman** **Poster Number:** 18
Home Institution: University of Puerto Rico - Rio Piedras
Program: LSSURP
Faculty Mentor: Dr. John Osborn
Research Advisor: Megan Schmidt
Poster Title: **Comparison of Epicardial Monophasic Action Potentials from Porcine**

Abstract: Monophasic action potentials (MAPs) are electrical signals recorded from a nerve impulse that represent the depolarization and repolarization of the myocardium. The form that is detected is representative of a transmembrane action potentials (TAPs). One field that has benefited greatly from this knowledge is electrophysiology. In cases of atrial fibrillation MAPs can tell us the viability of localized damage cardiac tissue. In this experiment the purpose is collect and see the difference in Monophasic Action Potentials in various locations in the ventricles. In this experiment, MAPs were measured at various positions in both ventricles using two catheters simultaneously. One catheter was placed on the epicardial surface of the Apex, the other started at the same location and was moved superiorly in increments of around 2 centimeters. Changes in ADP90, which is the time from the start of the action potential to where the action potential has dropped by 90% of its maximum voltage, can be seen in locations at or greater than 4 cm apart.

Presenter: **Annika King** **Poster Number:** 19
Home Institution: University of New Mexico
Program: LSSURP
Faculty Mentor: Dr. Mark Herzberg
Poster Title: **Generation of Free Oxygen Radicals in Epithelial Cells During Bacterial Invasion by *Porphyromonas gingivalis***

Abstract: *Porphyromonas gingivalis*, a gram negative mucosal bacterium, is one of the most common pathogens associated with periodontal disease. Cells infected with bacteria such as *P. gingivalis* often produce reactive oxygen species (ROS) in an attempt to eliminate the invading pathogens. This study seeks to determine whether epithelial cells will elicit ROS upon invasion of *P. gingivalis. P. gingivalis* was grown to log phase and then used to inoculate epithelial cells for various time increments. H2DCFDA, a fluorescent marker that is reduced and fluoresces green when it encounters ROS, was added to the epithelial cells after inoculation with *P. gingivalis*. Analysis was then performed using flow cytometry as well as fluorescence microscopy. Results suggested that *P. gingivalis* may increase ROS in epithelial cells. The findings of this study could contribute to further research into the mechanism with which epithelial cells use innate immunity to combat infections like periodontitis.

Presenter: **Kimberly Kupinski** **Poster Number:** 20
Home Institution: Smith College
Program: LSSURP
Faculty Mentor: Dr. Clifford Steer
Research Advisor: Emil Lou
Poster Title: **Suppression of Tunneling Nanotubes: A Potential Avenue for Chemotherapy**

Abstract: Pancreatic cancer is among the most common types of cancer in the world. It is the seventh most deadly form of cancer; in the United States alone, it accounts for nearly 40,000 deaths annually. Cancer cells proliferate and form tumors by communicating with one another and with surrounding stromal cells by various mechanisms, including membrane channels and vesicular exocytosis. A recently described form of intercellular communication in cancer is the tunneling nanotube (TnT). This actin-based, non-adherent heterocellular protrusion spans large distances between cells and has been shown to assist the transport of proteins, Golgi vesicles, and mitochondria, and support the spread of antibiotic resistance in bacteria. TnTs have recently been implicated in chemoresistance of ovarian and breast cancers. Metformin is a widely used oral antidiabetic drug that has been shown to have anticancer properties. We are exploring the connection between the mechanism of action of metformin and its chemotherapeutic potential by the possibility of suppression of TnT formation. To begin to answer this question, we exposed pancreatic cancer cells to a series of concentrations of metformin to determine the point at which cell survival is unaffected while TnT formation is suppressed. With this information, we will be able to more deeply understand whether TnTs play a role in increasing cancer cell survival during chemotherapy, which can eventually be used to improve the efficacy of pancreatic cancer treatments. We predict that suppression of TnT formation will restrict cancer cell cross-talk and thereby reduce the cancer aggression.

Presenter: **Elizabeth Lezama** **Poster Number:** 21
Home Institution: Milwaukee School of Engineering
Program: LSSURP
Faculty Mentor: Dr. Paul Iaizzo
Poster Title: **Case Based 3-D Modeling and Comparative Imaging of Congenital Defects**
Abstract: Knowledge of congenital heart defects, such as the Tetralogy of Fallot (TOF) or septal defects afflict an underserved population of children. Yet, there is a lack of readily available models for TOF because of the low prevalence, on the order of 5 in 10,000 for TOF. MRI and CT scans of human hearts not viable for transplant or from living patients with congenital defects were taken and made into 3-D models using Mimics software. These models will be added to the Atlas of Human Cardiac Anatomy and augment the free access content including congenital heart tutorial. This addition to the Atlas should not only enhance the cardiologists and cardiac surgeon's knowledge but also serve a resource for parents, students, researchers, and engineers. The continued improvement to the congenital heart defect section of the Atlas will subsequently allow for better understanding of malformations of the heart such as TOF that will be used for improvement of medical treatment and device design.

Presenter: **Monica Lopez-Islas** **Poster Number:** 22
Home Institution: Colorado State University
Program: LSSURP
Faculty Mentor: Dr. Bryan Williams
Poster Title: **The Effect of Agmatine on the Inflammatory Response in an Acute Pneumonia Model in BALB/c Mice**
Abstract: Increased levels of agmatine in the sputum of cystic fibrosis patients may be associated with decreased lung function and an increased inflammatory response. Agmatine concentration in sputum and its effect on inflammation was previously unknown. DNA sequencing revealed that an eleven base pair deletion in the aguA gene of approximately ten percent of the clinical isolates of Pseudomonas aeruginosa collected from cystic fibrosis patients was preventing agmatine from being metabolized, resulting in the secretion of agmatine. To further understand the role of agmatine and its metabolism, we knocked-in a functional aguA gene into three clinical isolates with a mutated aguA. Agmatine levels were measured using a biosensor constructed from the agmatine induced promoter system of the aguBA operon and the mini-CTX-lux bioluminescent reporter. The aguA knock-in caused secreted levels of agmatine to decrease, suggesting that the mutated aguA was previously unable to metabolize agmatine. To evaluate pathogenicity, mice were intratracheally inoculated with the aguA defective PA004 and compared to the aguA knock-in of the same isolate. The mice infected with the aguA defective PA004 appear to have a stronger inflammatory response; supporting our hypothesis that agmatine increases inflammation in an acute pneumonia model in BALB/c mice.

