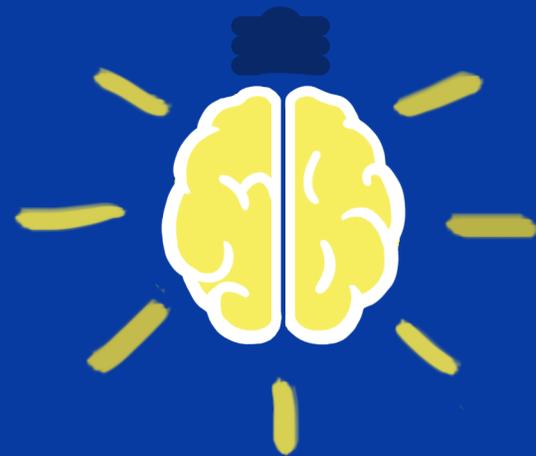




Intersections

Undergraduate Poster Session
Summer 2016

@ BRB Atrium
August 4, 2016



S O U R C E

SUPPORT OF UNDERGRADUATE RESEARCH AND CREATIVE ENDEAVORS



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Simultaneous Reduction and Functionalization of Graphene Oxide via Ritter Reaction

Laura Alonso, Department of Chemistry; **Al De Leon**, Department of Chemistry; **Joey Mangadlao**, Department of Chemistry; and **Emily B. Pentzer**, Department of Chemistry

The Ritter reaction has been reported to produce amides from tertiary alcohols and nitriles under strongly acidic conditions. Several bulky compounds have been successfully functionalized via the Ritter reaction, however no studies have demonstrated that this reaction can be used on the 2D nanosheet material graphene oxide (GO). Moreover, the mechanism for functionalization can also lead to GO being simultaneously reduced and functionalized under the conditions of the Ritter reaction. In this study, GO was reduced and functionalized through the surface of the nanosheets by the Ritter reaction with a nitrile. First, graphite flakes were exfoliated to produce highly oxidized graphene oxide (XOGO), nanosheets of carbon atoms containing epoxide rings, hydroxyl, and carboxyl groups. Then, XOGO was subjected to Ritter reaction conditions: the hydroxyl groups, located on the sheet, were protonated and removed to yield a highly stable carbocation, which could then undergo attack by the nitrogen of a nitrile, such as acrylonitrile to ultimately yield an amide after hydrolysis; alternatively, elimination of another functional group on XOGO would lead to a reduced version of the nanosheet (making it more conductive). Reduction and functionalization of XOGO was confirmed using a variety of functionalization methods, then different variables in the Ritter reaction, such as temperature, different nitriles, and time, were explored. As such, we were able to optimize the other conditions to simultaneously reduce and functionalize XOGO. The functionalization of XOGO by acrylonitrile allows for future studies to be conducted with this nitrile. Future studies include the simultaneous reduction and functionalization of graphene oxide using other reagents. This work lays the foundation of the 2D nanosheets based on XOGO to be used for a number of different applications in medicine, packaging, and electronics.

Project Mentor: Al De Leon, Department of Chemistry

Principal Investigator: Emily B. Pentzer, Department of Chemistry

Synthesis of Zn-doped Iron Oxide Nanocubes for Hyperthermia Studies and the Fabrication of a Magnetic Hydrogel Drug Carrier System

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Magnetic iron oxide nanoparticles have gained special interest as promising materials in the field of biomedical research because of its unique and tunable magnetic properties. In particular, iron oxide nanoparticles are being highly explored for their applications as drug carrier systems. In this study, we synthesized a series of zinc-doped iron oxide nanocubes (Zn-IONC) and optimized their magnetic hyperthermia properties by tuning their size and surface chemistry. Furthermore, we have fabricated a magnetic hydrogel system based on the synthesized zinc-doped nanocubes. The fabricated magnetic hydrogel is thermoresponsive and exhibits a sol-gel transition upon exposure to physiological temperatures. Moreover, the fabricated hydrogel is explored as a platform drug delivery system for anti-bacterial applications.

Mentor: Dr. Anna Samia, Chemistry Department



Preparation and Characterization of an Inverted Salt Bridge Mutant of hnRNP A1

Anise K. Bowman¹, Jeffrey D. Levensgood², Austin Allen³, and Blanton S. Tolbert²

¹Case Western Reserve University Department of Biochemistry, ²Case Western Reserve University Department of Chemistry, and ³Denison University Chemistry Department

The heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) protein is a multifunctional RNA binding protein with implications of alternative splice site selection, the regulation of telomere biogenesis, and mRNA transport/trafficking, and other RNA processing events. Mechanisms and hnRNP A1 interactions have been inferred primarily from the structure of its unwinding protein 1 (UP1) domain. The UP1 domain is composed of tandem RNA recognition motifs (RRMs) and an inter-RRM linker. The RRM s are held rigidly by two Arg-Asp salt bridges (R75:D155 and R88:D157). Though the structure of the UP1 domain has been determined, the essential components of the protein that affect RNA binding have not been fully investigated. In previous work, mutating the R75 and R88 to D75 and D88 affected binding affinity to HIV ESS3 stem loop. Based on this observation, we hypothesized that inverting the salt bridge of the UP1 (D75:R155 and D88:R157) would cause minimal change in binding affinity to ESS3. A gene block was prepared with the designated point mutation inversion of the Arg-Asp salt bridge in UP1 (ISB UP1). The gene block was inserted into a plasmid and the ISB mutant was over expressed in BL21 cells. The protein was isolated and then purified using metal affinity and gel filtration chromatography. An ¹⁵N labeled version of the protein was also prepared and an ¹H-¹⁵N HSQC spectrum was collected. Analysis of the spectrum revealed that the ISB UP1 adopts a similar structure to the WT UP1. However preliminary gel shift assays demonstrate ISB binds with a reduced affinity compared to WT UP1. Therefore, we conclude that inversion of the salt bridge destabilizes the RNA binding pocket. Additional experiments are underway to better understand the binding of ISB UP1 to ESS3.

