SOURCE

Intersections 2018

Summer Research Poster Session

Friday, August 3

10 a.m. to 12 p.m.

Biomedical Research Building

Atrium

Case Western Reserve University EST. 1826

think beyond the possible

Table of Content

Presenter Last Name	Presenter First Name	Home University/College	Major	Summer Program Name	Faculty Mentor	Faculty Mentor's Department
Anyaeche	Caleb	Fisk University	Computer Science	ACES+/NOA AGEP	Soumya Ray	Department of Electrical Engineering and Computer Science
Barral	Ann	Florida International University	Economics	Heart, Lung and Blood Minority research Training Program	Gaston, Benjamin	Pediatric Pulmonology
Cardona	Omar	University of Central Florida	Health Sciences Pre-Clinical Track	ACES+/NOA AGEP	Senyo, Sam	Biomedical Engineering
Carsey	Jane	Case Western Reserve Univeristy	Nutritional Biochemistry and Metabolism	Social Justice Institute Fellowship	Kam, Lynn	Department of Nutrition
Chin	Stephanie	Case Western Reserve University	Biomedical Engineering	Advanced Platform Technology Center Summer Internship	Majerus, Steve	Electrical Engineering and Computer Science, Biomedical Engineering
Connerton	Michael	Case Western Reserve University	Mechanical Engineering	SOURCE (PSURG, AHSS, STEM, SURES)	Quinn, Roger	Mechanical Engineering
Ellis	Kimberlyn	Spelman College	Biology	Heart, Lung and Blood Minority research Training Program	Peter MacFarlane	Neonatology
Folarin-Amode	Faridat	University of Illinois	Medical Student	Heart, Lung and Blood Minority research Training Program	Buck, Matthias	Physiology & Biophysics

Presenter Last Name	Presenter First Name	Home University/College	Major	Summer Program Name	Faculty Mentor	Faculty Mentor's Department
Fung	Kevin	Case Western Reserve University	Biology	SOURCE (PSURG, AHSS, STEM, SURES)	Brown, J. Mark	Department of Cellular and Molecular Medicine
Garb	Madeline	Case Western Reserve University	Nutritional Biochemistry & Metabolism	Wharton Summer Research Fellowship	Watowicz, Rosanna & Rogers, Catherine	Nutrition, Nutrition
Gillespie	Caitlyn	Fisk University	Biology	ACES+/NOA AGEP	Michiko Watanabe	Pediatrics
Grant	Joydan	Tuskegee University	Mechanical Engineering	ACES+/NOA AGEP	Bruckman, Laura	Materials Science and Engineering
Hegde	Pranav	Case Western Reserve University	Nutritional Biochemistry	Wharton Summer Research Fellowship	Swain, James	Nutrition
Huan	Yu	Case Western Reserve University	Biology and Statistics	SOURCE (PSURG, AHSS, STEM, SURES)	Chiel, Hillel	Biology, Neuroscience, Biomedical Engineering
Huang	Fanny	Case Western Reserve University	Nutritional Biochemistry and Metabolism	SOURCE (PSURG, AHSS, STEM, SURES)	Dombrowski, Stephen	Pediatric Neurosurgery
Jeansonne	Noel	Case Western Reserve University	Biomedical Engineering, Pre-Medicine	Advanced Platform Technology Center Summer Internship	Tyler, Dustin	Department of Biomedical Engineering
Kendall	Lee	Case Western Reserve University	Materials Science and Engineering	SOURCE (PSURG, AHSS, STEM, SURES)	Sehirlioglu, Alp	Materials Science and Engineering
Kendrick	NaShea	Oglethorpe University	Chemistry	ACES+/NOA AGEP	Tolbert, Blanton	Chemistry
Knight	Julia	Case Western Reserve University	Biochemistry and English	SOURCE (PSURG, AHSS, STEM, SURES)	Zaman, Khalequz	Biochemistry and Pediatrics
Koerwer	Hayden	case western reserve university	biomedical engineering	Advanced Platform Technology Center Summer Internship	Hess-Dunning, Allison	Biomedical Engineering

Presenter Last Name	Presenter First Name	Home University/College	Major	Summer Program Name	Faculty Mentor	Faculty Mentor's Department
Kong	Jessica	Case Western Reserve University	Communication Sciences	SOURCE (PSURG, AHSS, STEM, SURES)	Calandruccio, Lauren	Psychological Sciences
Martir Gonzalez	Sofia	San Juan Bautista School of Medicine	Medical Student	Heart, Lung and Blood Minority research Training Program	Levine, Alan	Departments of Molecular Biology and Microbiology, Pathology, Pharmacology, and Medicine
McMillen	Madelyn	Case Western Reserve University	Chemistry	The Pentzer Lab supported by NSF CAREER #1551943	Pentzer, Emily	Chemistry
Miller	Emiko	Marquette University	Biomedical Sciences	Independent of a Program/Working Directly With a Faculty Member	Dr. Andrew Pieper	Psychiatry
Mistry	Viral	Case Western Reserve University	Biology	Department of Physiology and Biophysics	LaManna, Joseph	Physiology and Biophysics
Moyer	Devlin	Case Western Reserve University	Biochemistry	SOURCE (PSURG, AHSS, STEM, SURES)	Padgett, Richard	Cellular and Molecular Medicine
Nelson	Melanie	Wesleyan University	Neuroscience and Behavior/Psychology	Heart, Lung and Blood Minority research Training Program	Maiseyeu, Andrei	Cardiovascular Research Institute
Nmai	Christopher	Harvard College	History and Science: Medicine and Society	Heart, Lung and Blood Minority research Training Program	Deiuliis, Jeffrey	Case Cardiovascular Research Institute
Noriega	Santiago	Tufts University	Cognitive and Brain Sciences	Heart, Lung and Blood Minority research Training Program	Lewis, Stephen	Pediatrics
Paranjape	Sanika	Case Western Reserve University	Biology and Cognitive Science	SOURCE (PSURG, AHSS, STEM, SURES)	Zigmond, Richard	Department of Neurosciences

Presenter Last Name	Presenter First Name	Home University/College	Major	Summer Program Name	Faculty Mentor	Faculty Mentor's Department
Pataroque	Kevin	Case Western Reserve University	Chemical Engineering	SOURCE (PSURG, AHSS, STEM, SURES)	Duval, Christine	Chemical Engineering
Qian	Samuel	Case Western Reserve University	Cognitive Science	SOURCE (PSURG, AHSS, STEM, SURES)	Tesar, Paul	Genetics and Genome Sciences
Reddy	Srija	Case Western Reserve University	Nutritional Biochemistry and Metabolism	Wharton Summer Research Fellowship	Brunengraber, Henri	Nutrition
Rosenberg	Joshua	Case Western Reserve University	Biomedical Engineering/ Electrical Engineering	Advanced Platform Technology Center Summer Internship	Schiefer	Matthew
Santiago	Raymond	The Ohio State University	Neuroscience	Heart, Lung and Blood Minority research Training Program	Thomas E. Dick	Division of Pulmonary, Critical Care and Sleep, Department of Medicine; CWRU
Sears	Ellen	Case Western Reserve University	Nutrition	Wharton Summer Research Fellowship	Hand, Rosa	Nutrition
Sedor-Schiffhauer	Zach	Case Western Reserve University	Nutritional Biochemistry and Metabolism	Wharton Summer Research Fellowship	Croniger, Colleen	Nutrition
Stamper	Michaela	Case Western Reserve University	Nutritional Biochemistry	Wharton Summer Research Fellowship	Manor, Danny	Nutrition, Pharmacology
Thompson	Austin	University of Cincinnati	Biology/ Pre-Med	Heart, Lung and Blood Minority research Training Program	Gerson, Stanton	Department of Medicine
Vance	Anastacia	Fisk University	Biology	ACES+/NOA AGEP	Milton, Adrianna	Department of Neurosciences
Vera-Cruz	Elfreda	University of Arkansas at Pine Bluff	Nursing	ACES+/NOA AGEP	Jolly, Emmitt	Biology

Presenter Last Name	Presenter First Name	Home University/College	Major	Summer Program Name	Faculty Mentor	Faculty Mentor's Department
Walker	Bradley	Case Western Reserve University	Chemistry	Heart, Lung and Blood Minority research Training Program	Sekaly, Rafick-Pierre	Pathology
Walker	Jasmine	Emory University	Human Health, Pre-Medicine	Heart, Lung and Blood Minority research Training Program	Dallas, Mari	Pathology
Wang	Louisa	Hathaway Brown	N/A	Hathaway Brown	Pentzer, Emily	Chemistry
White-Smith	Airreonna	Tuskegee University	Mechanical Engineering	ACES+/NOA AGEP	Carter, Jennifer	Material Science
Whittsette	Angela	Case Western Reserve University	Nutritional Biochemistry	Heart, Lung and Blood Minority research Training Program	Mu, Tingwei	Physiology and Biophysics
Williams	Jaylen	Miami University	Zoology	Independent of a Program/Working Directly With a Faculty Member	Wei, Peiran	CHemistry
Yu	Marina	Case Western Reserve University	Biomedical Engineering	Advanced Platform Technology Center Summer Internship	Capadona, Jeffrey	Biomedical Engineering

Predicting Treatment Success of Overactive Bladder Syndrome with Anti-Cholinergic Therapy

Caleb Anyaeche, Department of Computer Science; Dr. Soumya Ray, Department of Electrical Engineering and Computer Science; Dr. David Sheyn, Department of Obstetrics and Gynecology, University Hospitals

The purpose of this study is to use machine learning to accurately predict successful treatment of Overactive Bladder Syndrome with anti-cholinergic medications. Overactive bladder (OAB) is a condition characterized by urinary urgency, frequency, nocturia, and urinary incontinence. OAB is thought to affect as many as 17.5 million women in the US, with up to 30% of women over the age of 65 being affected. While some cases of OAB may be related to neurological disorders such as spinal cord injury and multiple sclerosis, or a result of prior urinary tract surgery, the majority of cases are idiopathic. The current standard of care for treating overactive bladder (OAB) is first to try medical management with a class of drugs that exert anticholinergic activity on the bladder to decrease bladder spasm. However, this medication is only effective in roughly half of patient with OAB with those failing medications moving on to more effective third line therapies such as injection of botulinum toxin into the bladder muscle or placement of a neuromodulation device, a sort of bladder pacemaker. The current treatment paradigm for OAB means that patients must fail one to two medications prior to moving on to these therapies. Often patients become frustrated with lack of treatment response and are lost to follow-up. This results in only 5% of patients eligible for third line therapy undergoing the treatment. Currently there is no way to predict who will respond to medications and who will not. If it was possible to have this information, physicians would be able to appropriately triage patients to effective therapies, leading to decreased times of relief of symptoms and potentially significant reduction in direct and indirect cost of OAB treatment. We are investigating the use of machine learning methods to do this. We are working with an anonymized dataset of 490 patients with various measurements such as history of neurologic disease and presence of diabetes. Our current models, which are based on tree structures, achieve an average predictive accuracy of 73.4%. Analyzing individual feature also leads to interesting insights about the efficacy of a medical test sometimes performed before prescribing medication.