Presenter: **Nicole Mandel** **Poster Number:** 23
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Lisa Peterson
Poster Title: **Inter-Individual Differences in Sensitivity to a Tobacco Carcinogen**
Abstract: Lung cancer is the leading cause of death by cancer in the United States. It is the second most common type of cancer, and is strongly associated with smoking. A combination of environmental and genetic factors has been proposed to play a role in higher risk of smoke-related lung cancer development in certain ethnic populations. The goal of this study was to determine the inter-individual variability in response to cytotoxic and DNA-damaging properties of a tobacco carcinogen. International HapMap Epstein-Barr virus (EBV)-transformed B-lymphocytes were used to investigate this difference in susceptibility. The cell lines were treated with N-nitrosomethylurethane (NMUr), a model reactive compound that mimics methylating effects of a tobacco carcinogen. Exposure to NMUr leads to generation of O6-methylguanine and 7-methylguanine DNA adducts. Cytotoxicity was measured using CellTiter-Glo® 48 hours after treatment. DNA was isolated to determine DNA methyl adduct levels. Samples were analyzed using LC-MS/MS and HPLC. It was found that cytotoxicity as well as rates of DNA adduct formation and repair varied among individuals. There was no strong correlation between cytotoxicity and DNA repair, suggesting that the individual susceptibility to cytotoxic effects of NMUr is determined by more than just DNA repair.

Presenter: **Tomaz Manzoni** **Poster Number:** 24
Home Institution: St. Olaf College
Program: LSSURP
Faculty Mentor: Dr. Timothy Griffin
Poster Title: **Assessing Microbiome Contributions to Oral Cancer**
Abstract: The proteome of the human salivary microbiome in the presence of oral cancer is not very well understood. To improve our understanding of this proteome we took two approaches, a bioinformatics analysis and an experimental method focused on sample preparation optimization. In the bioinformatics approach, previously collected data was used to investigate microbial proteins associated with oral cancer using mass spectrometry based metaproteomics. Microbial proteins were compared for samples from healthy tissue, oral premalignant tissue (OPML), oral squamous cell carcinoma (OSCC) and matched control tissue. Analysis of quantitative microbial peptide data allowed for the identification of several organisms whose protein quantities seemed to shift according to different stages of the cancer. Further studies must be done in order to assess the validity and interpretation of these findings. The sample optimization approach was used to investigate better ways to prepare human samples for metaproteomic analysis. Due to the nature of saliva, mammalian proteins vastly outnumber bacterial proteins. Work was done to procure a way to separate mammalian and bacterial proteins. In order to preferentially acquire better mass spectrometry data on the bacterial proteins, differential lysis procedures were tested. Results were inconclusive with unusually low yields for mammalian proteins.

Presenter: **William Marrero-Ortiz** **Poster Number:** 25
Home Institution: University of Puerto Rico - Rio Piedras
Program: LSSURP
Faculty Mentor: Dr. David Potter
Poster Title: **(±)-14,15-EET Regulation of Mitochondrial STAT3 in MCF-7 Breast Cancer Cells**

Abstract: Cytochrome P450 (CYP) monooxygenases are responsible for the metabolism of a variety of xenobiotics and endogenous substrates. Among the CYP enzymes, cytochrome P450 3A4 (CYP3A4) is a highly active arachidonic acid epoxygenase that has been shown to promote breast cancer proliferation through the biosynthesis of (±)-14,15-epoxyeicosatrienoic acid [(±)-14,15-EET]. Previous studies have demonstrated that exogenous (±)-14,15-EET induces the activation of c-src kinase and signal transducer of activation and transcription 3 (STAT3) at tyrosine 705 residue (Y705), leading to its translocation to the nucleus where it activates growth-related transcription in breast cancer cells. Recently, reports have demonstrated that phosphorylation of STAT3 on serine 727 (S727) causes it to translocate to mitochondria and regulate the complex I of the electron transport chain through non-transcriptional mechanisms, modulating bioenergetics of breast cancer cells. We hypothesize that (±)-14,15-EET promotes phosphorylation of STAT3 on S727 and induce its translocation to the mitochondria, thereby promoting the maintenance of mitochondrial oxidative phosphorylation. (±)-14,15-EET has also been shown to activate extracellular signal regulated kinase 1 (ERK1), a serine kinase that phosphorylates STAT3 on S727, thereby suggesting a mechanism by which EETs may promote mitochondrial function. In this study we test the hypothesis that CYP3A4-mediated (±)-14,15-EET biosynthesis promotes phosphorylation of STAT3 S727 in MCF-7 breast cancer cells, thereby stabilizing the mitochondrial membrane potential. This hypothesis will be tested using MCF-7 cells expressing dominant negative (SA) and constitutively active (SD) mutants of S727, CYP3A4 knock down MCF-7 cell lines and pharmacological inhibitors of CYP3A4 epoxygenase.

Presenter: **Kenechukwu Mbonu** **Poster Number:** 26
Home Institution: University of Maryland - Baltimore County
Program: LSSURP
Faculty Mentor: Dr. Harry Orr
Poster Title: **Cholecystokinin is Involved with Cerebellar Synaptic Pruning and Spinocerebellar Ataxia Type 1**

Abstract: Spinocerebellar Ataxia Type 1(SCA1) is a neurodegenerative disorder that involves the loss of pruning, which is the removal of unnecessary climbing fiber synapses on Purkinje cells in early postnatal development. A loss of pruning has been found in SCA1 mice models that may be due to high levels of Cholecystokinin (CCK). CCK knockout SCA1 mice models have been found to have a restoration of pruning. We crossed CCK knockout mice with mice that have high levels of CCK (D30 mice) to see whether pruning would be restored. My project dealt specifically with the heterozygous mice produced from the cross. We used qPCR to determine the levels of CCK and the IMARIS program to count the synapses on the cells. It was found that the CCK levels in the heterozygous mice were less than the D30 mice but more than the CCK knockout mice. The assessment of climbing fiber synapse numbers is underway but I hypothesize that the levels of pruning will correspond to the levels of CCK found in wild type, heterozygous knockout and the knockout mice. If the expected results are found this project will add to the suggestion that CCK is involved with pruning.

Presenter: **Nicholas Moeller** **Poster Number:** 27
Home Institution: University of Minnesota - Twin Cites
Program: LSSURP
Faculty Mentor: Dr. Daniel Bond
Poster Title: **Does a Motile *Geobacter sulfurreducens* Mutant Exhibit Chemotaxis?**
Abstract:

Geobacter spp. couple the oxidation of fermentation by-products to Fe(III) reduction, and inhabit environments where these reactions play a role in mineral cycling. Natural sediments contain insoluble Fe(III) oxides and it is hypothesized that motility confers an advantage to organisms in environments where metabolites do not readily diffuse. Although most members of the *Geobacter* genus are motile, *G. sulfurreducens* is not, despite possessing the required genes. The master regulator for flagellar genes, *fgrM*, is interrupted by a transposable element in *G. sulfurreducens* and removal of this element has restored swimming in a mutant used in this study. Comparative genomics have revealed a large number of chemotaxis genes in *G. sulfurreducens* that belong to pathways with various predicted functions. Here we characterize the growth of a motile mutant on chelated and insoluble Fe(III) oxide, and chemotactic swimming using a quantitative assay. We suggest that there may be metabolic costs associated with flagellar synthesis or motility, and that further examination of chemotaxis genes and behavior is warranted.