Project Mentor: Professor Blanton S. Tolbert, Department of Chemistry

Examination of diabetic associated genes in human pancreatic differentiation system

Grace Bugos, Department of Biochemistry; Haiyan Li, Department of Genetics and Genome Science, Zhou Fang, Department of Genetics and Genome Sciences; and Yan Li, Department of Genetics and Genome Science

According to the CDC, approximately 9.3% of the United States population has diabetes. Genome-Wide Association Studies (GWAS) have successfully identified common genetic variants associated with the risk of diabetes, but further studies are required to identify the functional significance of diabetes-associated genetic variants. This project seeks to characterize diabetes associated genetic variants by examining their effect on insulin production in vivo. CRISPR gene editing technology is used to make these characterizations. The CRISPR Cas-9 system is a targeted nuclease that can effectively knockout the function of specific genes. A decrease in insulin production following CRISPR gene knock out indicates that a gene serves a function in insulin production. So far, genome wide CRISPR screening has successfully identified insulin regulating genes in mouse beta cells, now the lab is transitioning its focus to human beta cells. This is an important transition since insulin production and insulin genes are not well conserved between mice and humans. This human study focuses on genes identified in the mouse study that have also been associated with diabetes risk in genome wide association studies. The effect of knocking out genes will be observed in a human pancreatic differentiation system. The successful characterization of insulin regulation genes will contribute to a better understanding of the genetic basis of diabetes.

Project Mentor: Dr. Yan Li, Department of Genetics and Genome Sciences



Macro-nutrient and Micro-nutrient Status in Division III Collegiate Athletes

Madeline Deabler, Nutritional Biochemistry and Metabolism, Nutrition Department; Dr. Lynn Cialdella Kam, Department of Nutrition

The American College of Sports Medicine (ACSM) has established guidelines for athletes outlining the recommendations for pre, during, and post-workout meals. These include specifications for timing, calories, protein, and carbohydrate intake at each stage of exercise. Failing to meet these requirements may lead to what is termed “low energy availability.” Low energy availability is defined as a state in which the body has not been nourished properly, resulting from a calorie deficit or improper distribution among macronutrient groups, and can occur with or without disordered eating. Low energy availability can lead to numerous complications. In females, a primary concern is the Female Athlete Triad, which can lead to both premature bone loss and amenorrhea. Less is known about low energy availability in males specifically. In both populations, it can alter hormone levels, decrease immune function, and negatively impact performance. Thus, it is important to examine athletes, a very active group of individuals, to ascertain whether this large population is meeting the requirements set out by the ACSM in order to ensure that collegiate athletes are optimizing both performance and health. Additionally, it is also important to assess the micronutrient status of these individuals to ensure that there are not significant deficiencies that could affect their overall health and performance.

Project Mentor: Dr. Lynn Kam, Department of Nutrition

Engineering polymer formulations with tunable delivery properties to treat osteoarthritis

Ashley Djuhadi, Department of Biomedical Engineering; Edgardo Rivera-Delgado, Department of Biomedical Engineering; Horst A. von Recum, Department of Biomedical Engineering, Jonathan Kenyon, Department of Biology, Arnold Caplan, Department of Biology

Osteoarthritis is a degenerative joint disease that degrades the articular cartilage between joints, which impedes on daily movement. Current intra-articular drug delivery provides only temporary pain relief because drugs escape rapidly from the knee joint. As new research emerges on injections of Mesenchymal Stem Cells that differentiate into chondrocytes to produce new cartilage or signal for the existing cartilage self repair, there is a need for classical drug delivery vehicles to aid in the survival, differentiation and proper function of these cells. Thus, our objective is to develop an injectable affinity drug vehicle with tunable small molecule release properties that is compatible with these cell-based treatments. Cyclodextrin derivatives (α , β , γ , and dextran) were crosslinked with a 1,6-hexamethylene diisocyanate or ethylene glycol diglycidyl ether to formulate viscous solutions and gel disks. Then, these geometries were loaded with anti-inflammatory corticosteroids (dexamethasone, hydrocortisone, and triamcinolone). After drug loading, drug release profiles were determined in aqueous solution for the viscous polymers and disks. The release solutions were analyzed by UV spectroscopy. Specifically with the viscous solutions, it was determined that the higher the concentration of affinity polymer, the slower the drug release profile. Human Mesenchymal Stem Cell aggregate assay incubated with 1mg/ml of polymer in the culture media resulted in no significant decrease in the viability of the aggregates.

Project Mentor: Dr. Horst von Recum, Department of Biomedical Engineering



PAR 3 is a Signal Regulator for PAR 1 and PAR 4

Valerie Flores Robles, Department of Biology

Protease-activated receptors (PARs) are a family of G protein coupled receptors (GPCRs) that are activated by cleavage of the N-terminal extracellular domain by serine proteases such as thrombin and trypsin. There are four PARs (PAR1-4), which are expressed in platelets, endothelial cells, myocytes, podocytes, and neurons. PAR3 appears to have no direct signaling role, therefore has not been well studied. However, recently it has been identified that PAR3 increases the sensitivity and fine tunes the signaling of other PARs. The goals of my project were to create a stable cell line expressing PAR3 and purify the extracellular domain of PAR3 for generating monoclonal antibodies. These tools will facilitate further studies that examine how PAR3 influences thrombin signaling from PAR1 and PAR4. To generate PAR3 expression cells the PAR3-pcDNA was generated by PCR and cloned into pcDNA5. Direct sequence analysis verified that there were no mutations in the PAR3-pcDNA. The PAR3-pcDNA5 vector was transfected into 293 FLIPin cells and hygromycin resistant clones were selected. PAR3 expression for 20 clones was determined by Western blot. Fourteen clones were positive for PAR3 expression. To purify the extracellular domain of PAR3, the pcDNA encoding for amino acids 25-100 were cloned into the vector pMAL. Direct sequence analysis verified the proper reading from and ensured that there were no mutations. The PAR3-maltose binding protein (PAR3-MBP) expressed in BL21. PAR3-MBP was purified with affinity chromatography using an amylose column. The next step of these projects are to induce and adaptive immune response in mice that would lead to the creation of a specific antibody, and study the interaction of PAR3 with other PARs.