Project Mentor: Dr. Soumya Ray, Department of Electrical Engineering and Computer Science

◆◆◆

Analysis of exhaled S-Nitrosoglutathione in children with Sickle Cell Disease

Ann Barral, Department of Pediatric Pulmonology; James Seckler, Nikki M. Meyer, Jennifer Yonkof, Justine Ade, Department of Pediatrics; Laura Smith, Benjamin Gaston, Department of Pulmonary Pediatrics

S-Nitrosoglutathione (GSNO) is a type of endogenous S-nitrosothiol (SNO) that plays a critical role in cell signaling. It is a source of biologically available nitrosonium (NO+) and nitric oxide (NO) radical. GSNO concentrations regulate respiratory function by controlling airway tone and inflammatory responses in the respiratory tract. Hemoglobin (Hb) iron in the 3+ oxidation state permits GSNO and NO production by somatic cell NO synthase, while Hb Fe2+ captures NO and promotes formation of inert nitrate (NO3-) (Nature 491:473-7, 2012; Ann Rev Physiol 2015; 77:431-52). Because

Hb is expressed in airway epithelial cells, we studied differences in the NO metabolism between children with Hb SS sickle cell disease and healthy children by analyzing the differences in airway GSNO, NO3- and nitrite (NO2-)using exhaled breath condensate samples. Sickle Cell Disease children frequently have airflow obstruction despite having minimal evidence for classical asthma. Instead, children with sickle cell disease acquire acute chest syndrome (ACS). ACS is caused by sickling in the pulmonary vasculature and/or a lung infection and the resulting inflammation and loss of oxygen saturation leads to further sickling of red cells. We hypothesized that at baseline, mean GSNO/ NO3- ratio would be lower in patients with SS disease because of inadequate oxidation of mutant Hb β iron in the airway epithelium, helping to explain the tendency of these patients to develop obstructive lung disease, and that this would be particularly true in those with a history of ACS. A carbon fibered tip nanosensor was used to measure GSNO concentrations; NO3- and NO2- were measured via colorimetric analysis. As the first step in this process, we measured levels in 8 healthy control children, [GSNO] is still in the process, NO3- is undetectable, GSNO/ NO3- is still in the process and NO2- is 0.13 μM. These values have not previously been measured in normal children, and the data will be an essential reference for study airway abnormalities in children with sickle cell disease.

Project Mentor: Dr. Benjamin Gaston, Department of Pulmonary Pediatrics

◆◆◆

Effects of ARNT and Hypoxia on Regeneration of Bone Marrow After 5-Fluorouracil Treatment

Nichola Bomani, Department of Biochemistry; Diana Ramirez-Bergeron PhD, Cardiovascular Research Institute, CWRU School of Medicine

The bone marrow (BM) houses hematopoietic stem and progenitor cells (HSPCs), which give rise to all mature blood cells. Normally quiescent, HSPCs rely on the BM vascular niche for activation and subsequent differentiation. Specific endothelial molecules have been shown to facilitate the maintenance and reconstitution of the bone marrow, including the Notch signaling pathway. Conditions of low oxygen, hypoxia, are one such activator of proliferation in the BM. Hypoxia Inducible Factors (HIFs) are master regulators of the cellular responses to hypoxia. In normoxic conditions, HIF1-α is degraded, but in hypoxia, it binds to HIF1-β (aryl hydrocarbon nuclear translocator, ARNT). The complex moves to the nucleus, acting as a dimeric transcription factor upregulating hundreds of genes, particularly those that promote angiogenesis, the formation of new bloods vessels. 5-Fluorouracil (5-FU) is a common chemotherapy drug used to target proliferative cells, including the BM's blood cells. However, 5-FU has been shown to have cardiotoxic properties. Data from the laboratory shows that conditional deletion of ARNT in the endothelium of mice leads to their increased susceptibility to 5-FU. We hypothesize that endothelial HIF signaling is critical for recovery of the vasculature of the BM niche in response to stress. Our objective was to generate experimental approaches to further address the role of HIF signaling in supporting the regeneration of BM vasculature after 5-FU treatment. First, we investigated the effects of hypoxia on the transcription of Notch molecules in EC cultures. Second, we examined how the in vivo loss of endothelial-ARNT affects the proliferation of BM cells following 5-FU treatment. Last, we developed a novel organ BM culture system that will permit us to carefully examine the effects of hypoxia and 5-FU using genetically or chemically modified specimens.

Project Mentor: Dr. Diana Ramirez-Bergeron, Cardiovascular Research Institute, CWRU School of Medicine

◆◆◆

Developing established cell-line models to identify adverse effects of a diabetic environment on fibroblast derived extracellular matrix and cardiomyocytes

Omar A Cardona, Burnett School of Biomedical Sciences, University of Central Florida, John C Bradford, Department of Biomedical Engineering, Stephan Nieuwoudt, Department of Biomedical Engineering, Samuel E Senyo, Department of Biomedical Engineering

Heart disease is a major cause of death in diabetic patients with complex pathological mechanisms that are not completely understood and distinguish diabetic cardiomyopathy from other cardiac disorders. We hypothesize that diabetic microenvironment (DM) plays a direct role in the adverse cellular signaling associated with diabetes. Though it is established that the extracellular matrix (ECM) provides biological cues that influence cell behavior; there is no explicit evidence that metabolic perturbations of diabetes mellitus can have an effect on disease progression in diabetic cardiomyopathy. The purpose of this study is to develop *in vitro* models of diabetic cardiomyopathy using established cell lines to generate functional ECM to then test direct effects of diabetes mellitus on cardiac insulin signaling. Using published approaches, visible yields of acellular ECM is generated by ascorbic acid-induced fibroblast ECM. Preliminary results from our lab have shown that primary neonatal cardiomyocytes cultured in low-glucose media on ECM produced by cardiac fibroblasts treated with high glucose exhibit reduced insulin sensitivity (pAkt). Establishing a similar model with cell lines would expand experimental manipulation while reducing costs. The following cell lines were used: H9c2, left ventricular *Rattus norvegicus* (rat) cardio-myoblasts; and NIH/3T3, *Mus musculus* (mouse) embryonic fibroblasts. Akt phosphorylation (pAkt), a key protein in glucose metabolism, was used as an indicator of insulin sensitivity and assessed via Western blot. We first showed that H9c2 cells demonstrate insulin sensitivity in a similar fashion to C2C12 cells, an insulin sensitive myoblast cell line derived from mouse skeletal muscle. Our experiments suggest that supra-physiological levels of glucose induce 3T3 cells or primary fibroblasts to deposit ECM exhibiting altered structure and biological activity. Collagen deposition was significantly greater in primary FDM under high glucose versus low glucose treatment (144.6±23.6 versus 98.5±19.1 Analine blue density), independent of decellularization solution used (NH_4OH, SDS, or TX100). Clinically, the DM has direct effects on cardiomyocytes, as indicated by an increase in the deposition of lipid metabolites into peripheral tissues such as the heart, leading to insulin resistance. As such, we have established a model for this specific diabetic phenotype using the H9c2 cell line based on insulin induced Akt signaling with an intriguing direct role for the ECM.

Project mentor: Samuel Senyo, Department of Biomedical Engineering

Investigation of the Role of Estrogen Receptors and Gut Hormones in Sensitivity to Eating Disorders and Menstrual Dysfunction in Female Collegiate Athletes

Jane C. Carsey, Nutritional Biochemistry and Metabolism

The prevalence of clinical and subclinical eating disorders is greater in athletes than in non-athletes. Additionally, females are at a higher risk due to increased prevalence of restrictive eating behaviors, participation in aesthetic and low body weight sports, and social pressures. Disordered eating can lead to a decrease in energy availability (EA), which is defined as energy intake minus the energy expended from exercise. Chronic low EA can cause health consequences including loss of muscle mass and bone density, increased injury risk, reproductive cycle disruptions, and immune system suppression. It remains unclear, however, why only some individuals with low EA may develop menstrual dysfunction or eating disorders and others may not. The primary purpose of this study is to determine whether subjects with higher risk for eating disorders have differences in estrogen receptors and gut hormones compared to those at low risk. A secondary aim is to explore the relationship between low EA, diet quality, menstrual status, estrogen receptor expression, and gut hormone levels. Disordered eating behaviors will be assessed using the sub-scores for perfectionism, drive for thinness, and body dissatisfaction from the Eating Disorder Inventory-2 (EDI-2). We will also use 3-day food and physical activity logs, resting metabolic rate (RMR), and body composition to evaluate energy status. Diet quality will be assessed using the Healthy Eating Index (HEI). Estrogen receptor polymorphisms will be determined by PCR and gut hormones will be assessed using ELISA kits. We hypothesize that lower EA will be associated with differences in the estrogen receptor genes, a higher EDI-2 sub-score, higher plasma ghrelin and peptide YY (PYY), and lower plasma estradiol. We also expect menstrual dysfunction to be associated with higher ghrelin and PYY and lower estradiol. We anticipate that this study will help determine possible risk factors for eating disorders in female athletes.

Project Mentor: Dr. Lynn Kam, Department of Nutrition

◆◆◆

Stenosis Characterization and Identification for Dialysis Vascular Access

Stephanie Chin, Department of Biomedical Engineering; Binit Panda, Department of Biomedical Engineering; and Steve Majerus, Department of Electrical Engineering and Computer Science, Department of Biomedical Engineering

The Veterans Health Administration cares for an increasing population of over 35,000 Veterans suffering from kidney failure. The cost of treatment and long-term dialysis success is directly related to a well-functioning vascular access, which is most commonly hindered by blood vessel narrowing (stenosis). Current screening methods to detect stenosis are both time consuming and costly. Because of this a noninvasive, real-time screening procedure of patients at risk of vascular access clotting or failure is desired. Previous research has shown that digital analysis of blood flow sounds (phonoangiograms or PAGs) can estimate the level of stenosis in a vascular access. This research is concerned with

determining specific spectral features of PAGs that accurately predict both the location and degree of stenosis (DOS). We have analyzed PAG recordings from six vascular access phantoms with varying DOS and hemodynamic flow types using blood-mimicking fluid. Our results indicate that out of the 14 spectral features tested, the Average Spectral Centroid of a PAG can accurately locate and estimate the DOS.

Project Mentor: Dr. Steve Majerus, Department of Electrical Engineering and Computer Science, Department of Biomedical Engineering

◆◆◆

Lower-Body Exoskeleton Planetary Style Power Unit Development

Michael Connerton, Department of Mechanical Engineering; Mark Nandor, Department of Mechanical Engineering

The Biologically Inspired Robotics Research Laboratory here at CWRU has been in the process of developing a Cyber-Physical Walking System (CPWS) that integrates: a person with a spinal cord injury but still has intact and excitable lower motor nerves, a mechanical exoskeleton, DC motors for powered joint assistance, and algorithms that learn to improve walking and standing stability. This research focused on the DC motor power units that actuate to provide joint assistance for the hips, knees, and ankles. In the past, the team of engineers working on this project explored the options of hydraulics and harmonic drive reduction but found these options too complicated and inefficient respectively. In an attempt to improve on these previous designs, this research developed a similar DC motor power unit based off a planetary gear system in place of the harmonic drive used in the past. This idea came from the fact that planetary gear systems are highly efficient as well as compact. This allowed the use of a smaller motor for the desired power output while allowing for a more compact overall design. In addition to the DC motor drivetrain, each power unit incorporates an electromechanical brake to aid in stability of the joints as well as a torsional feedback system allowing the control unit to monitor its output for more precise movements. This power unit has been designed in a way such that it is generalizable to all three major lower-body joints: hips, knees, and ankles, in an attempt to simplify the manufacturing process from previous versions of the exoskeleton.