Presenter: **Stephanie Morgan** **Poster Number:** 28
Home Institution: State College of Florida – Manatee - Sarasota
Program: LSSURP
Faculty Mentor: Dr. Karen Echeverri
Poster Title: **The Role Of miR-Ax6820 In Early Tail Regeneration Of The Axolotl**
Abstract:

The axolotl, Ambystoma mexicanum, demonstrates the ability to regenerate fully functional and structurally identical appendages following amputation. In the initial stages of regeneration the stump left over after amputation will form a mound of proliferating cells called a blastema. The exact origin of all cells in the blastema is unclear but some are known to come from dermis and dedifferentiation of mature muscle fibers. RNASeq previously performed by the Echeverri lab revealed differences in expression of microRNAs in the mature tissue versus the regenerative blastema, identified many highly conserved miRs and also identified putative novel miR's which may play an important role in regeneration. These differences in expression are indicative of potentially important roles certain microRNAs play in the regenerative process. miR-Ax6820; a putative, novel miR is highly up-regulated in the days immediately following tail amputation; however, at 8 days after amputation expression returns to normal levels. To better understand the role miR-Ax6820 plays in tail regeneration, axolotls were injected with either a miR-Ax6820 inhibitor or mimic following amputation to manipulate the levels of miR-Ax6820 during blastema formation. Immunofluroescent and immunohistochemical stains were used to observe the effect of modulating miR-Ax6820 had on blastema morphology, proliferation, innervation and vascularization.

Presenter: **Andrew Olinger** **Poster Number:** 29
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Mark Schleiss
Poster Title: **Analysis of Guinea Pig Cytomegalovirus (GPCMV) Glycoprotein gp129 as a Potential Vaccine Candidate**

Abstract: Cytomegalovirus is the most common congenital viral infection in the developed world, and can lead to sensorineural hearing loss, mental retardation, and cerebral palsy. Development of a CMV vaccine is a major public health priority, with the pentameric complex (PC) receiving recent attention as a vaccine candidate. The human CMV (HCMV) PC, composed of glycoproteins gL/gH/UL128/UL130/UL131, has proven essential for infecting epithelial and endothelial cells. The guinea pig protein homologues of HCMV UL128/130/131, designated GP129/131/133, facilitate infection of macrophages/monocytes, an important factor for viral dissemination *in vivo*. Recombinant protein was generated using a baculovirus construct. The 530 bp GP129 ORF, with a predicted molecular weight of 21 kDa, was cloned as a fusion protein in-frame with GFP and 6-His tags, and expressed in SF9 insect cells (resultant fusion protein size ~50 kDa). The protein was purified by affinity chromatography, and demonstrated on western blot to be of the predicted molecular weight when probed with anti-GFP antibodies. Notably, infected guinea pigs engendered an immune response that recognized the GP129 protein, authenticating this protein as a potential vaccine target. The guinea pig model will be used to test for protection against congenital infection and disease in gp129-vaccinated animals.

Presenter: **Spencer Oughton** **Poster Number:** 30
Home Institution: University of Minnesota - Twin Cites
Program: LSSURP
Faculty Mentor: Dr. Romas Kazlauskas
Research Advisor: Bryan Jones
Poster Title: **Catalytic Promiscuity of the Hydroxynitrile Lyase from *Manihot esculenta***

Abstract: Evolution creates families of enzymes from ancestral enzymes. Enzymes in such families often catalyze different reactions, so researchers hypothesize that the ancestral enzymes could catalyze several reactions. To test this hypothesis, we have reconstructed several ancestral enzymes within the α/β-hydrolase family. Modern members of this family include esterases, which catalyze hydrolysis of esters and hydroxynitrile lyases, which catalyze the removal of hydrogen cyanide from a cyanohydrins. We hypothesize that the ancestral enzymes can catalyze both reactions, while the modern enzymes catalyze only one. My goal this summer was to characterize one of the modern enzymes, HNL from cassava (*Manihot esculenta*) or *Me*HNL. Since it is a modern enzyme, I expected that it would catalyze the cyanohydrin cleavage, but not ester hydrolysis. I prepared *Me*HNL using a recombinant strain of *Escherichia coli* and measured its ability to catalyze cleavage of mandelonitrile and hydrolysis of p-nitrophenyl acetate using UV-vis spectrophotometry. As I expected, it efficiently cleaved mandelonitrile (k_{cat} = 5.7 s^{-1}), but was a poor esterase (k_{cat} = 0.016 s^{-1}).

Presenter: **Alberto Palacios-Carbajal** **Poster Number:** 31
Home Institution: University of California - San Diego
Program: LSSURP
Faculty Mentor: Dr. Colin Campbell
Poster Title: **Construction of Plasmid Containing Site-Specific for DNA Protein Cross-Link**
Abstract: DNA protein cross-links (DPCs) are DNA lesions caused by oxidative stress, ionizing radiation, and chemicals such as diepoxybutane, nitrogen mustard and other alkylating agents. Interestingly, chemotherapeutic drugs used in the treatment of cancer induce DPCs. It has been hypothesized that DPCs interfere with DNA replication and thus proliferation. In addition, cells have the ability to repair DPCs, however, the method by which cells recognize and remove DPCs is not fully understood. Current obstacles in the study of DPCs are the variations among the type and number of DNA-damage. Our objective was to create a controlled model by constructing a plasmid with a site specific modification where a DPC adduct could be introduced. The luciferase reporter vector pGL3 was the basis of the new plasmid and used to transform E. coli bacteria. Restriction enzymes were then used to confirm the identity of the modified pGL3 plasmid. Future experiments could monitor the expression of the luciferase gene as a function of the ability for a cell to remove DPC damage.

Presenter: **Breanna Pearson** **Poster Number:** 32
Home Institution: Central Michigan University
Program: LSSURP
Faculty Mentor: Dr. Lucy Vulchanova
Poster Title: **Antibiotic-Induced Visceral Hypersensitivity To Study Neuro-Immune Mechanisms In Mice**
Abstract: Irritable bowel syndrome (IBS) is a functional chronic pain syndrome that affects 5-10% of the world's population. Because the pathology is not known, it is difficult to treat. Interactions between the microflora and gastrointestinal mucosa are becoming increasingly understood, and past research indicates that the microflora in patients with IBS is altered. As a result, we expect to find that changes in the microflora after antibiotic treatment would result in changes in the immune and neural components of the gut causing visceral hypersensitivity. Sixteen C57BL/6 mice aged 6-8 weeks were gavaged either non-absorbable antibiotics or sterile saline for 10 days. Behavior measures included cutaneous mechanical sensitivity and horizontal grip force assays. Immunohistochemistry of colon mucosa using antisera for immune cell markers Iba1 and MHC-II revealed a trend of decreasing immune cells in antibiotic-treated mice and migration of the immune cells toward the lumen. Labeling in the dorsal root ganglia for neuronal markers TRPV1 and CGRP revealed a trend toward increased numbers of neurons positive for CGRP. These preliminary findings suggest that antibiotic-induced alterations in the microflora influence the immune cells and afferent innervation in the colon mucosa.