Project Mentor: Dr. Marvin Nieman, Department of Pharmacology

Defining the Role of Hypoxia Inducible Factor (HIF) in Non-pulmonary Vascular Smooth Muscle Cells

Bradley Frate, MSII Medical Student at the Jacobs School of Medicine at the University at Buffalo; Anna Henry, Cardiovascular Research Institute/ Department of Pathology

Peripheral vascular disease (PVD) affects tens of millions of people in the United States, and is a significant cause of morbidity. PVD is a pathological condition that is caused by insufficient tissue perfusion, which could lead to the loss of a limb or even death. Insufficient tissue perfusion leads to ischemia, which is characterized by low oxygen tension (hypoxia) and reduced nutrients. Hypoxia leads to the activation of Hypoxia Inducible Factor (HIF), which is a transcription factor regulating the expression of angiogenic genes. Vascular smooth muscle cells (VSMC), present in the tunica media layer of vessels, modulate their phenotype in response to physiological and pathological cues. We hypothesize that HIF-1 is an essential regulator of smooth muscle cell activation and phenotype in the peripheral vasculature required for effective angiogenic and arteriogenic responses to ischemia. Our research is concerned with testing the requirement for HIF in non-pulmonary VSMC responses to stresses including hypoxia and starvation by examining the expression of contractile genes in peripheral VSMC and subsequent phenotypic changes. Additionally, we utilized an in vivo carotid artery ligation model to examine the vascular remodeling in VSMC-HIF deficient mice. Our preliminary data depicts trends of differing levels of Alpha-actin-2 and Calponin-1 gene expression between starved and unstarved conditions, in arterial and venous VSMCs. However, we observed no difference in the remodeling of ligated carotids between the control and VSMC-HIF knocked out mice. These preliminary results will help further our understanding of the role of HIF in regulating peripheral VSMC phenotype.

Project Mentor: Dr. Diana L. Ramirez-Bergeron, Cardiovascular Research Institute/ Department of Medicine



Determination of surface markers of acutely activated CD8+ T cells using the Activation Induced Marker technique

Gianina C. Hernandez Marquez, Department of Biology; David H. Canaday, Division of Infectious Diseases; Htin Aung, Division of Infectious Disease; Kenny Golovan, Division of Infectious Disease; Ismail Sayin, Division of Infectious Disease

In the human immune system cells such as CD4+ T cells and CD8+ T cells play a crucial part in fighting infections. CD4+ T cells perform an important part in the adaptive immune system; these types of cells aid the antigen presenting cells that present the foreign peptides on MHC class II by creating cell-to-cell interactions and cytokine production. Whereas CD8+ T cells, also known as cytotoxic cells, take part in killing infected or damaged cells. Moreover, cytotoxic cells recognize the antigens presented on the surface by the MHC class I and bind to them in order to destroy an infected cell. As part of our project we have been evaluating the expression of candidate markers of acutely activated cells with the following surface markers: OX40, CD25, CD69, and PD-L1, in CD4+ T cells versus CD8+ T cells. As stated in a recently published paper focused only on CD4+ T cells, such markers were up regulated in what they call the Activation Induced Marker technique (AIM). The purpose of our project is to understand the use of this method for CD8+ T cells. We have been focused in comparing the efficiency of the following methods, the Intracellular Staining technique (ICS) and the Activation Induced Marker technique and acquiring the data on a BD Fortessa and analyzing it using Flowjo Software. Being able to efficiently identify vaccine specific CD8+ T cells and CD4+ T cells with surface markers will be of great benefit for those who are trying to improve the outcomes of different types of vaccines in adults.

Project Mentor: Dr. David H. Canaday, Division of Infectious Disease

Mitochondrial Dynamics in Tachypacing Induced Heart Failure

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Heart failure is the leading cause of death in the United States which has an increased associated risk of developing atrial fibrillation. Past studies have investigated mitochondrial structure and function in the left ventricle (LV) but not much is known of the mitochondrial changes that occur in the left atria (LA) in a heart failure model. We measured mitochondrial respiration and markers of mitochondrial dynamics in the LA utilizing a canine ventricular tachypacing (VTP) induced heart failure model. Mitochondrial respiration was assessed in the LA and LV of control (CON), 24-hr VTP, and 2-wk VTP. We found that mitochondrial respiration was decreased in the LA at 24-hr VTP whereas these changes occurred in the LV at 2-wk VTP. We then assessed mitochondrial dynamics (content, size and distribution) in electron microscopic images from the LA of these animals. The total mitochondrial area fraction was increased in response to VTP, largely due to increased abundance of interfibrillar mitochondria (IFM) (p=0.021). In addition, we found that the mean LA cross-sectional cell area was increased in 2-wk VTP. These data suggest that changes in mitochondrial dynamics underlie changes in respiration in the LA, and that significant mitochondrial remodeling occurs in VTP induced heart failure.