Project Mentor: Dr. Roger Quinn, Department of Mechanical Engineering

◆◆◆

Developing a neonatal rat model of airway hyperreactivity associated with bronchopulmonary dysplasia (BPD)

Kimberlyn Ellis[1], Catherine Mayer[2], Peter MacFarlane[2]

[1]Department of Biology, Spelman College
[2]Department of Pediatrics, Case Western Reserve University

Underdeveloped lungs pose a significant contribution to the morbidity of prematurity. Preterm infants in the NICU often require supplemental O_2 (ie. Hyperoxia) as an important life-saving mode of respiratory support to ensure healthy oxygen intake. One unfortunate consequence of supplemental O_2 therapy, however, relates to the adverse effects it has on lung development. This can manifest as airway (AW) hyperreactivity which is an underlying feature of lung disease (ie bronchopulmonary dysplasia) and various wheezing disorders including asthma. The neonatal mouse exposed to hyperoxia in the early postnatal period is a common animal model used to induce airway hyperreactivity and replicate a preterm infant with BPD. The purpose of this study, however, was to develop a rat model of airway hyperreactivity. Neonatal rats received hyperoxia (40% inspired O_2) 24 hours/day for their first five postnatal days (p1-p5). On the sixth day (p6), the rats were sacrificed and AW reactivity to increasing doses of methacholine (0.25μM-8μM) were measured using the precision cut lung slice method. It was found that 40% O_2 increased airway reactivity compared to age-matched rats raised in normoxia. We are beginning to explore the possibility that the airway hyperreactivity following hyperoxia exposure is associated with increased expression of lung hyaluronan, an important component of the extracellular matrix previously shown to play a role in AW hyperreactivity seen in other lung disease models. This suggests neonatal rats may also be a viable model of airway reactivity associated with BPD.

Project mentors: Dr. Peter M. MacFarlane

Funded by the National Heart, Lung, and Blood Institute, Grant# R25-HL03152 (Monica Montano, PI)

Characterizing Neuropilin-1(NRP1) co-receptor transmembrane region interaction and binding with with Plexin B1(PB1) transmembrane region

Faridat Folarin-Amode, UIC College of Medicine, Susan Kim, Department of Physiology & Biophysics; Jeannine Muller-Greven, Department of Physiology & Biophysics

Neuropilin-1 is a transmembrane coreceptor to Plexin transmembrane receptors and Semaphorin ligands. Neuropilin plays a diverse role in human physiology and pathology including normal organogenesis and cancer progression. The purpose of this research project is to detail the protein-protein interactions between the Plexin-B1 transmembrane region and the Neuropilin-1 co-receptor transmembrane region leading to the formation of protein complexes. In order to study this topic, we are purifying Semaphorin (ligand of Plexin-B1), plexin B1 and Neuropilin using Nickel beads to bind to protein His-tag and High Pressure Liquid Chromatography. The next steps will then be identifying protein structure and interactions using Nuclear Magnetic Resonance spectroscopy. This project can then provide further insight on the signalling mechanisms in the cell's hydrophobic transmembrane region.

Project Mentor: Professor Matthias Buck, Department of Physiology & Biophysics

◆◆◆

Understanding the Role of the Bile Acid Activated Farnesoid X Receptor (FXR) in the Pathogenesis of Obesity and Cardiovascular Disease

Kevin Fung, Candidate for B.S. in Biology, Department of Cellular and Molecular Medicine; Dr. Rebecca Schugar, Department of Cellular and Molecular Medicine; Dr. Anagha Kadam, Department of Cellular and Molecular Medicine; Chelsea Finney, Department of Cellular and Molecular Medicine; J. Mark Brown, Department of Cellular and Molecular Medicine

Flavin Monooxygenase 3 (FMO3) is a hepatic enzyme that converts the gut microbial metabolite trimethylamine (TMA) into trimethylamine n-oxide (TMAO). Clinical studies have shown that elevated TMAO levels in plasma are also associated with cardiovascular disease (CVD) and obesity in humans and mice. The exact mechanism by which the TMA/FMO3/TMAO pathway affects the pathogenesis of obesity and CVD is unknown. Previous studies have demonstrated that FMO3 is a direct transcriptional target of the Farnesoid X Receptor (FXR), a bile acid activated nuclear receptor that plays a critical role in metabolism and gut microbial community structure. This research is focused on how the FMO3 pathway and FXR interact to cause changes in the host metabolism. Using mouse models, we have investigated the effects of the FXR agonist GW4064 on metabolite levels in the intestines, plasma, and bile, hypothesizing that the activation of FXR increases the secretion of TMAO into the bile and out of the body. This proposed increased excretion

of TMAO may lead to lower plasma TMAO levels, decreasing adverse cardiovascular outcomes in patients. These studies will be the first to elucidate whether FXR agonism can be used therapeutically to reduce the risk of developing metabolic syndrome.

Project Mentor: Dr. J. Mark Brown, Department of Cellular and Molecular Medicine

◆◆◆

Pediatric Primary Care Providers' Perception and Documentation of Obesity

Madeline Garb, Department of Nutrition

A barrier to implementing a successful pediatric obesity intervention program is the low percentage of patients with obesity who have diagnoses consistent with obesity in the electronic medical record (EMR.) Diagnosed patients are more likely to receive obesity-related counseling (diet, exercise, weight management) than those who remain undiagnosed. Previous studies of retrospective chart reviews from primary care centers found the majority of overweight children and approximately half of obese children remain unidentified by primary care providers. Aside from time constraints, literature indicates the greatest obstacle to pediatric obesity screening is primary care providers' apprehension discussing sensitive topics which may offend children and their primary caregivers and adversely affect clinician-patient relationships. The outpatient Rainbow Ambulatory Practice (RAP) clinic at Rainbow Babies, and Children's Hospital (RBC) has identified the need for obesity interventions among their patient population. This study aims to quantitatively characterize the RAP clinic primary care providers' rate of documented obesity diagnosis and qualitatively examine barriers to diagnosis and strategies to circumvent barriers. The purpose of this abstract is to present the methodology that will be used in the future for this study. We will conduct a retrospective chart review of the EMR to identify the proportion of children 2-18 with obesity (defined as a BMI ≥ 95th percentile for their age and sex according to CDC 2000 growth charts) seen for well-children visits in the past 2 years, and assess documentation of diagnoses as well as interventions. We will also recruit n=54 RAP clinic clinicians (60% of total) to complete electronic surveys designed through REDCap. Of the electronic survey participants, a subsample of n=10 physicians will be recruited to complete one-on-one-telephone interviews. Interviews will be transcribed, coded, and analyzed. We anticipate the present study will help improve pediatric obesity identification and management practices in the RAP clinic, as well as other primary care offices.

Project Mentors: Dr. Catherine Rogers, Department of Nutrition; Dr. Rosanna Watowicz, Department of Nutrition

Effects of Prenatal Alcohol Exposure on The Innervation of The Heart

Caitlyn Gillespie, Department of Biology; Angela Zhu, Lab Investigator; Nikhita Kumar, Lab Investigator; Jun Kim, Department of Biology; Safdar Jawaid, Department Pediatrics; Michael Jenkins, Department of Pediatrics; Meredith Broberg, Department of Pediatrics; Michiko Watanabe, Ph.D., Department of Pediatrics

Fetal Alcohol Syndrome, a lifelong illness that includes physical, behavioral, or learning problems, occurs when a mother consumes alcohol while the baby is still *in utero*. The risk of alcohol exposure *in utero* is extremely high for pregnant women in the first trimester because many of them are unaware of their pregnancy. Previous research in the field has shown that neural crest cell ablation leads to an abnormal neuronal innervation of the heart, and alcohol adversely affects neural crest cells. In the current experiment, the exposure of alcohol and its effects on the neuronal innervation of the embryonic heart was studied using an avian model, quail as its autonomic innervation of the heart is similar to those of humans. Quail eggs were injected with 50% ethanol solution during gastrulation to simulate a binge drinking episode during the first few weeks of pregnancy. Control eggs were injected with phosphate buffered saline. These eggs were then incubated for eight days until development of a four chambered heart and neuronal innervation similar to those of humans. The embryo was then dissected, and the whole hearts were immunostained with anti-TUJ1, which is specific for neurons. Our results show that the neuronal innervation of ethanol injected hearts appeared distorted, displaying shorter and irregular branching compared to control hearts. Additionally, the thoracic sympathetic cardiac branch on one of the ethanol injected hearts appeared to be split into two branches. Our study concludes that ethanol exposure results in embryonic heart defects and abnormal neuronal innervation in the quail model, thus strongly suggesting that prenatal alcohol exposure leads to serious congenital defects.

Project Mentor: Dr. Michiko Watanabe Ph.D., Pediatric Cardiology Research Laboratory

◆◆◆

Cracking of Polymer Backsheets

Joydan Grant, Mechanical Engineering

Polymer backsheets are 3 layers of poly laminate film that provide electrical insulation. They also act as environmental barriers against electrical shock. In this study, different multilayer backsheets with varying material combinations were exposed to real-world and accelerated weathering conditions to deduce the breakdown of these polymer materials. There are five different types of cracking degradation: parallel, mudflat, localized, transverse branching, and longitudinal branching. Parallel is the orientation in which all the cracks are going in the same direction; contrary to parallel cracking, mudflat cracking is when the cracks are oriented in different directions. These two types can also be localized, meaning they do not cover the entire width of the backsheet. Transverse branching, one of the least common

types of cracks, has perpendicular branches stemming from parallel cracking. Last but not least, longitudinal branching are finely spaced parallel cracks which are often difficult to notice. There are two types of de-adhesion degradation: blistering and delamination. Blistering is the localized loss of adhesion between the layers, which causes the backsheet to bubble upwards or curl if a crack is present. Delamination is the large-scale loss of adhesion which causes bits and pieces of the inner layer of the backsheet to fall off. In order for continuing growth in this industry, it is important to understand and quantify the forms of degradation that can compromise the integrity and performance of the varying backsheets.

Project Mentor: Laura S. Bruckman, Department of Materials Science and Engineering

◆◆◆

The Effects of Excess Dietary Iron on Intestinal Tumorigenesis

Pranav Hegde, Department of Nutrition; Isaac Jang, Orange High School; Maria Linda Burola, Department of Nutrition; and James Swain, Department of Nutrition

The link between diet and cancer has been shown in general nutrition and biomedical studies; however, the effects of specific nutrients on tumor development and growth is still poorly understood. While prior research studies indicate that minerals in the diet can accelerate cancer cell proliferation, most of these studies do not correlate with the amount of normal human dietary mineral consumption. The goal of this study was to determine the effect of excess dietary iron – in high, yet realistic amounts - on tumorigenesis using cell proliferation markers as indicators of potentially abnormal growth patterns in normal and intestinal tumor tissue. In this study, weanling (4-6 weeks old) male $Apc^{min/+}$ mice were fed one of three different concentrations of dietary iron: adequate iron (AFe; 45 mg Fe/kg diet), moderately high iron (MHFe; 250 mg Fe/kg diet), and excessively high iron (HFe; 450 mg Fe/ kg diet) as ferrous sulfate monohydrate ($FeSO_4 \bullet H_2O$). After 10 weeks, intestines were excised and placed on slides, then stained with ki-67, an immunohistochemical (IHC) marker for cell proliferation. IHC slide samples were examined using the automated Leica DM6000B microscope. A Leica SCN 400B Rapid scanner was used to produce high-res images. After imaging intact crypt-villi segments, data were analyzed using SPSS statistical software. Our data show that there was a significant effect of excess dietary iron on ki67 expression in full-length crypts and villi crypt-to-tip sections [$F(2, 122) = 22.11$, $p < 0.05$; and $F(2, 122) = 4.84$, $p < 0.05$]; however, no correlation was found on the effect of excess dietary iron on ki-67 expression on only the villi [$F(2, 122) = 1.85$, $p = 0.16$]. Findings suggest that excess dietary iron leads to increased cell proliferation in the crypts of this mouse model of human intestinal tumorigenesis. These results

are clinically significant in that such acceleration may lead to a greater risk of uncontrolled polyp growth and hence increased cancer mortality.