Presenter: **Vanessa Phuong** **Poster Number:** 33
Home Institution: Johns Hopkins University
Program: LSSURP
Faculty Mentor: Dr. Kristin Hogquist
Research Advisor: You-Jeong Lee
Poster Title: **The lymphoid localization of natural killer T-cell subsets within the spleen of BALB/c mice**

Abstract: Natural killer T-cells (NKT cells) make up a distinct group of cells that express both T-cell receptors and markers associated with normal natural killer cells. It is increasingly important to understand these specialized cells because their dysfunction or deficiency can lead to several autoimmune diseases, like diabetes and atherosclerosis. Recently, there has been evidence that invariant NKT cells eventually diverge into three separate lineages: NKT1, NKT2, and NKT17, which are differentiated by their expression of proteins unique to their subset. In particular, NKT1 cells alone express t-bet; likewise for NKT2 cells and GATA-3, and NKT17 cells and RORɣt. However, the abundance and localization of the three subsets in relation to anatomical compartments within immune system glands like the spleen is unknown. By staining serial spleen sections from BALB/c strain mice and then viewing them through immunofluorescence, these two qualities were determined for NKT1 cells and NKT2 cells. The results showed that the majority of NKT2 cells reside in the T-cell zones of the spleen, whereas the NKT1 cells are more dispersed throughout the whole of the spleen. Understanding where these subsets typically reside in the spleen of BALB/c mice is important because their localization can link to their functional properties and more specifically which APCs cause their activation along with what kinds of cells NKT1 and NKT2 cytokines could interact with.

Presenter: **Zavier Pope** **Poster Number:** 34
Home Institution: University of Maryland - Baltimore County
Program: LSSURP
Faculty Mentor: Dr. Wensheng Lin
Poster Title: **The Role of VEGF in Neurodegeneration Observed in Multiple Sclerosis**

Abstract: Multiple Sclerosis (MS) is an autoimmune disease that affects the central nervous system. Initially, MS was believed to be a demyelinating disease; however, recent studies have discovered its neurodegenerative properties as well. Vascular endothelial growth factor (VEGF) is a signal protein that plays an essential role in cell survival. This project studied the function of VEGF in neuron survival in experimental autoimmune encephalitis (EAE), an animal model of MS. C57BL/6J mice with EAE were injected with vehicle DMSO or SU5416, a VEGF receptor inhibitor, from day 10 to 21 after initial EAE induction. Lumbar spinal cords were collected and used for Neuronal nuclei staining. The number of neurons in the gray matter of the spinal cord was then counted. Both groups of mice suffered similar degrees of mobility loss. The neuron number decreased significantly in the EAE mice that received SU5416 compared to DMSO. These results suggest disrupted VEGF function as a potential factor underlying cell loss in MS in humans.

Presenter: **Luis Rivera-Perez** **Poster Number:** 35
Home Institution: University of Puerto Rico - Ponce
Program: LSSURP
Faculty Mentor: Dr. Mark Masino
Research Advisor: Jacob Montgomery
Poster Title: **Role of Intraspinal Serotonergic Neurons in the Modulation of Locomotion in Zebrafish**

Abstract: Intraspinal serotonergic neurons (ISNs) are cells that produce serotonin, a neurotransmitter shown to be important in locomotion and other behaviors in vertebrates. Even though this is known about serotonin, there is no evidence that ISNs have a role in locomotion. The morphological properties and soma locations of ISNs have led us to hypothesize that they may be involved in the modulation of motor activity in zebrafish. However, previous studies have shown that it is difficult to immunolabel the ISNs at later larval stages. We hypothesize that at these stages, the zebrafish's skin acts as a barrier to immunolabeling, which makes ISN labeling and quantification difficult. As a preliminary step in our experiment, we performed immunohistochemistry in 5 days post-fertilization (dpf) larvae using Proteinase K (PK), an enzyme that digests proteins. The objective of this treatment was to improve antibody penetration by permeabilizing the skin. We found that a PK concentration of 40µg/ml and 40 minutes of exposure improved immunolabeling in the spinal cord of 5dpf zebrafish, showing that the skin acted as a barrier against antibody penetration. By using this immunolabeling protocol we may be able to study the role of ISNs in the modulation of locomotion in zebrafish thoroughly.

Presenter: **Erik Rodriguez** **Poster Number:** 36
Home Institution: Montreat College
Program: LSSURP
Faculty Mentor: Dr. Margaret Titus
Poster Title: **Study of Myosin 7 Motor Protein in *Dictyostelium discoideum***

Abstract: Myosin is a superfamily of proteins that have various functions within cells such as organelle transport and generating forces for cells migration. Some cells move about through a specialized form of motility known as amoeboid movement. Our objective is to characterize the biochemical activity of Myosin 7 motor protein in the model organism *Dictyostelium discoideum*, to determine its function in the cell. A His tag GFP marker was fused to the myosin 7 motor protein and transformed into the *Dictyostelium* cells, then verified via a cytoskeleton prep followed by Western Blot analysis. Once it was determined that the cells contained the desired protein, a protein purification can then be conducted for further isolation and characterization. The His tag is essential in the protein purification as it has a high affinity to the talon resins. At a small scale the His-GFP myosin 7 motor protein was found in the supernatant of the cell lysate, confirming that the *Dictyostelium* cells express the desired protein and that the His tag is present. However at a larger scale it is not determined whether this is the case. With the purification of this His tagged protein the biochemical activity of the protein can be better characterized through other means of experiments, giving a better understanding of its function in the cell.