Project Mentor: Dr. Julie Rennison, Department of Molecular Cardiology

Project Faculty: Dr. David van Wagoner, Department of Molecular Cardiology



Cyclodextrin Polymer Cytotoxicity during In Situ Loading for Treating Osteoarthritis

Dominic Kizek, Edgardo Rivera-Delgado, Horst A. von Recum
Department of Biomedical Engineering

Osteoarthritis (OA) is a disease in which the cartilage in healthy joints in the body breaks down causing the individual to experience pain during movement. The overall goal of our lab is to use cyclodextrin (CD) polymers to increase the residence time of drugs to treat OA. My first objective is to test the capacity of in situ drug loading of CD polymers. Secondly, because we aim to have a high residence time of the polymer in the knee, factors such as body temperature, inflammation and mechanical loading can lead to polymer degradation. Thus, the second objective of my project is to determine how the degradation byproducts of our polymers affect the viability of an engineered stem cell sensor line. To simulate reloading of CD polymers, 0.25% (w/v) agarose gels were implanted with CD disks. Methylene blue and phenol red were chosen as the two model dyes because of different affinities with CD polymers. Area scans were run at 668 nm to detect methylene blue and 560 nm for phenol red. β -CD and γ -CD polymers were shown to have a higher loading for phenol red as compared to dextran and α -CD polymers during loading with no noticeable difference for methylene blue. To determine polymer cytotoxicity, pluripotent stem cells containing Oct4-GFP plasmid were exposed to different polymers and byproducts and then fluorescence sorted. Less fluorescence after polymer exposure indicates a differentiated phenotype, which in the literature correlates to poor health outcomes. Future work aims to verify long-term effects by accelerated heat failure and compressive loading.

Project Mentor: Dr. Edgardo Rivera, Department of Biomedical Engineering
Faculty Sponsor: Dr. Horst A. von Recum, Department of Biomedical Engineering

Site-Directed Mutagenesis of Ribonucleotide Reductase on loop 2

Marie-Louise Kloster (Department of Chemistry), Andrew Knappenberger (Department of Biochemistry), Michael Harris (Department of Biochemistry)

The enzyme ribonucleotide reductase (RR) synthesizes the four deoxynucleotide diphosphates (dNDP) necessary for the replication and repair of DNA. Both the specificity and the activity of RR are regulated allosterically, therefore, this enzyme is a point of interest for studies of allostery. The specificity of RR is regulated by the binding of dNTP effectors on the allosteric specificity site so that it may maintain a proportional nucleotide pool. The effector and substrate binding sites are connected via loop 2, which is known to be of importance due to its conservation among eukaryotes, as well as evidence from studies *in crystallo*, *in vitro*, and *in vivo*. The mutation of loop 2 will help determine the chemical groups that contribute to the regulation of hRR specificity. Six mutations were chosen based upon hypotheses made from *in silico* models and information from previous studies. One such study, from Kumar *et al.* in 2012 observed the effects of RR loop 2 mutations in *Saccharomyces cerevisiae* on dNTP pools. The data gathered for these mutations provided a foundation for the hypotheses regarding the Y285 and D287 mutants. The mutations are: N270A, Y285D, Y285E, Y285H, D287H, and D287N. These six mutations were successfully created using site directed mutagenesis, based upon the sequencing results. Additionally, they are able to be used in the protein preparation stage, so that the resulting protein may be tested to gain insight into the regulation of hRR specificity.

Project Mentor: Andrew Knappenberger, Department of Biochemistry
Faculty Sponsor: Dr. Michael Harris, Department of Biochemistry



Heart rate variability and cardiorespiratory coupling in mice with inducible-hypertrophy in cardiac myocytes

Christina Lewis, Department of Nursing, University of Florida; Kenneth Gresham & Julian Stelzer, Department of Physiology and Biophysics; Khyati Pandya & Michiko Watanabe, Division of Cardiology, Department of Pediatrics; Monica Montano, Department of Pharmacology; David Nethery, Yee-Hsee Hsieh and Thomas E. Dick, Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine

HEXIM1 (Hexamethylene-bis-acetamide-inducible protein 1) is a transcription factor expressed primarily during development. Re-expression of HEXIM1 in adult mice cardiomyocytes improves stroke volume and coronary vascularization and slows heart rate. Thus, cardiac function improves similar to that in athletes, so we hypothesized that heart rate variability (HRV) would be highly modulated with ventilation. To test this hypothesis, we recorded and analyzed HRV in conscious, transgenic mice in which expression of HEXIM1 in cardiomyocytes depends on doxycycline. Transgenic mice were instrumented with miniature amplifiers and transmitters to record ECG via radio telemetry. Breathing patterns of the mice were recorded using a flow-through, whole-animal, plethysmographic chamber. An initial study compared 3 mice with and 3 without HEXIM1 expression and found no difference in the Power Spectral Density (PSD) of heart rate. To resolve how this occurred given the difference in heart rate between the two groups, we recorded from a mouse before, during and after 30 days of doxycycline administration. Data analysis for HRV involved PSD of HR and ventilation. We selected 3 epochs (1-5 min) to analyze from each day of recording. Cardiac cycles were identified from the peak of the R-wave (Spike 2 software). Respiratory cycles were identified using a threshold-crossing algorithm. The relative power was determined by comparing the absolute PSD in the following 3 frequency bands: very low frequency (VLF) which was between 0.078-0.15 Hz; low frequency (LF), 0.15-1.5 Hz; and high frequency (HF), 1.5-5 Hz. The respiratory frequency varied between 2 and 3 Hz and overlapped with HF band. For the first 10 days after doxycycline, the relative power spectrum was similar to that at baseline; but between Day 10 and 25, the relative power in the HF and LF bands oscillated alternating between a dominate HF and LF relative power expression. By Day 30, the relative power was similar to that at baseline. We anticipate that the autonomic control of heart rate in these mice *adapted* to their stronger hearts and that the sympathetic and parasympathetic tone became balanced similar to that during baseline. This process of adaptation in mice may be due to sympathetic withdrawal rather than enhanced parasympathetic activity.