Faculty Mentor: Dr. James Swain, Department of Nutrition

◆◆◆

Neural Network Dynamics Controlling Egestive Feeding Behavior in *Aplysia californica*

Yu Huan, Department of Biology; Jingyi Yang, Department of Biology; Nathan Kodama, Department of Electrical Engineering and Computer Science; Hui Lu, Department of Biology; Dr. Roberto F. Galán, Department of Electrical Engineering and Computer Science; Dr. Hillel J. Chiel, Department of Biology

A major goal in current neuroscience is to understand how constantly changing neural activity allows animals to adjust their behaviors to accommodate to the environment. The motor expression of feeding behaviors in *Aplysia californica* is controlled by a neural network located within the buccal ganglia and therefore provides a valuable model to address this question. In previous studies, the identity and behavior-related function of interneurons and motor neurons in the buccal ganglia have been characterized individually, but to understand the dynamics of the neural circuit, studying interactions between a large population of neurons and their coordinated activities that contribute to the feeding behavior is as critical as individual neural activity. In this study, we simultaneously recorded the nerve activities on the I2 nerve, the radular nerve, buccal nerve 2 and buccal nerve 3, and activity from a large population of key neurons via a microelectrode array (MEA, 120 electrodes) over time. We also investigated the effects of interneurons B4/B5 on other motor neurons and the motor output by stimulating the neurons directly through MEA. We identified multiple key neurons simultaneously on the array through their one-to-one projections to the nerves. In response to stimulation of nerve branch BN2-a, their activity was consistent with an egestive motor program. Our data showed that interneurons B4/B5 have an inhibitory effect on several motor neurons such as B6/B9, consistent with what is known from intracellular studies. Showing this as the neurons receive synaptic input from many other neurons provides evidence for the dominant role of B4/B5 in shaping egestive motor patterns.

Project Mentor: Dr. Hillel Chiel, Department of Biology

◆◆◆

Choroid Plexus Carcinoma (CPC): Model Development and Characterization

Fanny Huang, Christopher Slater, Tamara Abou-Anton, Stephen Dombrowski, Violette Recinos

Overall pediatric brain tumors now represent the greatest cause of death among all childhood cancers. Choroid plexus

tumors, which account for approximately 2-4% of all pediatric brain tumors, can be further classified as choroid plexus papilloma (CPP) that have 90% survival rate, and the more aggressive choroid plexus carcinoma (CPC), WHO grade III tumor are recurrent and ultimately fatal, with less than 50% survival rate in children younger than 5 years old. Current therapies for CPC typically include surgical resection, chemotherapy, radiotherapy, or a combination, often are associated with transient improvement but no long term patient survival. Thus, there is an unmet need to develop new treatments that can be used independently or in combination with current clinical therapies. In order to support these efforts, an experimental model is greatly needed to better understand the underlying genetic and molecular characteristics during tumor development and progression. In this investigation, we have developed working experimental models of primary and recurrent CPC tumors obtained from a single patient (2y.o, male). From primary tumor specimens, and primary and patient-derived xenograft (PDX) cell lines, we characterize these tumors using DNA sequencing and methylation, histology/immunohistochemistry, and in vitro functional assays including tumor cell proliferation, migration and sphere formation, as well as in vivo (orthotopic) model survival. We hypothesize that the CPC xenograft models will retain the same genetic mutations as the primary tumor cell lines; however the recurrent CPC cell line will have more genetic alterations than the primary cell line, perhaps contributing to tumor growth and/or drug resistance. In addition, we report findings for functional properties including tumor cell migration and sphere formation (in vitro) and survival (in vivo). Overall, we have successfully established pediatric CPC models (primary, recurrent, and xenograft) which have different genetic and molecular profiles to help better understand etiology and pathological progression, as well as identify more effective treatments for children with this deadly brain tumor.

Project Mentor: Dr. Stephen Dombrowski, Department of Pediatric Neurosurgery

◆◆◆

Design and Analysis of a 3D Printed Magnetic Breakaway Connector for Use in Implanted Stimulation Devices

Noel Jeansonne, Biomedical Engineering & Pre-Medicine; Ivana Cuberovic, B.S., Department of Biomedical Engineering; Dustin Tyler, Ph. D., Department of Biomedical Engineering

The Magnetic Breakaway Connector (MBC) is designed to reduce risk of injury during at-home use with a neural prosthesis system. The neural prosthesis system contains percutaneous leads from the limb of the subject which are connected to various sensors on the prosthetic surface. Snagging of these cables could pull the percutaneous leads out of the skin, causing injury to the subject, and potentially harming the implanted system. Tension on the wires of the neural prosthesis system causes the MBC to open, thus disconnecting the internal electrical components by counteracting the magnetic connective force. The internal magnetic system is composed of a ring of magnets around the electrical components to compress the internal connector spring pins with 2.0 pounds of pull force required for complete separation of the electrical components. The MBC has a 2-keyed directional design to prevent improper connection of the male and female pieces when used by the subject at home. The housing was printed in Think[box] using the 3D printers to create a cost effective and easy to manufacture housing.

Project Mentor: Professor Dustin Tyler, Ph. D.

Exfoliation of $LiCoO_2$ into CoO_2 Nanosheets Investigated by XPS

Lee Kendall, Department of Materials Science and Engineering; Kevin Pachuta, Department of Materials Science and Engineering

$LiCoO_2$ is currently the most widely utilized positive electrode material for commercial Li-ion batteries, and its bulk properties have been extensively studied. However, there is a lack of current research in the two-dimensional properties of $LiCoO_2$, and the CoO_2 phase that lies between the Li layers. To investigate this CoO_2 layer, lithium deintercalation of $LiCoO_2$ to CoO_2 has been carried out chemically through the exfoliation of an ion-exchangeable layer. The exfoliation of the layered $LiCoO_2$ into CoO_2-nanosheets is done by breaking the weak interaction between the layers by intercalation. The electronic structure of $LiCoO_2$ to CoO_2 has been investigated by X-ray photoelectron spectroscopy (XPS) to aid in the new developments surrounding the exfoliation of CoO_2 nanosheets from bulk $LiCoO_2$. All available core peaks and valence spectra have been analyzed throughout the chemical exfoliation treatment process. It is found that both cobalt and oxygen undergo an oxidation process during the exfoliation of bulk $LiCoO_2$ into CoO2-nanosheets.

Project Mentor: Professor Alp Sehirlioglu, Department of Materials Science and Engineering; Professor Emily Pentzer, Department of Chemistry

◆◆◆

Solution Structure of ESS2P and its Interactions with Heterogeneous Nuclear Ribonucleoprotein (hnRNP) R

NaShea Kendrick, Department of Chemistry; Andrew Sugarman, Department of Chemistry; Liang Yuan Chiu, Department of Chemistry

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a family of RNA binding proteins that are multifunctional. They are the most abundant in eukaryotic nuclei, therefore they are largely responsible for alternative splicing mechanisms in human immunodeficiency virus type 1 (HIV-1). They process heterogeneous RNA's into mature mRNA, and they act as trans-factors in regulating gene expression, making them important factors in mRNA export, translation, and stability. hnRNP R is one of the several multifunctional proteins that are composed of RNA recognition motifs (RRMs) and dictate functionality in RNA processing. According to the literature, hnRNP R has been found to recognize AU rich regions of RNA. The second exonic splicing silencer (ESS2P) shows an AU-rich looped region on its secondary structure, therefore we hypothesize that hnRNP R will bind with ESS2P to repress splicesite A3 of HIV-1, which is required for Tat mRNA production. However, little is known about the structure that allows for these mechanisms to take place. In this project, we aim to characterize the structure of hnRNP R as it interacts with the ESS2P through the study of RNA recognition motifs such as RRM1, RRM2, and RRM12. The proteins cultured within BL21 E. coli cells were overexpressed, purified by nickel affinity chromatography, and characterized by NMR spectroscopy. ESS2P was characterized using various a combination of XPLOR-NIH and AMBER software to produce a structure.
Project Mentors: Liang Yuan Chiu and Blanton S. Tolbert, Department of Chemistry

Characterizing the novel interaction of S-nitrosothiols with co-chaperones CHIP and Aha1 in stabilizing cell surface F508del CFTR

Julia Knight, Departments of Biochemistry and English; Dr. Khalequz Zaman, Departments of Biochemistry and Pediatrics; Dr. Stephen Lewis, Departments of Pharmacology and Pediatrics, and Dr. Benjamin Gaston, Departments of Physiology and Pediatrics, Case Western Reserve University School of Medicine.

Cystic fibrosis (CF) is a heritable disorder caused by mutations in the gene which encodes the Cystic Fibrosis Transmembrane Regulator (CFTR) protein, an epithelial chloride channel. CF causes progressive multi-organ damage due to the accumulation of thick mucus in the lungs and digestive tract. S-Nitrosothiols (SNOs) are small, native, endogenously-produced cell signaling compounds which are S-nitrosylating agents; levels of SNOs are lower in CF airways than in healthy airways. Here, we are studying the interaction of SNOs with two similar proteins, co-chaperone C-terminus Hsc70 interacting protein (CHIP) and Hsp90/Hsp70 co-chaperone (Aha1), which are known to inhibit CFTR and target it for degradation. First, we showed that both CHIP and Aha1 are expressed in primary human bronchial epithelial and CFBE41o$^-$ cells, expressing either wild type or F508del CFTR. We then demonstrated that SNOs significantly reduce the expression of both CHIP and Aha1 and subsequently increase F508del CFTR cell surface stabilization and maturation. In addition, we demonstrated that GSNO, a particular SNO compound, S-nitrosylated CFTR-associated CHIP and decreased levels of CFTR ubiquitination. These data suggest that S-nitrosylation of CHIP by GSNO inhibits CHIP's E3 ubiquitin ligase function, preventing F508del CFTR degradation. We also demonstrated cellular co-localization of CFTR and CHIP, as well as CFTR and Aha1, in CFBE41o$^-$ cells using confocal microscopy and immunoprecipitation assay, showing once again that GSNO reduced the levels of CHIP and Aha1. We conclude that SNOs significantly reduce CFTR degradation by CHIP and Aha1, resulting in functional, mature CFTR at the surface of bronchial epithelial cells.

Project Mentor: Dr. Khalequz Zaman, Departments of Biochemistry and Pediatrics

◆◆◆

Micro Contact Printing of Reduced Graphene Oxide on Microelectrodes

Hayden Koerwer, Department of Biomedical Engineering; Allison Hess-Dunning, APT Center, Louis Stokes Cleveland Department of Veterans Affairs Medical Center

Neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease are strongly tied to neurochemical dysregulation. Measuring spatiotemporal fluctuations in neurochemical concentrations can give insight into the functions of the brain and the upstream causes of these debilitating disorders. Current industry standard electrodes lack long term efficacy (> 20 years) which is crucial to these studies. They become dysfunctional after a few months due to glial scarring. Our solution to this is to use a nanocomposite, inspired by a sea cucumber dermis, consisting of a cellulose nanofiber network embedded in a poly(vinyl acetate) matrix. This composite has a high stiffness when dry and a lower stiffness when wet. This allows for an effective dry insertion and improved mechanical matching in the brain due to the relaxed stiffness. Our goal is to use reduced graphene oxide to coat the electrode surface in order

to increase sensitivity to byproducts of brain activity. High sensitivity is desired since it allows for smaller, less invasive electrodes to be used. Applying the graphene oxide is a challenge due to the micro--scale size sites that need to be coated without overlap. To do so, we used micro-contact printing with a PDMS stamp in order to accurately stamp the electrode sites. The stamp is manufactured using a laser-micro machined acrylic mold. After stamping, the graphene oxide is reduced using electrochemical reduction and we found that doing so greatly increased the sensitivity. The sensitivity is measured running varied concentration of a hydrogen peroxide-PBS solution over the electrode setup (which consists of a Ag-Cl reference electrode, the electrode being tested & a Pt counter electrode). A steady voltage is run through the system and the resulting current is measured. The sensitivity is then determined by the current divided by hydrogen peroxide concentration.