Presenter: **Edaris Rodriguez-Izquierdo** **Poster Number:** 37
Home Institution: University of Turabo
Program: LSSURP
Faculty Mentor: Dr. Michael Sadowsky
Poster Title: **Characterizing the Symbiotically Important Components of the Type IV Secretion System in the *Sinorhizobium*-Medicago Symbiosis**

Abstract: Nitrogen is essential for all agricultural crops, but biologically-useful nitrogen is the limiting resource in fields. Agriculture is dependent upon biologically-fixed nitrogen form symbiotic association between rhizobia and plants. Research has focused on understanding this symbiosis with the goal of enhancing biological nitrogen fixation by increasing the number of nodules formed or enhancing the efficiency of nitrogen fixation. A previous work on *Medicago truncatula* genotypes against 48 strains of *Sinorhizobium* indicated that some Type IV secretion genes (T4SS) are symbiosis-related and involved in nitrogen fixation efficiency. The three strains KH46b, KH46c, and KH46c Δ*virB6-9* (key difference being KH46c possesses the T4SSb), will be inoculated in a pair-wise mixture with two *Medicago* genotypes HM003 and HM005. Each strain was genetically transformed site-specifically with either GFP or mCherry, in a location that is stable and has no effect on nodulation or fitness. Removing the T4SSb confers a competitive advantage or disadvantage in nodule formation depending on host. The presents of the T4SSb accounts for most of the variation in the number of nodules form between KH46c and KH46b. After five weeks of the growth the plants, nodules were harvested from the plants and were surface-sterlized. The bacterial strains were recovered from individual surface-sterilized nodules. Each strain was identified by multiplex Colony PCR for mCherry and GFP, so the total number of nodules formed by each strain can be determined. The findings of this study will improve our understanding of *Sinorhizobium* mechanism for nodulation and explore possibilities for increasing biologically-fixed nitrogen.

Presenter: **Natalia Rodriguez-Sosa** **Poster Number:** 38
Home Institution: University of Puerto Rico – Rio Pierdras
Program: LSSURP
Faculty Mentor: Dr. Robert Meisel
Poster Title: **Plasticity in Brain and Behavior following Aggressive Experience in Female Syrian Hamsters**

Abstract: Pathological aggression is a serious clinical problem that can lead to violence and criminal behavior. Although both males and females display aggression, research examining the neurobiology of aggression has focused on males. Previous research has demonstrated that aggressive experience in female hamsters increases dendritic spines in nucleus accumbens (NAc) neurons. As dendritic spines are the site of excitatory inputs to the NAc, we hypothesize that glutamate receptors in the NAc mediate brain and behavior plasticity after aggressive experience. Adult female Syrian hamsters were given 5 daily aggressive experiences. Prior to each experience, females were treated with mGluR1 or mGluR5 glutamate receptor antagonists, or a vehicle control. One week after the last aggressive experience, females were sacrificed and tissue punches were taken from the NAc and evaluated for gene expression of PSD-95, a marker of dendritic spines. Inhibition of mGluR did not attenuate aggressive behavior. Blocking mGluRs prevented PSD-95 from increasing in the NAc. This finding is difficult to interpret, however, as PSD-95 did not increase in control groups as previously demonstrated. In the future, manipulating PSD-95 expression may be beneficial. Understanding the neurobiology of aggression in females will help identify novel therapeutic targets for treating pathological aggression in women.

Presenter: **Nicolle Rosa-Mercado** **Poster Number:** 39
Home Institution: University of Puerto Rico - Cayey
Program: LSSURP
Faculty Mentor: Dr. Sundaram Ramakrishnan
Poster Title: **Hypoxia-Induced Changes on Iron-Regulatory Proteins in Ovarian Cancer Cells**
Abstract:

Ovarian cancers develop from the single layer of epithelial cells surrounding the ovaries or from the tubal epithelium and metastasize to the peritoneum. A majority of ovarian cancer patients present malignant ascites at later stages of tumor progression. As ascites is stagnant the oxygen concentration in the fluid is very low when compared to arterial blood. Thus, ovarian cancer cells are continuously exposed to hypoxia (reduced oxygen). Tumor cells reprogram metabolic pathways during hypoxic stress. These changes are dependent on the availability of iron. Intracellular iron levels are tightly controlled by modulating either transferrin mediated iron uptake, storage by ferritin caging, or by exporting iron through an efflux pump, ferroportin. Perturbing any of these regulatory proteins will impact iron homeostasis and can cause ROS-mediated cellular damage. In this study we evaluated the expression of proteins involved in iron homeostasis in epithelial ovarian cancer cell lines exposed to hypoxia. It is hypothesized that by perturbing iron homeostatic mechanisms, tumor cells can be induced to undergo apoptosis through oxidative stress. Preliminary studies suggest that, under hypoxia, ferritin is up regulated in ovarian cancer cell lines. RNA interference methods were then used to knockdown ferritin heavy chain. Knockdown was verified by qPCR and western blots. Effects of ferritin knockdown on cell proliferation and migration during hypoxia will be presented.

Presenter: **Sarah Rudasill** **Poster Number:** 40
Home Institution: Wake Forest University
Program: LSSURP
Faculty Mentor: Dr. Karen Echeverri
Poster Title: **Membrane Depolarization Following Spinal Cord Injury Regulates Regeneration in the Axolotl**
Abstract:

Mammalian spinal cord injuries, which can result in permanent loss of motor and sensory function, disable 250,000 people annually. Remarkably, other vertebrates like the salamander are capable of complete structural and functional spinal cord regeneration throughout their entire lives. Recent breakthroughs indicate that dynamic changes in cellular membrane potentials regulate regeneration. However, the exact mechanism remains uncharacterized, so the Mexican axolotl is a model that can elucidate the possible regulatory role of the observed membrane depolarization following injury. Axolotl membrane potentials were manipulated with Ivermectin, a drug that potentiates glycine gated chloride channels (GlyCl) to hyperpolarize cells and prevent depolarization after injury. Membrane polarization was modulated in vivo by spinal cord microinjection of Ivermectin or glycine prior to spinal cord ablation. Wholemount immunohistochemistry and stainings were performed at various intervals post injury to assess the extent of axonal regeneration and response of glial cells. It was observed that activation of GlyCl significantly blocked spinal cord regeneration by decreasing cell migration and inhibiting glial cell proliferation as compared to controls. Axons did not grow across the lesion site in Ivermectin or glycine injected axolotls. Therefore, membrane depolarization following injury is critical in promoting faithful spinal cord regeneration through cellular proliferation, migration, and axon regrowth across the lesion. Harnessing the membrane potential to develop novel spinal cord therapies holds promise in improving the prognosis for an otherwise permanent injury.