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



Effects of Baroreceptor and Cardiopulmonary Reflex Activation on Lumbar Sympathetic Nerve Activity, Blood Pressure and Heart Rate in Anesthetized and Conscious Rats

Jackquelin M. Loera, Department of Biology, University of Rochester; **Stephen J. Lewis**, Department of Pediatrics, Case Western Reserve University School of Medicine; **Martin S. Muntzel**, Department of Biological Sciences, Lehman College

Hypertension is a primary risk factor for heart disease and stroke that affects over a billion people worldwide. Since human essential hypertension involves increases in sympathetic nerve activity (SNA) to the heart and vasculature, it is vital to chronically record SNA in animal models of hypertension to gain insight into relevant pathophysiological mechanisms. To do this, we developed methods to simultaneously record lumbar SNA (LSNA), blood pressure (BP) and heart rate (HR) in normotensive, adult Sprague-Dawley rats using implantable telemetry devices. We first recorded LSNA, BP and HR in anesthetized rats and then in conscious, unrestrained rats. The quality of nerve signal was assessed by quantifying responses to activation of baroreceptor and cardiopulmonary reflexes. We recorded the following events in both anesthetized and conscious rats, (1) baroreceptor-mediated increases in LSNA in response to decreases in MAP elicited by bolus intravenous (IV) injections of a vasodepressor/vasodilator agent, the nitric oxide-liberating drug, sodium nitroprusside (2.5-20 $\mu\text{g}/\text{kg}$), (2) baroreceptor-mediated decreases in LSNA in response to increases in MAP elicited by IV injections of the pressor/vasoconstrictor agent, the α_1 -adrenoceptor agonist, phenylephrine (2.5-20 $\mu\text{g}/\text{kg}$), and (3) abrupt decreases in LSNA induced via activation of 5-hydroxytryptamine (5-HT)₃ receptors on cardiopulmonary vagal afferents elicited by IV injections of 5-HT (5-50 $\mu\text{g}/\text{kg}$). In anesthetized rats, we also demonstrated marked increases in LSNA in response to systemic hypoxia, induced by mechanical closure of the upper airway. In summary, these results suggest that our technology will provide a reliable method to study mechanisms underlying the expression of hypertension in anesthetized and in conscious, freely-moving rats.

Project Mentor: Dr. Stephen J. Lewis, Department of Pediatrics

Isolating and Cloning Voltage Gated Sodium Channels to Study its Molecular Structures

Khalid Manzoul, Department of Physiology and Biophysics; **Soumili Chatterjee**, Department of Physiology and Biophysics; **Rajan Vyas**, Department of Physiology and Biophysics

Voltage gated sodium (Na) channels are one of the most important and omnipresent channels in the human body. They are involved in a number of various cellular functions. A prime example would be in electrical signaling such as helping drive action potentials in cells. Na channels are most prevalent in excitable cells such as ones in the nervous system and heart. In order to study the structure of this channel, we must be able to isolate and purify the protein. Unfortunately, it is very difficult to isolate and analyze Na channels from human cells or any eukaryotic cells. So, our lab uses bacterial voltage gated sodium channels since they share a lot of the major characteristics as eukaryotic channels. Because it is easier to isolate sodium channels from bacteria, we can clone many channels that can be structurally analyzed. To better understand the structures of voltage gated Na channels, we clone the channel from bacteria that are mutants and wild types. Understanding the molecular structures from the mutants and wild types will help us later in understanding the dynamics of voltage gated Na channels. The research conducted on these channels can help us in understanding channelopathies and future drug developments.

Project Mentor: Dr. Sudha Chakrapani, Department of Physiology and Biophysics



Culturing of Human Mesenchymal Stem Cells (HMSC) for Glucose Uptake Analysis and Innovative Cartilage Regenerative Applications

J. Anthony Morris, Department of Chemical and Biomolecular Engineering; Yi Zhong, Department of Chemical and Biomolecular Engineering; and Anna Wertheim, Department of Chemical and Biomolecular Engineering

Mesenchymal Stem Cells (MSCs) can be used to create tissue engineered cartilage constructs to treat osteoarthritis. MSCs can be induced to undergo chondrogenesis to form chondrocyte like cells, the principal cells in cartilage. The strategic objective of this research is to develop a non-invasive methodology to interrogate the process of chondrogenesis. Towards this objective, a goal of the research is to analyze the glucose uptake rate of MSCs undergoing chondrogenesis *in vitro* under various conditions. Cultured MSCs were subjected to chondrogenesis and the resultant aggregates of the cells were cultured for three weeks. The glucose uptake rate of the aggregates was analyzed. The uptake rate and histology data will be presented.

Project Mentor: Dr. Harihara Baskaran, Department of Chemical and Biomolecular Engineering

Synthetic Method for Covalent Linkage of Small Molecule Thiols to the Surface of Graphene Oxide Nanosheets

Eric Mosher, Department of Chemistry; Bradley Rodier, Department of Chemistry; and Emily Pentzer, Department of Chemistry

Janus materials, particles functionalized with different chemical functionalities on opposite sides, are of increasing interest due to the diverse applications which are complimentary to those of particles that are homogeneously functionalized. The specific applications of Janus particles are dictated by the identity of their chemical composition, and include stabilization of interfaces, directional particle propulsion, and electronically responsive inks. Our aim is to prepare Janus nanosheets based on graphene oxide (GO) with the hydrophilic polymer poly(N-isopropylacrylamide) (PNIPAM) on one side and a hydrophobic polymer or alkyl chain on the opposing face. GO is a plane of carbon atoms decorated with oxygen functional groups and is a particularly interesting substrate to use for Janus particles, as the material has good gas barrier and antimicrobial properties, is mechanically robust, and is a precursor to conductive materials. In order to prepare Janus GO nanosheets, we must first develop an effective route for the covalent functionalization of the surface of graphene oxide. We demonstrate that propargylamine is used to functionalize GO by nucleophilic ring opening of the epoxide groups, then explore the reaction of small molecule thiols with the pendant alkyne groups. Successful functionalization of GO with small molecules is supported by FTIR and XPS characterization. Now that a model route for functionalization of GO has been developed, we are exploring the functionalization of GO with PNIPAM and other polymers to make the desired Janus nanosheets. Complete analysis and characterization of these novel systems will reveal their unique properties and establish their ideal applications.