Project Mentor: Dr. Allison Hess-Dunning, APT Center, Louis Stokes Cleveland Department of Veterans Affairs Medical Center

◆◆◆

The effect of sentence-level prosody on speech-in-speech recognition for bilinguals and monolinguals

Jessica Kong, Department of Psychological Sciences

The focus of our lab is to better understand factors that impact speech recognition in noisy environments. We assess both intrinsic and extrinsic factors, or factors related to the listener and the auditory environment, respectively. Specifically, this study examines how prosodic cues within a speech signal facilitate speech recognition in competing background noise. Sentence recognition was assessed for three groups of listeners: sequential Mandarin-English bilinguals (native Mandarin speakers with English acquired as a second language), simultaneous Mandarin-English bilinguals (Mandarin and English acquired at the same time), and monolingual speakers of English. The stimuli used for this project included a spoken corpus of sentences each recorded in three prosodic speaking styles: normal prosody (conversational speech), flat prosody (monotone speech), and exaggerated prosody (very happy/excited speech). These recordings were produced by three different female talkers. The experimental conditions included target sentences (the speech the listeners were instructed to repeat back) spoken using the three prosodic speaking styles. Background speech, also spoken in all three speaking styles, was presented on every trial. Data are presented for 20 sequential bilinguals, 19 simultaneous bilinguals, and 8 monolinguals. Sequential bilinguals had the poorest performance. No difference was observed between the simultaneous bilinguals and the monolinguals. Performance was worse for all three groups when the target and masker speech were matched in the exaggerated or flat speaking style. Overall, performance was best for the flat target condition, which does not support our original hypothesis. We predicted that the exaggerated target would be the easiest condition (as it would help focus the listener's attention) and the flat target would be the most difficult (as it would be more difficult to segregate from the competing speech signals).

Project Mentor: Dr. Lauren Calandruccio, Department of Psychological Sciences

A role for Sirt 1 and Sirt 2 in modulating the intestinal epithelial barrier

Sofia Martir Gonzalez, BS, San Juan Bautista School of Medicine; Nga Le, Department of Molecular Biology and Microbiology; Dr. Alan D. Levine, Departments of Molecular Biology and Microbiology

The intestinal epithelium creates a barrier between the external environment and the body, providing the first line of defense preventing the entry of pathogens to the tissues. It is formed by a monolayer of cells sealed by intercellular tight junctions that regulate selective paracellular permeability. Disruption of these tight junctions increases permeability, which is related to diseases such as inflammatory bowel disease, diabetes, and obesity. The goal of this project is to identify molecules or pathways that improve barrier integrity. Preliminary data from our laboratory has indicated that inhibiting tubulin polymerization increases barrier integrity, possibly indicating a role for protein acetylases and deacetylases in tight junction assembly or function. We chose to target the deacetylases Sirt 1 and Sirt 2 using the inhibitor cambinol. The human intestinal epithelial cell line Caco-2 BBe, grown on transwell permeable supports, was used as an *in vitro* model of the barrier monolayer. When cells were treated with 50 uM and 100 uM of cambinol, a known Sirt 1 and Sirt 2 inhibitor, a time-dependent increase in transepithelial electrical resistance (TER) was observed after 4 hours, reflecting a strengthening of the epithelial barrier. Understanding the mechanism by which cambinol affects the epithelium may lead to the identification of key compounds or pathways that play a therapeutic role in protecting or repairing the intestinal barrier.

Project mentor: Dr. Alan D. Levine, Departments of Molecular Biology and Microbiology, Pathology, Pharmacology, and Medicine

◆◆◆

Distinct Properties of Surface Modified Graphene Oxide Nanosheets via ATRP

Madelyn McMillen, Department of Chemistry; Peiran Wei, Department of Chemistry

Graphene oxide (GO) has received widespread attention due to its distinct properties and applications including its controllable aspect ratio, antimicrobial properties, and its function as a precursor to electrically and thermally conductive materials. In recent years, GO has been modified to have distinct properties compared to unmodified GO and therefore garner interest in applications as diverse as drug delivery, sensing, optics, and interfacial modification. However, the structure-property relationship isn't fully understood due to the inability to control chain length. Herein, we used atom-transfer radical polymerization (ATRP) to modify the surface of GO by first functionalizing GO with an initiator and then grafting polymer from the surface using methyl methacrylate as the monomer. A sacrificial initiator, ethyl α-bromoisobutyrate (EBIB), was used in the reaction in order to measure the polymer brush length from floating polymer in the solution. Different chain lengths were then polymerized by changing the monomer to initiator ratio to 10:1, 100:1, and 500:1. It was found that larger monomer to initiator ratios produced longer polymer

chains, which were measured by gel permeation chromatography (GPC). Then, the properties of surface modified GO were characterized by X-ray Photoelectron Spectroscopy (XPS), Fourier Transfer Infrared Spectroscopy (FTIR), Atomic Force Microscopy (AFM), and Contact Angle. We found that the polymers had significant differences in their properties including hydrophobicity, solubility and more. These results allow us to better understand the structure-property relationship of GO. Future work should focus on synthesizing Janus nanosheets and exploring how different polymers impact its properties.

Project Mentor: Peiran Wei, Department of Chemistry
Principal Investigator: Dr. Emily Pentzer, Department of Chemistry

◆◆◆

Understanding the Effects of Blast Traumatic Brain Injury on Mitochondrial Dynamics and Activity

Emiko Miller, Biomedical Sciences and Neuroscience; Preethy Sridharan, BS, Department of Psychiatry; Andrew Pieper, MD, PHD, Department of Psychiatry

Models of Traumatic Brain Injury (TBI) have shown that mitochondria play a prominent role in TBI pathophysiology because of the reduced cellular respiration, increased ROS, and apoptotic cell death following injury. In addition, there is some evidence that mitochondrial fission and fusion dynamics may be disrupted after concussive TBI. Our objective was to characterize these fission and fusion dynamics in a blast mediated model of TBI. In this study, blast TBI was induced by placing a mouse in an enclosed blast chamber that generated a blast wave that traveled through the mouse's head. Cortical tissue was collected 24 hours and 2 weeks after injury. Western blots were done to probe for the proteins that regulate mitochondrial fission and fusion. Our results suggest that although protein expression of fission protein is unaltered, expression of fusion proteins is reduced in the cortex after blast TBI. Further experimentation needs to be done in order to further understand how these changes affect neuronal survival, how these results may vary in other regions of the brain, how these changes may be manipulated to uncover potential therapeutic targets.

Project Mentor: Andrew Pieper, MD, PHD, Department of Psychiatry

◆◆◆

HIF1α-2α cerebral knockout effects on cognitive function following environmental enrichment in mice

Viral Mistry, Department of Physiology and Biophysics; Dr. Kui Xu, Department of Physiology and Biophysics; Alireza Abdollahifar, Department of Physiology and Biophysics; Sahej Bindra, Department of Physiology and Biophysics

Summary: Hypoxia-inducible factor-1α & 2α (HIF1α-2α) are transcription factors critical to the neurobiological response to hypoxia, a condition when the body is low in oxygen, by inducing short-term vasodilation and long-term angiogenesis. Angiogenesis is also strongly related to cognitive improvements made through environmental enrichment in mice. Since HIF1α-2α are crucial to angiogenesis, it is of interest if cerebral knockouts for the HIF1α-2α transcription factors would prevent gains in cognitive function from environmental enrichment. Experiments are currently underway to see how the HIF1α-2α knockout mice, which have been bred in-house, perform on a Y-maze working memory test compared to control HIF1α-2α floxed mice prior to and after one week of environmental enrichment. After the behavioral experiments are complete, relevant regions of the brain will be compared for differences in capillary density, to establish quantitative differences in capillary density as it relates to rodent activity and cognitive performance.

Project Mentor: *Joseph LaManna, Ph.D, Department of Physiology and Biophysics*

◆◆◆

IntronicDB: A Search Engine for Intron Annotation and Orthology Information

Devlin Moyer[1, 2], Graham Larue[3], Courtney Hershberger[2], Scott Roy[3], Richard Padgett[2,4]
[1] Department of Biochemistry, School of Medicine, Case Western Reserve University
[2] Department of Cellular and Molecular Medicine, Lerner Research Institute, Cleveland Clinic
[3] Department of Biology, San Francisco State University
[4] Center for RNA Science and Therapeutics, School of Medicine, Case Western Reserve University

In most protein-coding eukaryotic genes, the protein-coding sequences within the genes are interrupted by a series of non-coding sequences, diving genes into exonic, i.e. protein-coding, and intronic, i.e. non-coding, regions. After genes are transcribed into pre-messenger RNA, introns must be spliced out in order to generate mature messenger RNA transcripts that encode functional proteins. A small subset of introns, known as the minor-class introns, present in most eukaryotic genomes are spliced by a distinct splicing apparatus. Many questions remain about the evolutionary history of these two distinct mechanisms of intron splicing. Mutations linked to errors in intron splicing have been associated with hundreds of human diseases, and much remains unknown about regulation of splicing.

Many databases cataloguing intron annotation data have been created in the past 20 years, but very few of these databases have been maintained, and most are no longer accessible. To remedy this situation, a website was

implemented that provides a robust and user-friendly search engine of intron annotation data in a wide variety of genomes. Users can search for introns using Ensembl accession numbers, gene names, or genomic coordinates. Users can also simply acquire lists of all introns meeting specific criteria, including intron class, which is neglected in many genomic databases. The website uniquely provides an intron ortholog search engine, allowing users to find orthologous introns in the genomes of many model organisms. The website will be useful for a variety of investigations into the evolutionary history of introns and the pathologies of splicing-associated diseases.

Project Mentor: Richard A. Padgett, Center for RNA Science and Therapeutics

◆◆◆

Itaconate as a New Target for Atherosclerosis Regression

Melanie Nelson, Departments of Neuroscience and Behavior and Psychology (Wesleyan University, Middletown, CT); Matt Mignery, Galina Pylypiv, Alice Chaplin, Andrei Maiseyeu (Case Western Reserve University School of Medicine, Cleveland, OH)

Background:
Atherosclerosis is a cardiovascular disease that occurs when plaque narrows in arteries, which can lead to coronary heart disease and death due to heart attack or stroke. Our lab found that the metabolite itaconate (ITA) is expressed in regressing atherosclerosis and was also shown in literature to have an "off-switch" for inflammation in macrophages. Because macrophages play an important role in atherosclerosis inflammation, we hypothesized that ITA could be a new molecule for targeted treatment of cardiovascular diseases.

Methods:
In this study, a drug delivery system was developed to either embed ITA into the membrane of the liposomes (OI-Lip) or encapsulate ITA into the liposome interior (ITA-Lip). Liposomes are microscopic vesicles frequently used for drug delivery. The liposomes were tested *in vitro* in six groups of Bone Marrow Derived Macrophages (BMDM) using lipopolysaccharide (LPS) as a model of inflammation, using OI concentrations of 5 μM, 25 μM, and 50 μM and an LPS concentration of 100 ng/mL. qPCR was completed to look at expression of Interleukin 1 beta (IL1β), a marker of inflammation, and Immune-Responsive gene 1 (IRG1), an enzyme that produces ITA inside of the cell.