Presenter: **Albert Santiago-DeVeer** **Poster Number:** 41
Home Institution: Interamerican University of Puerto Rico
Program: LSSURP
Faculty Mentor: Dr. Betsy Martinez-Vaz
Poster Title: **Survey of ACC Deaminase Genes Present in the Rhizosphere of Soybean, Alfalfa, and Clover**

Abstract: High levels of ethylene inhibit the symbiotic association between rhizobia and their host legumes. This inhibition prevents root infection and nitrogen fixation. Bacteria containing ACC deaminases have the ability to reduce ethylene levels and promote plant growth by enhancing the conversion of nitrogen gas to ammonia. The goals of this study were to investigate the distribution of the ACC deaminase genes in the rhizosphere of different legumes. The first part of the project consisted of isolating DNA and ACC -degrading bacteria from the rhizosphere of alfalfa, clover and soybean plants. Bacteria that could degrade and utilize ACC as a sole nitrogen source were isolated using M9 medium containing this compound. These organisms were characterized by 16SrRNA analysis. The results showed that most ACC-degrading bacteria isolated from the rhizosphere of legumes were Pseudomonas, Advenella and Comamonas species. PCR analyses were performed in the microbial isolates and soil samples using primers specific for three different ACC deaminse genes (acdS1, acdS2, acdS3). None of the ACC-degrading bacteria isolated from the rhizosphere contained these genes. This observation suggests that these organisms might contain novel ACC deaminases that could not be amplified by the primers utilized in this study. PCR analyses of soil samples showed that AcdS1 was only detected in the rhizosphere of soybean, no amplification was observed in clover or alfafa; acdS3 gene was found in three out of the eight soils samples analyzed. The acdS2 gene was not present in any of the soil samples investigated. These results suggest that ACC deaminase genes are not highly abundant in the legume rhizosphere. A possible reason for the lack of detection of acdS genes is that they might be plasmid-encoded and difficult to amplify using total soil DNA or bacterial lysates as templates for PCR. Experiments are currently in progress to address this possibility.

Presenter: **Chidyaonga Shalita** **Poster Number:** 42
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Timothy Walseth
Poster Title: **Development of Biotin Modified NAADP Analogues for the Indentification of the NAADP Receptor**

Abstract: Calcium is an essential intracellular messenger in all cell types. Calcium signaling regulates a vast array of cellular functions including fertilization, T-cell activation, muscle contraction, enzyme function, and gene expression. NAADP (Nicotinic Acid Adenine Dinucleotide Phosphate) is a molecule that activates calcium release from lysosomal stores. Calcium release mediated by NAADP can result in both local and global signaling cascades within a cell, triggering other calcium signaling pathways. Currently, the receptor protein for NAADP has not been identified. Two-pore channels, a family of ion channels have been implicated in the NAADP pathway but have not been convincingly shown to bind specifically to NAADP. This study highlights the design and functional characterization of monofunctional NAADP probes which may have utility in the isolation and identification of the NAADP receptor protein by affinity chromatography techniques. Biotin was introduced into NAADP analogues containing a propylazido or an alkyne at the 5-position of the nicotinic acid ring (5-propylazido-NAADP or 5-alkyne-NAADP) using click chemistry methods. The linker between biotin and NAADP contains a diazo group which is cleavable when treated with sodium dithionite. Two biotin modified NAADP analogues were successfully synthesized and characterized for their ability to mimic NAADP binding to the receptor protein. Both analogues were able to compete with ^{32}P-NAADP in a competition binding assay with IC_{50} values of 215nM for the 5-biotin-diazo-DBCO-NAADP and 941nm for 5-Alkyl-diazo-NAADP. The fact that both analogues were able to successfully compete with ^{32}P-NAADP indicates that they are able to specifically bind with the NAADP receptor protein. In addition, both biotin modified analogues were able to successfully bind to the strepadvidin agarose beads and subsequently detach upon treatment with sodium dithionite solution. The ability of 5-biotin-NAADPs to bind to both the NAADP protein receptor and strepadvidin agarose beads should provide an extremely powerful affinity technique to enrich the NAADP receptor protein.

Presenter: **Joanna Silverman** **Poster Number:** 43
Home Institution: Grinnell College
Program: LSSURP
Faculty Mentor: Dr. Patrick Arndt
Poster Title: **Hematologic Expression Patterns of RIP140 in Critically Ill Patients**

Abstract: Sepsis is an inflammatory PAMP-induced infection characterized by the presence of proinflammatory cytokines. PAMP-infected neutrophils varyingly express RIP140, an anti-inflammatory protein. Recently, RIP140 has been shown to counter-regulate the production of proinflammatory cytokines in healthy neutrophils exposed to PAMPs. We sought to determine if RIP140 elicited a similar counter-regulatory effect in neutrophils compromised by sepsis or other critical illnesses. Our goal was to determine if disparities in RIP140 and pro-inflammatory cytokine expression exists between patients admitted with various critical illness. To determine RIP140's regulatory effects in compromised neutrophils, plasma and neutrophil populations were isolated from ICU donor blood and exposed to LPS and LTA (PAMPs). Subsequent use of flow cytometry and ELISA analysis were utilized to determine RIP 140 and pro-inflammatory cytokine expression, respectively, before and after PAMP stimulation. IL-6 cytokine expression was detected in elevated quantities in sepsis-compromised plasma and uniformly decreased upon recovery in all sepsis donors compared to non-sepsis donors. Conversely, TNFα and IL-10 exhibited no uniform expression trend in sepsis patient plasma. Due to the IL-6 expression pattern, it was deemed an appropriate cytokine to monitor and diagnose sepsis recovery and progression. Future experiments will utilize flow cytometry to determine RIP140 expressions in collected neutrophil samples.

Presenter: **Nathaniel Soto-Rosado** **Poster Number:** 44
Home Institution: University of Puerto Rico - Mayagüez
Program: LSSURP
Faculty Mentor: Dr. Louis Mansky
Research Advisor: Jessica L. Martin
Poster Title: **Identification of Residues of the HTLV-1 Capsid Protein N-Terminal Domain Involved in Gag-Gag Interactions**

Abstract: HTLV-1 is a deltaretrovirus responsible for a number of diseases, the most devastating of which is an aggressive adult T-cell lymphoma. Viral assembly is a noteworthy target for inhibiting HTLV-1 replication. Viral assembly is predominantly driven by the Gag polyprotein precursor, which is essential for the formation of viral particles and expression of it alone is known to form virus-like particles. Cleavage of Gag by the viral protease, divides Gag into three subunits: matrix (MA), nucleocapsid (NC) and capsid (CA). These Gag subunits are structurally discrete, but have overlapping functional domains. CA is thought to primarily drive Gag-Gag interactions and it is composed of two domains: the N-terminal domain (NTD) and the C-terminal domain (CTD). In HTLV-1, the NTD is thought to contain the residues responsible for indirect and direct Gag-Gag in-teractions. This is in contrast with HIV-1 where the CTD is responsible for these interactions. De-spite the functional differences, structural conservation among these domains is high, particularly in the α-helices; therefore, it has been hypothesized that Gag-Gag interaction in HTLV-1 can be at-tributed to residues located in the loops of the NTD. Therefore, in order to determine the amino ac-ids responsible for Gag-Gag binding in HTLV-1 I am mutating amino acid residues in diverse loca-tions of the loops of the NTD using alanine scanning site-directed mutagenesis. The mutants that are created by this method will be analyzed for phenotypic differences by confocal microscopy analysis and by immunoblotting in order to access the binding of the Gag polyprotein.