Project Mentor: Dr. Emily Pentzer, Department of Chemistry



Effects of in utero lipopolysaccharide (LPS) and Postnatal Sustained Hypoxia in a Rat Model of Sudden Infant Death Syndrome

Tien Nguyen, Department of Biology; Peter MacFarlane, Department of Pediatrics

Previous research has shown that respiratory abnormalities and vulnerability to infection during a critical window of development are connected to Sudden Infant Death Syndrome (SIDS). Preterm infants, commonly born in association with or as a consequence of in utero infection, are at increased risk of SIDS. In the present study, we investigated whether in utero treatment with the endotoxin lipopolysaccharide (LPS) heightens the vulnerability of the respiratory neural control in response to a later hypoxia challenge. Pregnant rats received an intraperitoneal injection of lipopolysaccharide (LPS) ~2 days prior to giving birth and the pups were then exposed to 5 days of hypoxia (SH) exposure ~10 days later. The ventilatory response to an acute hypoxic challenge (HVR) was then measured as an index of respiratory neural control. Compared to untreated control rats, SH attenuated the acute HVR; however, prior treatment to in utero LPS did not worsen the effects of SH on the HVR. Ongoing experiments are aimed to determine whether other LPS increases the vulnerability of other aspects of respiratory control and to determine if they could confer major risk factors for SIDS.

Project Mentor: Dr. Peter MacFarlane, PhD, Department of Pediatrics, Dr. Richard Martin, MD, Department of Pediatrics

Inhibition of S-Nitrosogluthathione Reductase in Asthma

Floyd Nichols, Department of Biology; Laura Smith, Pediatrics; Dr. Benjamin Gaston, Pediatric Pulmonology

S-Nitrosothiols (SNOs) are a bioactive form of nitric oxide. S-Nitrosogluthathione (GSNO), a form of SNO, is an endogenous bronchodilator present in the epithelium of the airways produced, in part, by inducible nitric oxide synthase (iNOS). In many cases of severe asthma and cystic fibrosis, there is a lack of GSNO. Studies have shown that this reduction in GSNO is caused, in part, by a protein known as S-Nitrosogluthathione Reductase (GSNOR), which catabolizes GSNO, forming inactive ammonia and oxidized glutathione. Many patients with severe asthma or cystic fibrosis have an abundance of the GSNOR protein which results in bronchoconstriction. Our research is concerned with expression, activity and inhibition of GSNOR. Two types of studies were performed. First, proof of concept studies were done in baseline and activated Raw 264 cells with several characteristics of airway macrophages. In the second set of experiments, we used human asthmatic bronchoalveolar lavage (BAL) samples from Severe Asthma Research Program (SARP). We performed immunoblots for GSNOR and iNOS – as well as control β actin – at baseline and after stimulation with $TNF\alpha$, Lipopolysaccharide (LPS), $INF\alpha$, and $IL\beta$, with a final concentration of 10 ng/ μ l each (“cytomix”) or a GSNOR inhibitor (30 μ M). Using a standard spectrophotometric assay, we also measured GSNOR activity under these conditions. In Raw 264 cells we found that cytomix decreased GSNOR expression (Control vs Cytomix; n= 4; p=0.006). As expected, cytomix increased the expression of iNOS (n= 4; p= 0.006), an effect that was not changed by co-incubation with GSNORi (n=4; p =0.004). Cytomix caused a minor decrease on GSNOR expression (p = NS). There was variability in GSNOR expression in the SARP samples, which may have been caused from degradation of the GSNOR protein (n= 8; p=0.004). Likely because of this degradation, there was no GSNOR activity in the SARP BAL samples. Using the results we obtain from the experiment, we hope to predict if a new therapeutic way of treating severe asthma is possible.

Project Mentor: Dr. Benjamin Gaston, Department of Pulmonology



Inhibition of EGFR as Novel Treatment Against Ocular Toxoplasmosis

Jared Ong, Nutritional Biochemistry & Metabolism; Dr. Carlos Subauste, Department of Medicine; Dr. Jose-Andres Portillo, Department of Medicine; Yalitza Lopez Corcino, Department of Medicine; and Yanling Miao, Department of Medicine

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects approximately one-third of the world's population and is the leading cause of infectious retinochoroiditis (Ocular Toxoplasmosis). Current treatment options are suboptimal, with loss of visual acuity in both immunocompetent and immunosuppressed individuals. In addition to loss of visual acuity, *T. gondii* is capable of causing encephalitis in immunosuppressed patients. *T. gondii* survives in host cells by residing in a non-fusogenic, parasitophorous vacuole that lacks the necessary transmembrane proteins to fuse with lysosomes. By residing within a non-fusogenic vacuole, *T. gondii* avoids autophagic targeting and therefore lysosomal degradation. This is accomplished during contact with the host cell, when micronemal proteins (MICs) secreted from *T. gondii* bind to domains on Epidermal Growth Factor Receptors (EGFRs) in the plasma membrane and induce activation of EGFR-Akt signalling. It is hypothesized that induced blockade of the EGFR-Akt signaling cascade results in parasite death via autophagy-dependent lysosomal degradation. We believe that EGFR tyrosine-kinase inhibitors, such as Gefitinib, provide a protective effect against *T. gondii* and antimicrobial agents, such as Pyrimethamine, reduce the parasite load after infection. By combining an EGFR tyrosine-kinase inhibitor and antimicrobial agent, we aim to provide a synergistic effect and improved eradication of *T. gondii in vitro*.