Results:
When examining the liposomes, the concentration of ITA in the liposomes was 0.315-0.423 mM, and the size of the liposomes was 98.8-105.8 nm. qPCR results showed a statistically significant difference in IRG1 expression between the 6.2 μM OI-Lip group and the +LPS control group ($p=0.017$). A difference was also seen in IRG1 expression between the two groups at 3.7 μM ($p=0.004$). A statistically significant difference was also found in IL1β and IRG1 expression when comparing 50 μM OI-Lip to the +LPS control group (IL1β: $p=0.016$; IRG1: $p=0.003$). A difference was also seen at 25 μM

(IL1β: p=0.019; IRG1: p=0.006). Using metabolomics analysis to determine other metabolites that may be involved in atherosclerosis regression, metabolites including glucose, lactate, and pyruvate were seen in the regression group.

Conclusions:
It can be concluded that the ITA-embedded liposomes dose-dependently reduce inflammation. The downregulation of IL1β and IRG1 may indicate that the delivery of ITA is more efficient with liposomes. The high expression of lactate in atherosclerosis progression could be the result of a higher level of glycolysis in progression than in control or regression.

Project Mentor: Andrei Maiseyeu, Cardiovascular Research Institute

◆◆◆

Cholesterol transporter ABCA1 levels are regulated by microRNA 223

Christopher Nmai, History and Science: Medicine and Society; Jeffrey A. Deiuliis, Case Cardiovascular Research Institute

The mechanisms that regulate uptake and efflux of sterols by macrophages are not fully understood and affect the development and progression of atherosclerosis in humans and animals. Using primary mouse macrophages, we show that microRNA 223 (miR-223) regulates cholesterol metabolism and efflux, with multiple abnormalities in cholesterol metabolic pathways, compared to wild type. miR-223$^{-/-}$foam cells have impaired efflux to both apoA1 and HDL. Our findings demonstrate that ABCA1 is largely degraded by lysosomal and proteasomal processes in wild type cells and that the loss of miR-223 accelerates ABCA1 degradation rates via these processes. Further, our data suggests an important role for miR-223 in macrophage cholesterol metabolism; therapeutics that increase miR-223 expression will potentiate cholesterol efflux and reverse cholesterol transport *in vivo*, with implications for decreasing atherosclerotic disease.

Project Mentor: Jeffrey A. Deiuliis, Ph.D., Case Cardiovascular Research Institute

◆◆◆

Degradation of S-Nitrosothiols by Superoxide Dismutase and Exploration of Novel Cofactors in the Reaction

Santiago Noriega, Cognitive and Brain Sciences, Tufts University; James Seckler, Department of Pediatrics, Case Western Reserve University School of Medicine; Stephen Lewis, Department of Pediatrics, Case Western Reserve University School of Medicine

Superoxide dismutases (SODs) are enzymes that convert superoxide radicals (O_2-) into molecular oxygen and hydrogen peroxide to minimize the toxic effects of high concentrations of the radical. SOD1 is a copper and zinc binding protein found in the cytoplasm which also has been shown to reduce the S-nitrosothiols (SNOs) S-nitrosocysteine (SNOC) and S-nitrosoglutathione (GSNO). SOD1 has also been shown to oxidize nitrite (NO_2) to nitrate (NO_3). We hypothesized that the combination of SNO reduction by SOD1 with nitrite oxidation will stimulate both reactions, increasing their Km and Vmax. To measure this, absorption spectroscopy was used to measure the degradation of various SNOs by SOD1 in the presence of varying levels of nitrite. Without nitrite, SOD1 reduced L-SNOC, but not D-SNOC, GSNO, S-nitrosocysteamine (ScysA) or S-nitrosocysteinylgycine (SGC). SCysA and SGC had significantly higher Vmax and lower Km values when reacting with SOD1, showing that it has a much higher affinity for these compounds than for L-SNOC and GSNO. Upon the addition of nitrite, the Vmax and Km of most tested compounds improved with the most dramatic result being that SOD1 gained the ability to reduce D-SNOC. This suggests that nitrite oxidation stimulates the degradation of certain SNOs into nitric oxide and that endogenous nitrite levels within the cytosol affect the production of nitric oxide from intracellular SNOs.

Project Mentor: Dr. Stephen Lewis, Department of Pediatrics, Case Western Reserve University School of Medicine

◆◆◆

Characterize Cells Showing Conditioning Lesion Effect in Axon Regeneration Using TrkA, TrkC, TH, and Somatostatin Markers

Sanika Paranjape, Department of Biology and Department of Cognitive Science; Jeong Seo, Department of Neurosciences; Richard Zigmond, Department of Neurosciences

The central nervous system (CNS) exhibits minimal axonal regeneration because mature adult CNS neurons have a very low growth capacity. However, in the peripheral nervous system, axons have the ability to regenerate after damage more easily. If a conditioning lesion is performed before an injury in the peripheral axons of dorsal root ganglion (DRG), that regeneration ability is improved and there is a greater growth capacity. The conditioning lesion effect is seen when previously injured neurons have an increased ability for new axonal growth compared to neurons that have not been injured before. Our study aims to differentiate between and characterize the cells that display this conditioning lesion effect versus those that do not. To do so, we tested markers such as tropomyosin-related kinase A (TrkA), tropomyosin-related kinase C (TrkC), tyrosine hydroxylase (TH), and somatostatin, which are each known to characterize subpopulations of sensory DRG neurons. Through immunocytochemical staining and imaging, we analyzed the data by observing cell staining and measuring neurite growth. In the future, this research can hopefully be used to increase axonal regeneration in the peripheral nervous system, and ultimately in the central nervous system as well.

Project Mentor: Richard Zigmond PhD, Department of Neurosciences

Characterizing Vanishing White Matter Disease Astrocytes

Samuel Qian, Department of Cognitive Science

Leukodystrophies are a category of rare, genetic diseases that affect the white matter of the brain. Specifically, Vanishing White Matter Disease (VWMD) is a type of fatal leukodystrophy that has been linked to mutations in the genes which encode for any of the five eukaryotic translation initiation factor 2B (eIF2b) subunits. These mutations have been associated with oligodendrocyte and astrocyte dysfunction. However, the mechanisms behind these dysfunctions which cause myelin loss are unclear. In order to uncover the mechanisms and phenotypes characteristic of VWMD pathology in astrocytes, I used induced-pluripotent stem cell (iPSC)-derived oligodendrocyte progenitor cells (OPCs) from a VWMD mouse model. OPCs are stem cells capable of differentiating into oligodendrocytes or astrocytes. Specifically, I tested for the *in vitro* genetic and functional phenotypes which have been associated with the "A1" astrocyte subtype, a recently described subtype that is toxic to mature oligodendrocytes. To test whether VWMD astrocytes secret cytokines harmful to oligodendrocytes, I tested the ability of OPCs to differentiate into oligodendrocytes when co-cultured with VWMD astrocytes. I also tested for oligodendrocyte differentiation in OPCs cultured in VWMD astrocyte-conditioned media. Preliminary data suggests that neither of these two conditions has an effect on the ability of OPCs to differentiate into oligodendrocytes. I have also tested for the phagocytic ability of VWMD astrocyte cultures. This assay suggests that VWMD astrocytes have decreased ability to uptake fluorescently-tagged bioparticles when compared to wild-type astrocytes. Finally, I used real-time quantitative Polymerase Chain Reaction (rt qPCR) analysis to look for parallels between the sets of transcripts regulated by VWMD and A1 astrocytes. Our data suggests that SERPING1 (a target gene identified for the A1 subtype) is upregulated about two-fold in VWMD astrocytes when compared to wild-type astrocytes. However, assays on other A1 target genes do not indicate a discernible difference in regulation between VWMD and wild-type lines. These findings show that VWMD astrocytes may not be fully polarized to the A1 subtype. Future tests will be to see if the addition of an ER stressor has an effect on the above assays, as previous literature has shown that the symptoms of VWMD onset following acute trauma.

Project Mentor: Dr. Paul Tesar, Genetics and Genome Sciences, Dr. Ben Clayton, Genetics and Genome Sciences

◆◆◆

Impact of β-Alanine Gavage on Carnosine and Anserine Concentrations in Rat Muscle

Srija Reddy, Department of Nutrition; Maioqi Zhang, Department of Nutrition; Kirkland A. Wilson, Department of Nutrition; Henri Brunengraber, Department of Nutrition; Charandeep Singh, Cleveland Clinic

β-Alanine is marketed to athletes as a nutritional supplement. Muscle tissue converts β-alanine to carnosine and anserine, both of which are β-alanyl-histidine dipeptides. Carnosine and anserine act as strong pH buffering agents and have suspected roles as antioxidants. Some athletes ingest β-alanine in hopes of increasing the pH buffering capacity of their muscles, thus preventing fatigue and increasing performance and endurance However, a major side effect of β-

alanine ingestion is intense paresthesia in some subjects. The mechanism of paresthesia is not known.
We investigated the impact of gavaging rats with β–alanine on the concentrations of carnosine and anserine in muscle. Rats were sacrificed at different times after gavage with unlabeled or β-[^{15}N, $^{13}C_3$]alanine. Muscles were quick-frozen before analysis by liquid chromatography-mass spectrometry. Carnosine and anserine were assayed by isotope dilution with an internal standard of [$^{15}N_2$, $^{13}C_6$]lysine. This protocol allowed calculating (i) the concentrations of carnosine and anserine, and (ii) their labeling from β-[^{15}N, $^{13}C_3$]alanine. This project will shed light on the effects of β-alanine on the buffering capacity of carnosine and anserine in muscle. We hope that metabolomic analyses of rat organs after β–alanine gavage will detect alterations in neurotransmitters possibly related to the triggering of paresthesia. This study was partly supported by a Wharton Fellowship awarded to Srija Reddy, and by a grant from the National Cancer Institute.

Project Mentor: Dr. Henri Brunengraber, Department of Nutrition

◆◆◆

A Fast Linear Approximation Model to Predict Action Potentials in Sensory Axons

Josh Rosenberg, Department of Biomedical Engineering; Dr. Matthew Schiefer, Louis Stokes VA Medical Center APT Center

Electrical stimulation is a valuable tool for rehabilitation in patients who have experienced strokes, spinal cord injuries, and other neurological injuries. By applying an electric field to nerve fibers, it is possible to induce action potentials. These electrically-induced action potentials can restore movement, relieve pain, or restore sensation. As with drugs, the optimal therapeutic effects are achieved on a patient-by-patient basis. Designing an optimal stimulus is a time consuming, but can be achieved through computer simulations. The double-cable axon model serves as a gold-standard in computer nerve models. However, its use of nonlinear methods and small solver step sizes means that single-fiber simulations can take seconds to complete. This is compounded in simulations accounting for thousands of fibers within a nerve in which several stimulus parameters may need to be tested. As the number of independent parameters increases, the stimulus search space quickly and simulation time quickly becomes intractable. To reduce simulation time, we are building a linear approximation model that will predict the axon's response to stimulation without the necessity of solving nonlinear differential equations at every time step. The linear approximation model will account for the voltage (potential) along the axon and the duration of stimulation as well as eliminate the integration of nonlinear channel dynamics. Output from the model will be binary, suggesting that either an action potential did or did not propagate. The new approximation method will significantly reduce run time and computational complexity, and as a consequence, improve the rate of optimization.
Project Mentor: Dr. Matthew Schiefer, Louis VA Medical Center APT Center

◆◆◆

Determining the Dynamics of Ventilatory Pattern Variability (VPV) in Healthy and Septicemic Rats

Raymond Y. Santiago, Department of Neurosciences; Ohio State University
David Nethery*, Shiloh Tackett*, Neha Solanki*, Frank J. Jacono*, and Thomas E. Dick, Division of Pulmonary, Critical Care and Sleep, Department of Medicine; CWRU and *VA

Biologic variability arises from random and deterministic sources. General measures of variability do not distinguish between these types, but magnitude of sources can be estimated by comparing the original data to surrogate data sets that preserve the randomness and destroy time-dependent properties of the original data set. VPV measures the *time-dependent* variability in the length and waveform of breaths in a continuous series of cycles. A healthy breathing pattern is variable. We hypothesize that the nonlinear component of VPV increases in sickness. To test this, adult (bw~300 g), male Sprague rats (n=22) were implanted with either a sterile (sham) or inoculated fibrinogen/thrombin pellet containing *Escherichia coli* (1, 10, 25, 50, or 100 ($x10^6$) cells). Between 18-24 h after the implant, we recorded the ventilatory pattern using whole-body plethysmography. VPV was measured from 'stationary' epochs (n=100-200 breaths) by calculating: 1) mean, standard deviation and coefficient of variation (CV), 2) temporal Poincaré variability (TPV), 3) predictability of the waveform of the breathing pattern. The systemic inflammatory response increased with increasing doses *E. coli*. Specifically, as *E. coli* dosage increased, total variability decreased which was reflected in measures of CV and TPV but TPV also revealed time-dependent variability. Even though variability decreased, the deterministic component of VPV increased. Thus, the waveform became more predictable leading to an increase in the nonlinear component of variability. These studies formed the basis to test how cardio-respiratory interaction is affected by systemic infection and the development of sepsis. In these studies, we implant sterile and pellets with $25x10^6$ *E. coli* cells. In preliminary data, cardioventilatory coupling CVC, defined as a preferential occurrence of the last heart beat in expiration was present at 18h but not at 24h after inoculation. In summary, last summer's experiments supplied the data necessary to justify VPV analysis as an analytical approach to track and to choose an effective dose of *E. coli* with which to infect and track a rat for survival at 24h.