Presenter: **Benjamin Tittle** **Poster Number:** 45
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Gregory Vercellotti
Poster Title: **Spectrophotometry as a Technique for Evaluating Hemopexin in Plasma from Transgenic Sickle Mice**

Abstract: Sickle cell disease (SCD) is characterized by hemolysis. The subsequent release of heme into the vasculature promotes vaso-occlusion and ischemic organ damage. Hemopexin (Hpx) is a high-affinity heme scavenger in plasma. This project uses spectrophotometry to research circulating free Hpx levels. We hypothesize that heme clearance in SCD mice (HbSS) is depressed as compared to normal (HbAA) mice. Blood plasma collected from 5 transgenic strains of mice was combined in solution with an excess of 10 uM heme. A spectrophotometric assay captured the wavelength shift and corresponding change in absorbance at 414 nm that indicates Hpx-Heme binding activity. The experimental groups showed no significant difference in absorbance values. Additional studies are underway to optimize the assay and to compare against an enzyme-linked immunosorbent assay (ELISA) designed to determine Hpx content of the plasma samples. Free Hpx binding capacity was unable to discriminate between genetic lines of mice. Further murine testing should put an emphasis on controlling hemolysis during plasma collection. Measurement of plasma Hpx by ELISA or western blot may be a better test than the spectrophotometric assay. Despite the unexpected findings, Hpx transgene therapy has potential therapeutic application in SCD.

Presenter: **Cristina Torres-Cabán** **Poster Number:** 46
Home Institution: University of Puerto Rico - Aguadilla
Program: LSSURP
Faculty Mentor: Dr. Deanna Koepp
Poster Title: **Effects of Dia2 degradation on Checkpoint Recovery in *Saccharomyces cerevisiae***

Abstract: Accurate DNA replication is essential for cell viability. During this process, cell division can be blocked by replication stress. This occurs after DNA damage has been encountered by the replication complex, which initiates a fork stall that causes activation of a checkpoint kinases cascade. When this takes place, a higher probability of accumulating mutations exists, which may transmit defects to daughter cells. The *S. cerevisiae* F-box protein Dia2 is involved in the degradation of a checkpoint protein, Mrc1, after checkpoint activation during recovery. Since the Dia2 protein has multiple roles in the cell cycle, we needed an approach to remove Dia2 function specifically in checkpoint recovery. We hypothesized that a Dia2 degron strain would delay checkpoint recovery by failing to target Mrc1 for degradation after checkpoint activation. Studying this process would help achieve better understanding of the importance of an F-box protein during recovery. The generation of a Dia2 degron strain was done with the addition of a Myc9 tag and Auxin Inducible Degron to the DIA2 gene via PCR amplification. The purpose was to control protein expression of the Dia2 protein with the exposure of auxin. In the future, the insertion of our product will be done into dia2Δ strain to ensure the only copy of DIA2 in the genome is the degron-tagged version. After verifying that the strain functions correctly, cells can be tested during checkpoint recovery in order to observe the effects of the absence of Dia2 in the system.

Presenter: **César Torres-Gutierrez** **Poster Number:** 47
Home Institution: University of Puerto Rico - Ponce
Program: LSSURP
Faculty Mentor: Dr. Yaniv Brandvain
Poster Title: **Admixture in Horse Breeds Illustrated from Single Nucleotide Polymorphism Data**

Abstract: More than 300 horse breeds exist in the modern world. Many of these horse breeds are thought to have closely related evolutionary paths, but with the horse genome being sequenced recently (2007), genome-wide single nucleotide polymorphism (SNP) studies confirming evolutionary relationships are lacking. This project determines whether there is admixture between horse breeds that are thought to be evolutionary related by using the program Treemix to create a phylogenic tree, which illustrates admixture in the multiple horse breeds. Allele frequencies across 34,648 SNP sites formatted from the genotype of 37 breeds (795 individuals in total) obtained with an Illumina Beadchip, were used as the input data for the program. We found evidence for admixture events between several breeds of horses, which was confirmed with F3 and F4 statistics tests (ABBA/BABA test). These findings will facilitate future studies of the origin of the domestic horse and its evolutionary path by providing new insights on each breed and how they are related to each other. In the future we will use denser chips to obtain more SNP sites allowing us to find regions of introgression and determine whether or not they are coding sequences.

Presenter: **Alejandra Torres-Marrero** **Poster Number:** 48
Home Institution: University of Puerto Rico - Mayaguez
Program: LSSURP
Faculty Mentor: Dr. Michael Sadowsky
Research Advisor: Chanlan Chun, Matthew Nelson
Poster Title: **Identifying Essential Genes Involved in Nodulation By Random Transposon Mutagenesis in the Medicago-Sinorhizobia Symbiosis**

Abstract: Nitrogen is a primary nutrient of plants, a necessary part of all proteins, enzymes, as well as metabolic pathways. Plants, however, are not able to use abundant atmospheric nitrogen and are dependent of common soil bacteria, rhizobia to provide nitrogen for plant growth. The symbiosis between rhizobia and legume plants gives way to the formation of nodules, contributing large amounts of fixed nitrogen to the plant. Previous studies in the model legume-rhizobium symbiosis, Sinorhizobium-Medicago symbiosis have shown a large degree of variation in nodule number depending on both the genotype of Medicago and the strain of Sinorhizobium. Furthermore, the Sinorhizobium strains are able to evade plant immune responses through multiple regulatory pathways and provide the plant with the necessary nitrogen uptake. To fully understand keygenes involved in nodulation and nitrogen-fixing activities, we incorporate random transposon mutagenesis into a S.medicae strain and observe the nodule formation in Medicago truncatula. The discovery of these genes will give insight into mechanisms involved in nodulation and explore possibilities for increasing biologically-fixed nitrogen.