Project Mentor: Dr. Carlos Subauste, Department of Medicine, Division of Infectious Diseases and HIV Medicine

Are Photochemical Side Effects Induced by the Azathioprine Drug or Its Metabolites?

Luis A. Ortiz-Rodríguez, Department of Biology, University of Puerto Rico at Humacao; Glesmarie Ortiz-Zayas, Marvin Pollum and Carlos E. Crespo-Hernández, Department of Chemistry and Center for Chemical Dynamics, Case Western Reserve University.

Azathioprine (Aza) has been prescribed as an immunosuppressive prodrug to prevent rejection following organ transplantation, and for an array of autoimmune diseases, since the 1960s. In order to be clinically effective, Aza requires enzymatic conversion. In this process, Aza is metabolized to 6-mercaptopurine (6-MP; primary) and 6-thioguanine (6tGua), both of which are further metabolized to 6-thioguanosine. Prolonged treatment of patients with Aza has been correlated with a 10-fold increase in basal cell carcinomas and a 65- to 250-fold increase in squamous cell carcinomas. These side effects have been attributed to photochemically-induced side effects leading to cell damage, and eventually carcinomas. In an effort to understand if these side effects come primarily from the drug itself and/or from its metabolites, we have investigated Aza, 6-MP, and 6tGua using a combination of spectroscopic and computational techniques. We will present molar absorptivity coefficients, vertical excitation energies, excited-state decay lifetimes, and triplet state and singlet oxygen quantum yields, and discuss their ramifications in the observed photochemical side effects.

Project Mentor: Professor Carlos E. Crespo-Hernández, Department of Chemistry



Toward developing a cell culture model for pathogenic GABAA receptors

Julisha Patten, Department of Physiology and Biophysics; Xiaojing Di, Department of Physiology and Biophysics; Kate Fu, Department of Physiology and Biophysics

Genetic mutations most often lead to malformed or misfolded proteins. A change in the structure of an encoded protein causes a decrease of fully functional proteins in the cell membrane or complete loss of gene expression. This research is geared toward understanding the protein homeostasis (proteostasis) of ion channels. Loss of function or proteostasis of ion channels are one of the principle factors causing numerous diseases, including neurodegenerative and cardiovascular diseases. We use Gamma-aminobutyric acid type A (GABAA) receptors as the model ion channel for our research. They are the primary inhibitory ion channels in the human central nervous system. Mutations of these receptors lead to idiopathic epilepsies. Experiments are mainly focused on the mirroring of the physiology relevant expression level of GABAA receptors. The most common types of subunits of GABAA receptors in the human brain contains two $\alpha 1$ subunits, two $\beta 2$ subunits, and one $\gamma 2$ subunit. Through the use of a standard PCR, which amplifies target DNA, the CMV promoter of each subunit was replaced with a weaker TK promoter for $\alpha 1$ and $\beta 2$ subunits. This would allow the generation of model neuronal cells that express pathogenic GABAA receptors at the endogenous protein level. Such model neurons will allow the elucidation of the molecular mechanism underlying the pathology. Furthermore, novel therapeutic strategies could be developed using these model cells to correct protein misfoldings and their underlying diseases.

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

Isolation of 4-Hydroxy Acetyl-CoA Kinase Via a Functional Assay

Rachel Polak, Biochemistry; Jeremy Hess, Department of Chemistry

Lipid peroxidation (LPO) products are byproducts formed by the reaction of biological lipids and reactive oxygen species. Such products have been implicated in multiple disease states, including Alzheimer's and liver disease. It has been shown that 4-hydroxynonenal, the most ubiquitous LPO product, undergoes a novel isomerization of the hydroxyl group from the 4-position to the 3-position catalyzed by a 4-hydroxy acetyl-CoA kinase. Successful identification of this enzyme via a functional assay would allow a broader understanding of how the body processes LPO products and why their accumulation corresponds to disease. To prepare for the assay, γ -hydroxypentanoyl-CoA was synthesized to mimic the substrate of the kinase. In addition, d9-pentanoyl-CoA was synthesized to act as an internal standard and allow quantitative analysis of the assay. Separation of pig liver proteins by ammonium sulfate precipitation was previously performed, and further separation of these fractions by fast protein liquid chromatography utilizing a hydrophobic interaction column is in progress. Purity of the synthetic GHP-CoA and d9-pentanoyl-CoA was confirmed by NMR and mass spectrometry. The next step of this investigation is performing the functional assay with the synthesized compounds to further isolate the 4-hydroxy acetyl-CoA kinase.

Project Mentor: Dr. Gregory Tochtrop, Department of Chemistry



Drug discovery against infections of *Acinetobacter baumannii*

Pranoti Pradhan, Department of Biochemistry and Department of Economics; David Kuo, Department of Biochemistry; Dr. Michael Greenberg, Department of Biochemistry; Dr. Menachem Shoham, Department of Biochemistry

Infections of *Acinetobacter baumannii*, a highly resistant bacterial pathogen, have become more prevalent over the past few years, especially in hospitals. *A. baumannii* is a coccobacillus Gram-negative bacterium found in soil and water samples which causes disease by attacking the immune systems of patients. *A. baumannii* is resistant to all antibiotics except polymyxins, which have severe side effects and cause kidney damage. Thus, there is an imminent need to develop new therapeutic agents to treat multi-drug resistant infections of *A. baumannii*. The main objective is to discover potential drugs to inhibit virulence. Such agents are not antibiotics; they do not result in pathogen death, but inhibit the formation of disease-causing toxins. The drug target is the transcription factor GacA, which is part of a two component regulatory system for the transcription of virulence factors. GacA, the response regulator, is a small soluble protein that has been identified as a global virulence factor in *A. baumannii*. This response regulator functions by controlling the transcription of various virulence factors, one being Phospholipase D (PLD). PLD attacks the membranes of the host cells in the immune system. Several potential inhibitors of PLD were identified by virtual screening of a small molecule binding site on GacA with the diversity small molecule set library from The Developmental Therapeutics Program of the National Cancer Institute consisting of 1500 compounds. Certain compounds have exhibited significant inhibition of PLD at concentrations of at 10ug/mL. To determine their efficacy, these compounds are being tested in insect larvae at 10ug/mL. Future plans include testing the compounds in animal models at lower concentrations as well as searching for similar compounds.