Project Mentor: Thomas E. Dick, Division of Pulmonary, Critical Care and Sleep, Department of Medicine; CWRU

◆◆◆

Influence of CWRU's Combined Dietetic Internship/Master's Degree Program (CDI/MDP) on research involvement among its graduates.

Ellen Sears, Department of Nutrition; Dr. Rosa Hand, Department of Nutrition; and Dr. Stephanie Harris, Department of Nutrition

Various barriers prevent registered dietitian nutritionists (RDNs) from becoming involved in research in their practice settings, including lack of research training and/or skills. Although all dietetic interns are required to meet certain research competencies, there is a lack of more in depth research education among ACEND-accredited dietetic

internships. Case Western Reserve University (CWRU) offers a unique Combined Dietetic Internship/Master's Degree Program (CDI/MDP), which has included an emphasis on research experience since the early 2000s. A survey with 90 total possible questions will be administered to graduates of CWRU's CDI/MDP to determine research involvement, outputs, and self-efficacy levels. The survey includes the following validated tools: the Research Involvement Questionnaire (RIQ), the Clinical Research Appraisal Inventory-19 (CRAI-19), a portion of the Research Capacity and Context tool (RCC), and some questions from the Dietetics Practice Based Research Network (DPBRN) survey. Results from each tool will be compared to published data from previous studies that used the respective tools to measure research involvement, outputs, and self-efficacy among the general RDN population. A separate online search will be conducted to compare research involvement and outputs of CDI/MDP graduates to the data collected in the survey. Graduates will be searched for on LinkedIn and/or Google to determine whether or not they are currently involved in research in the practice setting. Publications from each graduate will be searched for on PubMed and Researchgate as evidence of research outputs. From the data collected in the online search, a descriptive analysis will be done to make generalizations about graduates of CWRU's CDI/MDP. We hypothesize that graduates from CWRU's CDI/MDP will demonstrate greater involvement in research in the practice setting, greater research-self efficacy levels, and a greater number of research outputs compared to the general RDN population. This poster will describe the methods as IRB approval is still pending.

Project Mentors: Rosa Hand and Stephanie Harris, Department of Nutrition

◆◆◆

Mice with a Deletion for the Phosphoenolpyruvate Carboxykinase Gene (Pck1) in Myeloid Cells Protect Mice from Developing Liver Fibrosis

Zach Sedor-Schiffhauer, Department of Nutrition; Daniel Counihan, Department of Nutrition

Obesity has become a major worldwide health problem and has been associated with diseases including insulin resistance, type 2 diabetes (T2D), atherosclerosis, heart disease, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). NAFLD is a term used to label a spectrum of liver diseases ranging from early stage fatty liver (steatosis) to advanced cirrhosis of the liver and hepatocellular carcinoma. This disease is aptly named as it occurs in individuals who consume little to no alcohol and the NAFLD pathology closely resembles that of a diseased liver attributable to alcohol abuse. NAFLD is believed to originate with the accumulation of fatty acids within the liver due to insulin resistance.

We have discovered that phosphoenolpyruvate carboxykinase (Pck1) is expressed in myeloid cells. Pck1 converts oxaloacetate (OAA) to phosphoenolpyruvate (PEP) and is an integral enzyme for gluconeogenesis, glyceroneogenesis, and refilling of the TCA cycle. We have shown that deletion of Pck1 in myeloid cells promotes a more pro-inflammatory (M1) phenotype. Our hypothesis was that the macrophage cells are integral to the development of carbon tetrachloride induced liver fibrosis. We have generated mice with a myeloid-specific deletion of Pck1 ($Pck1^{MC\text{-}KO}$). Using our novel $Pck1^{MC\text{-}KO}$ mice, we investigated if the altered macrophage phenotype modulated liver injury. We treated $Pck1^{MC\text{-}KO}$ mice and control mice with CCl_4 to induce liver fibrosis for 5 weeks. We found that the deletion of Pck1 in the myeloid cells

protected the Pck1$^{MC\text{-}KO}$ mice from developing liver injury.

Project Mentor: Dr. Colleen Croniger, Department of Nutrition

◆◆◆

Confirming Novel Functionalities of Vitamin E *in vitro* and Determining Molecular Mechanisms

Michaela Stamper, Nutritional Biochemistry, Department of Nutrition; Stephen Valentino, Department of Nutrition

Alpha-tocopherol, the active form of the plant-derived lipid Vitamin E, is a well-known for its antioxidant function within the body. Its function in reducing and preventing damage caused by Reactive Oxygen Species (ROS) oxidation in plasma membranes and lipoproteins has been well established. However, it has been recently suggested that Vitamin E has novel functions outside this antioxidant role, particularly in its ability to regulate the expression of the gene encoding the vitamin E transporter Tocopherol Transfer Protein (*TTPA*). My goal is to finalize these observations using a modified analog of vitamin which is defective in its antioxidant function. In addition, I will decipher the molecular mechanisms by which this activity regulates gene expression. The future implications of this research are broad at this point, but beyond shining a light on novel functions of an over 100-year-old vitamin, these findings can be applied to studies examining neurological diseases and various cancer types to be better understand the mechanisms behind both the prevention and promotion of these conditions by vitamin E.

Project Mentor: Danny Manor, Department of Nutrition, Division of General Medical Sciences, Department of Pharmacology

◆◆◆

Utilizing CRISPR/Cas9 to create Uracil DNA N-Glycosylase (UNG) knockout cell lines

Austin Thompson, Biology/ Pre-Med; Yan Yan, Department of Pharmacology

Uracil DNA N-glycosylase (UNG) is a DNA glycosylase that catalyzes the removal of uracil and 5-FU in DNA and initiates the Base Excision Repair (BER) pathway. Previously the lab found depletion of UNG by shRNA sensitizes chemotherapeutic agents pemetrexed or fluxuridine which induce uracil and 5-FU incorporation into DNA in human cancer cell lines. This served as a model to knockdown the gene at the RNA level. We now aim to utilize Crispr-cas9 system to knockout(KO) UNG at the genomic level. Crispr-cas9 is a genome editing system in which we can use to target Exon 2 on UNG to increase the efficiency of mutagenesis which allows knockout of the gene expression. Our objectives were to first construct the px330 vector containing our 5 guiding RNAs. Following this step our second objective was the screen the guiding RNAs efficiency by T7E1 cutting assay. After constructing our vector, we validated by colony

PCR (indicating the insertion of the guide RNA in the vector) as well by DNA sequencing using the U6 promoter primer. To test for guiding RNAs efficiency our 5 constructs were transfected by lipofectamine in 293T cells. Genomic DNA was extracted and target site around 550bp was PCR amplified. The PCR product was then denatured and reannealed. We incubated further with T7E1(which cuts the non-perfectly matched sequence) in order to analyze efficiency. We validated that all guiding RNAs exhibited cutting at expected size with comparable efficiency. Continuation of this research in the future will allow us to transfect our constructs into cancer cell lines to identify positive clones that KO UNG on the gene level.

Project Mentor: Dr. Stanton Gerson

◆◆◆

Promoting Functional and Anatomical Recovery after a Chronic Spinal Cord Injury

Anastacia Vance, Department of Biology

Spinal cord injuries (SCI) can block communication between the brain and the body. The spinal cord responds to an injury by increasing inhibitory molecules in the extracellular space, cause axon regeneration failure. Additionally, the spinal cord undergoes extensive remodeling that can persist up to years following a SCI. In our study, rats received a lateral cervical level four hemitransection (LC4H) injury to impair the left forelimb. The rats received a treatment either 0 days or 12 weeks after the injury. Each rat was randomly injected with either saline or Chondroitinase ABC (ChABC), an enzyme shown to digest the extracellular inhibitory molecules. The rats also received exercise rehabilitation in the form of swimming. To track the recovery of the forelimb use over time, the rats underwent weekly behavioral tests in order to measure their progress empirically. By combining the five-day weekly exercise regimen and enzymatic treatment, we predicted that the rats will improve in forelimb use when treated with ChABC 12 weeks after the LC4H, compared to the saline treated animals, and rats treated with ChABC at the time of the injury.

Project Mentor: Adrianna Milton, Department of Neurosciences, Case Western Reserve University, School of Medicine, Cleveland OH, USA

◆◆◆

Neurogenic Differentiation Factor and Neurogenin Homologs in Schistosomes

Elfreda Vera-cruz, Department of Biology; James Hagerty, Department of Biology; Emmitt Jolly, Department of Biology

Schistosomiasis is a neglected tropical disease affecting over 200 million people worldwide. The causative agents of

human schistosomiasis are parasitic worms of the genus Schistosoma. Schistosomes are obligate parasites and have complex lives fluctuating between an invertebrate and vertebrate host. Short-lived and free-swimming larvae, called cercariae, exit the snail host in fresh water and invade the mammalian host. Cercariae infect a human host by directly penetrating human skin using proteases and entering the bloodstream, where they migrate to the mesentery of the liver or bladder, grow, expand their nervous and muscular systems and develop into adult worms over several weeks. The lab has previously identified a neurogenic differentiation factor(NeuroD)/neurogenin homolog in schistosomes. Neurogenin and NeuroD are basic helix-loop-helix transcription factors that are involved in the development of the nervous system of mammalians, but their functional roles have not been explored in flatworms. E47, an early development protein, forms a heterodimer with NeuroD in order to bind to E-box DNA elements. This study further characterizes the role of neurogenin during early development in larval schistosomes. Characterization of these genes is important as they may be potential targets for disruption of developmental function of the nervous system in schistosomes.

Project Mentor: Dr. Emmitt Jolly, Department of Biology

◆◆◆

Design and development of a Tool to Identify Stem Cell Memory T Cells Through High Dimensional Flow Cytometry

Bradley (Howard) Walker, Department of Chemistry; Filipa Blasco Tavares Pereira Lopes, Laboratory of Immunology,Department of Pathology; Dr. Ashish Arunkumar Sharma, Laboratory of Immunology,Department of Pathology, Dr. Rafick-Pierre Sekaly; Laboratory of Immunology,Department of Pathology.