Presenter: **Ashley Velez-Delgado** **Poster Number:** 49
Home Institution: University of Puerto Rico - Humacao
Program: LSSURP
Faculty Mentor: Dr. James McCarthy
Research Advisor: Leah Colvin Wanshura
Poster Title: **Focal Adhesion Kinase (FAK) is Required for CSPG4-driven Cell Survival and AKT Phosphorylation in Response to Dabrafenib**

Abstract: The resistance of melanoma to chemotherapeutics, such as the RAF inhibitor Dabrafenib, is associated with the activation of cell surface proteins (e.g. integrin) and receptor tyrosine kinase (RTK) pathways. Chondroitin Sulfate Proteoglycan 4 (CSPG4) is a transmembrane proteoglycan found in high concentrations in melanoma cells. This proteoglycan leads cellular function that drive tumorigenesis by the activation of the integrin signaling pathway, through Focal Adhesion Kinase (FAK), and RTKs. We have previously found that CSPG4 is responsible for increasing AKT phosphorylation (P-AKT) and cell survival in response to Dabrafenib. This project consists of two aims, to study the inhibition of FAK to decrease P-AKT and to study the CSPG4 knockdown to see an increase in Dabrafenib-resistant cells vulnerability to Dabrafenib. In the first aim, we hypothesized that FAK is required for P-AKT in response to Dabrafenib. We inhibited FAK pharmacologically, and found that P-AKT decreased but using it in combination with Dabrafenib could not decrease the P-AKT. In the second part, we hypothesized that the loss of CSPG4 would re-sensitize Dabrafenib-resistant cells to Dabrafenib. CSPG4 was knocked down using siRNA, and cell survival analysis was used to generate a dose-response curve to SB590885, a Dabrafenib parent compound. It was determined that the cells with CSPG4 knock down were more vulnerable to the drug. However, only a partial knockdown was obtained, therefore future experiments will be focused on increasing knockdown efficacy by viral infection. These results suggest that targeting CSPG4 could improve melanoma treatment using a combination of therapies.

Presenter: **Ashley Wallin** **Poster Number:** 50
Home Institution: Arizona State University
Program: LSSURP
Faculty Mentor: Dr. Colin Campbell
Poster Title: **Investigating the Cellular Response to 1,2,3,4-diepoxybutane**

Abstract: Alkylating agents are a mainstay class of chemotherapeutic drugs, working by binding to DNA and preventing DNA replication and transcription. The chemistry behind how alkylating agents such as 1,2,3,4-diepoxybutane (DEB) work is known, however the cellular response is not. DEB is a carcinogenic metabolite of 1,3-butadiene, a prevalent occupational and environmental pollutant found in cigarette smoke, urban air and automobile exhaust. To further understand the cellular response to DEB, this study seeks to find a correlation between adduct formation, double strand breaks, and cytotoxicity in response to DEB-induced DNA damage among three Chinese hamster lung fibroblast cells lines: wild type (V79), Nucleotide Excision Repair deficient (VH-1), and FANCA deficient (VH-4). Following quantification of guanine-guanine adduct formation via mass spectrometry, double strand break formation and repair were examined by DNA repair foci measured by phosphorylated H2AX (γH2AX) immunocytochemistry. Additionally, clonogenic and direct-counting cytotoxicity assays were performed to measure the survival rate and the ability of the cells to proliferate at different concentrations of DEB. By understanding the cellular response to commonly used chemotherapeutic agents, novel drugs could be developed that make previously designed drugs more effective.

Presenter: **Hannah Whitis** **Poster Number:** 51
Home Institution: Macalester College
Program: LSSURP
Faculty Mentor: Dr. Lisa Schimmenti
Poster Title: **Genomic Engineering and Pharmacotherapeutics to Model Human Deafness**
Abstract: The goal of our research is to model hereditary human deafness in zebrafish, *Danio rerio*, in order to better diagnose and treat the condition in humans. In one experiment, we used a relatively new genomic engineering technique called TALENs, Transcription Activator-Like Effector Nucleases, to create a line of zebrafish with a mutation in Gap Junction Beta2 (GJB2), a gene whose mutations are responsible for Nonsyndromic Hearing Loss and Deafness (DFNB1). In a second experiment, we studied mutations in Myosin 7a, a motor protein, which are known to result in the degeneration of sensory cells in the eye and ear leading to a condition known as Usher Syndrome. We conducted a drug toxicity study in which we administered drugs that were hypothesized to target the mechanoelectrical transduction (MET) channel in the inner ear hair cells to zebrafish carrying a mutation in Myosin 7a in order to identify the lethal dose for these drugs. The efficacy of these drugs will be tested by future studies in the lab in hopes of identifying a drug-based therapy for Usher Syndrome.

Presenter: **Pauline Xu** **Poster Number:** 52
Home Institution: University of Maryland - Baltimore County
Program: LSSURP
Faculty Mentor: Dr. Gregory Vercellotti
Poster Title: **Dimethyl Fumarate Induces Cytoprotection and Inhibits Vaso-occlusion in Transgenic Sickle Mice**
Abstract: Sickle cell disease (SCD) is one of the most commonly inherited hematological diseases in the world. Patients with SCD undergo unrelenting hemolysis, leading to the release of heme into the vasculature that promotes oxidative stress, inflammation, vaso-occlusion, and ischemia-reperfusion injury. Induction of intracellular antioxidants such as heme oxygenase-1 (HO-1) and ferritin induce cytoprotective responses that inhibit oxidative stress, inflammation, vaso-occlusion and organ damage in transgenic sickle mice. The master regulator of these responses is nuclear factor-erythroid 2 p45-related factor 2 (Nrf2). Recent studies have indicated that dimethyl fumarate (DMF), a recently FDA-approved drug for the treatment of multiple sclerosis, is an activator of Nrf2. Given the importance of developing new agents to treat sickle cell disease, we evaluated DMF cytoprotective responses in transgenic sickle mice by investigating the physiological and molecular effects of treatment. DMF-treated sickle mice had less microvascular stasis (a measure of vaso-occlusion) compared to vehicle-treated mice, and a marked increase in HO-1 activity and HO-1 mRNA in liver extracts. Using this knowledge, we hope to further elucidate the effects of DMF in transgenic sickle mice and be able to utilize DMF as a clinical treatment for SCD in the future.

Presenter: **Nebiyat Zewdie** **Poster Number:** 53
Home Institution: University of Maryland - Baltimore County
Program: LSSURP
Faculty Mentor: Dr. Claudia Schmidt-Dannert
Research Advisor:
Poster Title: **Purifying Novel Terpene Synthase HMGS gene fusion from *Stereum hirsutum***
Abstract: Mushrooms are a plentiful source of sesquiterpenoids with known therapeutic effects. Despite their pharmacological potential, they are a class of organic compounds rarely synthesized because of their complex structure, and their biosynthetic enzymes were unknown. Recently, a novel terpene synthase, the first isolated hirsutene synthase, had been identified in *Stereum hirsutum*. Furthermore, this hirsutene synthase unexpectedly exists as a gene fusion with a putative HMG-CoA synthase subunit. HMG-CoA synthase catalyzes an early step in synthesis of the hirsutene synthase substrate. We set out to confirm the function of this hirsutene synthase and to measure the activity of the putative HMG-CoA synthase subunit. As a result, the gene was cloned with an N-terminal his6x tag, expressed, and its protein product was purified by Ni2+ Column purification, currently underway. After purification, the novel protein's enzymatic activities will be analyzed in vitro to determine the kinetics of both the hirsutene synthase and the HMG-CoA synthase subunits. If confirmed, this would be the first gene fusion to catalyze non-consecutive steps in the isoprenoid biosynthetic pathway.