Stem cells are an emerging choice in cell therapy due to their pluripotency, ability to self-renew and to repair multiple tissues. These characteristics are highly sought-after in treating a broad range of pathologies such as cancer and HIV. Recent data from Sekaly lab has identified a novel stem cell-like subset of T cells that express $CD45RA^{int}CD45RO^{int}$ and are $CD95^{+}CD127^{+}$. Consistent with the stem-like phenotype, transcriptomic profiling revealed that these cells express genes associated with a quiescent metabolic phenotype and Wnt signaling pathway. In addition, this new cell subset is capable of eliciting an enhanced long-lasting response in HIV. Standardized identification and quantification of the $CD45RA^{int}CD45RO^{int}$ subset will not only allow researchers to better develop and optimize a wide variety of cell therapies but physicians will also have a tool to predict the success of engineered cell therapies.

My project focuses on the design, development and optimization of a high dimensional flow cytometry assay (consisting of markers that differentiate T cell subsets and define stemness) to measure the frequency of this population in blood or therapeutic product. The design of the flow cytometry panel was optimized to reduce the spillover of fluorochromes into neighboring channels. Antibody testing and titration was done to decrease non-specific binding. Furthermore, the use of FMOs (fluorescence minus one controls), a thorough optimization of the compensation and extensive testing of cells of different origins (frozen PBMCs, whole blood and purified populations) was done to abolish

the presence of false positive events.

This validated standard operating procedure can be universally used to measure the frequency of $CD45RA^{int}CD45RO^{int}$ in blood under 2 hours, using a 8-color flow cytometer.

Project mentor: Filipa Blasco Tavares Pereira Lopes, Department of Pathology

◆◆◆

Examining the Relationship Between DRAK2 and γδ T cells using γδ Leukemia Cells

Jasmine Walker, Department of Human Health, Emory University; Suzanne Tomchuck, Ph.D., Pathology; Brooke Holland, MS, Pathology; Mari Dallas, MD, Pathology

Death-associated protein-kinase-related 2 (DRAK2) is a kinase expressed in lymphocytes that induces apoptosis, and serves as a negative regulator of T cell activation. There is little information known about the importance of DRAK2 in γδ T cells specifically. The ability of γδ T cells to directly recognize antigens from viruses, microbes and tumors and contribute to their destruction make these T cells ideal candidates for immunotherapy. Therefore, identifying potential negative regulators of γδ T cells may further improve their use in cell-based therapies.

This project focused on discovering the role of DRAK2 in γδ T cell biology. We first examined the expression of the DRAK2 protein in several γδ T cell leukemia cell lines using Western blotting, and found robust expression of DRAK2 in these leukemias. We then treated those cells for 24 to 72 hours with Concanavalin A, a T cell stimulant, and/or the DRAK2 small molecule inhibitor, TRD-0257, to investigate the relationship between DRAK2 and the apoptosis signaling pathways in activated γδ T cells. Analysis using Western blotting showed expressions of several proteins involved in the apoptosis signaling pathway, including caspase 3, caspase 9, and cleaved caspase 9, after treatment. We also used flow cytometry to examine apoptosis markers on the treated cells, and found no significant differences in the proportion of live healthy cells in apoptotic ones between treatment groups in the Jukat and Peer cell lines. However, the Loucy cells showed a strong increase in apoptosis markers, and a decrease in healthy live cells at the 48 and 72 hour time points. Lastly, we used quantitative polymerase chain reactions (qPCR) to look at DRAK2 mRNA transcript expression after treatment, and thus far have found expression of DRAK2 across all treatment groups. The Loucy cells specifically showed a strong decrease in DRAK2 mRNA expression at the 48 and 72 hour time points. Overall, we see a difference in apoptosis signaling pathways in the Loucy cell lines. We will further explore this relationship by treating for different time lengths, and utilizing western blotting and qPCR techniques for analysis.

Further understanding the role of DRAK2 in γδ T cell function and survival could potentially be applied to cancer immunotherapy. More research is needed to further explore the role of DRAK2 in γδ T cell biology.

Project Mentor: Dr. Mari Dallas, MD, Department of Pathology/ Department of Pediatric Hematology and Oncology

◆◆◆

Pickering Emulsions Stabilized by 2D Particles

Louisa Wang, Hathaway Brown; Katelynn Edgehouse, Department of Chemistry; and Emily Pentzer, Department of Chemistry

This research project focuses on the effects of various organic salts and salt amounts on graphene oxide (GO) – cobalt oxide (CO) emulsion stability. Emulsions are mixtures of two or more immiscible liquids in which the continuous phase of one fluid contains dispersed droplets of the other. An interesting class of emulsions are Pickering emulsions, which are stabilized with particles located at the fluid-fluid interface, resulting in a reduction of the interfacial energy. Adding a flocculating agent can further increase the stability of Pickering emulsions. Two-dimensional (2D) particles such as GO nanosheets are one such example and their effects on stability are attributed to oxygen functionalities such as hydroxyl groups, epoxy bridges, and carboxylic acids that give the material amphiphilic properties. These properties allow GO to stabilize the emulsion droplets by acting as a surfactant for the two phases. Pickering emulsions were also studied with CO, another 2D material. Interestingly, emulsions containing both nanosheets are possible, but are less stable than the GO emulsions. The instability may arise because both nanosheets have partial negative charges causing them to repel. However, this repulsion can be mitigated by adding a cation source as a flocculating agent, such as an organic salt.

Project Mentor: Professor Emily Pentzer, Department of Chemistry

◆◆◆

The Quantification of Surface Roughness on Aluminum Sheet Metal

Airreonna White-Smith, Mechanical Engineering, Tuskegee University

The objective of this summer work was to examine the hypothesis that the roughness of Aluminum sheet metal was not independent of the side. For example, aluminum foil used in your home has a shiny side and a dull side. The optical properties of the foil are caused by the interaction of light with the surface roughness. The question was: does thicker sheet show the same dependency of roughness with sides? For this work, a Nanovea Profilometer was used to measure surface roughness using the ISO4287 standard. Surface roughness was measured on both sides of the sheet from line profiles (60mm long). Parameters for roughness amplitude and spacing (frequency) were quantified from the line profiles after removing the low frequency distortions due to sheet warping. This work supports a project exploring the degradation of polymer sheet metal forming tools formed by fused deposition modelling, an additive manufacturing technique.

Project Mentor: Dr. Jennifer Carter, Department of Material Science and Engineering

Small molecules rescue epilepsy-associated gamma-amino butyric type A receptors

Angela Whittsette, Department of Nutritional Biochemistry; Meng Wang, Department of Physiology and Biophysics; Kate Fu, Department of Physiology and Biophysics; Dr. Tingwei Mu, Department of Physiology and Biophysics

Recent advances in genetics identified many mutations in gamma-amino butyric type A (GABAA) receptors that are associated with Idiopathic Epilepsy. Such mutations lead to loss of their function on the plasma membrane and thus disrupt neural circuits in the brain. Last summer, we aimed to elucidate the molecular mechanism of how these mutations lose their function and Identify mutations that lead to their misfolding and excessive protein degradation in cells and thus loss of their protein levels in their functional location, the plasma membrane. This summer we looked at small molecules ability to rescue trafficking efficiency of the mutant receptors. The molecules we observed effects for were compound 147 and compound 263.We used cellular assays that evaluate protein aggregation and total protein levels. In the experiment we cultured human embryonic kidney cells also known as HEK293 cells, Neuro2A cells, and SH-SY5Y Cells, performed transfection, performed SDS-PAGE protein electrophoresis, and concluded with a Western blot analysis. This was done to analyzing the bands produced by the Western blot that were displayed on the films. The amounts of proteins were compared; information was taken from relative protein content in the supernatant of drug treated cells. We demonstrated that drugs 147 and 263 consistently enhanced protein content when treated with increasing concentrations universally on all of the cell cultures. Our work will contribute to drug treatments used as new therapeutic strategies to ameliorate idiopathic epilepsy.

Project Mentor: Dr. Tingwei Mu, Department of Physiology and Biophysics

◆◆◆

Exploring the Use of Graphene Oxide as a Surfactant for Ultrasound Contrast Agent

Jaylen Williams, Department of Chemistry; Agata Exner, Department of Radiology, Houming Leng, Department of Chemistry; Al de Leon, Department of Radiology; Emily Pentzer, Department of Chemistry, Department of Chemistry; and Peiran Wei, Department of Chemistry

Ultrasonography is a frequently used practice in the medical field, which utilizes high-frequency sound waves that echo as they hit dense objects to capture live images. B-Mode imaging, which is widely used across the globe for performing functions such as fetal ultrasonography, uses bright dots to represent the ultrasonic echoes, thus creating an image. Although B-Mode imaging has clearly been proven to be very useful, it has a major flaw that when performing the ultrasonic scan, the image is not clear enough to use as a diagnosis for situations that require the ability to be able to view specific regions, such as internal organs. Contrast-Mode uses a contrast agent (microbubbles), in which a solid surfactant (the graphene oxide) can be used. These contrast agents respond as microbubbles to ultrasound pulses, in which technology allows for contrast mode to only receive responses from the contrast agent. The problem with using current contrasting agents is that when undergoing a lot of stress, the microbubble will lose its shape and burst.

Graphene oxide (GO) is a compound that is derived from graphene, which is useful for contrast mode because of a more stable microbubble. The microbubble is more stable due to structured GO nanosheets that trap the GO in the bubble and line up at the interphace as a result of a Pickering emulsion. Although GO is a very suitable contrast agent, it has to be small enough to be able to remain in the microbubble and different applications must be made to improve the holding capacity of the G) nanosheets. Various experiments are being conducted in which applications such as polymers or lipids and techniques for decreasing GO size have been tested.

Project Mentors: Al de Leon, Department of Radiology; and Peiran Wei, Department of Chemistry

◆◆◆

Mosquito Inspired Insertion Strategy to Improve Microelectrode Implantation

Marina Yu*, Department of Biomedical Engineering; Rachel Welscott*, Department of Biomedical Engineering; Seth Meade, Department of Biomedical Engineering; **Carmen Toth**, Department of Biomedical Engineering

* indicates co-first authors

Mosquitos are very efficient at successfully biting humans and animals. To successfully bite, they use numerous strategies to insert their thin needle-like mouth, called a proboscis, through layers of skin. The mosquito was an inspiration for new insertion techniques for microelectrodes in the brain. Improving insertion techniques can create opportunities for thinner, more flexible microelectrodes. This is important because smaller electrodes could mean less trauma and inflammation in the brain. The mosquito's strategies were used as inspiration because of the mosquito's ability to insert long, flexible needles successfully. Specifically, when the proboscis penetrates the skin, the sheath around it, called the labium, remains as a guide at the surface. The labium, used as a guide, effectively increases the critical buckling load of the proboscis. This allows the proboscis to be successfully inserted without bending or breaking. The Capadona lab had previously developed a mosquito labium-inspired guide as an insertion strategy for microelectrodes that significantly improved insertion success. Currently, the lab is exploring an oscillation inspired strategy. Mosquitos oscillate the proboscis at different frequencies as it penetrates through layers of skin. A piezo motor was created to apply a desired frequency of oscillation to the microelectrode as we insert it. Testing was completed with dummy electrodes and agar gels to mimic *in vivo* use. While the oscillating method used in this study did not produce a significant difference in the amount of successful implantations with the piezo motor versus without the piezo motor, additional studies are ongoing to explore additional variables within the parameter space: frequency, insertion speed, and probe dimensions. As the field is driving toward smaller and smaller electrodes, these strategies could be used to successfully implant smaller, more flexible electrodes.

Project Mentor: Dr. Andrew Shoffstall, Department of Biomedical Engineering
Faculty Sponsor: Dr. Jeffrey Capadona, Department of Biomedical Engineering