Project Mentor: Dr. Menachem Shoham, Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH

Folding of the Intrinsically Disordered Domain in Estrogen Receptor (N-terminal Domain, NTD)

Ling Qi, Department of Nutrition; Dr. Sichun Yang, Department of Nutrition; Dr. Yi Peng, Department of Nutrition; Dr. Blake Crochet, Department of Nutrition

Estrogen belongs to a class of steroid hormones that control the development and maintenance of normal sexual and reproductive function. The estrogen receptors (ERs) are ligand-inducible transcription factors that mediate the biological effects of estrogen. It was found that in breast cancer patients, the ERs are over-expressed or “ER-positive”. ERs contain two major transcription activation functions: the N-terminal region (AF1) and the C-terminal Ligand Binding Domain (AF2). The AF2 function requires the presence of hormone (e.g. estrogen) to activate the gene expression. The AF1-related N-terminal domain (NTD), however, is hormone-independent and has been shown to be unstructured in aqueous solution, but undergoes a conformational change to a folded state upon binding with co-modulators such as TATA box-binding protein (TBP). This research is to focus on investigating the folding of an intrinsically disordered NTD of the ER, responsible uniquely for ER activation function. The TBP has been indicated to induce the folding of ER (NTD). Experiments are under the way to understand how this intrinsically disordered NTD starts to fold in the presence of TBP.

Project Mentor: Dr. Sichun Yang, Department of Nutrition, Center for Proteomics



Florid osseous dysplasia: a pictorial review

Marija Rowane, Department of Biology, Department of Music; Ali Syed, B.D.S., M.S., Oral and Maxillofacial Medicine and Diagnostic Sciences, School of Dental Medicine

Florid osseous dysplasia (FOD) is characterized by clinically asymptomatic, non-neoplastic, fibro-osseous lesions, occupying multiple quadrants of the maxilla and mandible within the alveolar process. Fibro-osseous lesions ensue from a haphazard arrangement of fibrous connective tissue with abnormal bone, replacing lamellar bone. Often radiographically classified as an incidental finding, FOD predominantly manifests in middle-aged women of African descent, yet the etiology remains unknown. Imaging is essential to accurately distinguish pathological stages and diagnose these fibro-osseous lesions.

This literary review and radiographic pictorial presentation of FOD postulated five stages in the development and complications of FOD, from the three recognized maturation phases of fibro-osseous lesions, complicating to the multi-quadrant, complete FOD and subsequent atrophy: radiolucent, mixed, radiopaque, FOD and simple bone cavities, and diffuse sclerosing osteomyelitis. Radiographic features essential to accurate diagnoses and effective management were defined. Periodic clinical and radiographic examinations and proper oral hygiene, are recommended for the asymptomatic patient. Precarious outcomes of unnecessary surgical measures were identified, as noted in current literature. Therefore, elective antibiotic therapies, surgical procedures, and biopsies are contradicted, unless the fibro-osseous lesions are secondarily infected and present sclerosing osteomyelitis. Cognizance of various maxillary and mandibular maladies may potentially prevent misdiagnoses and deleterious surgical intervention.

Project Mentor: Ali Syed, B.D.S., M.S., Oral and Maxillofacial Medicine and Diagnostic Sciences, School of Dental Medicine

Faculty Sponsor: Barbara Kuemerle, Ph.D., Department of Biology

Prevalence of oral-antral communication in oral maxillofacial radiographic diagnosis: a cone-beam computed tomography study

Marija Rowane, Department of Biology, Department of Music; Ali Syed, B.D.S., M.S., Oral and Maxillofacial Medicine and Diagnostic Sciences, School of Dental Medicine

This descriptive cone-beam computed tomography (CBCT) study investigated the prevalence, dimensions, and patient demographics of oral-antral communication (OAC), a pathological condition characterized by discontinuity of the maxillary sinus. OAC derives from posterior tooth extraction but, otherwise, may arise from maxillary infectious and inflammatory processes, trauma, cysts developing from the mucosa of the maxillary sinus, implant surgery, or benign or malignant neoplasms.

CBCT cases between 2015 and 2016 were evaluated for OAC incidence. A 7.14% (11) incidence of OAC among 154 cases was identified. Patient demographics included a mean age of 61 years and male predilection of 55% (6). Approximately 60% (7) of patients were referred from periodontics regarding dental implant surgical errors or follow-up examinations. The OAC area ranged from 3.46 mm² to 106.11 mm².

Incidence, patient demographics, and dimensions of OAC reflected previous studies, as well as inconsistencies in current literature. A broader sample size of OAC is needed to more accurately assess prevalence of OAC among the general patient population. The practicality of CBCT imaging, providing a broader field of view and reduced radiation dosage, has been demonstrated in diagnosing oral pathologies. Communication of the common incidence, patient demographics, and dimensions associated with OAC substantiates current literature with consistent findings and may serve as an educational effort in facilitating appropriate referrals.

Project Mentor: Ali Syed, B.D.S., M.S., Oral and Maxillofacial Medicine and Diagnostic Sciences, Dental Medicine

Faculty Sponsor: Barbara Kuemerle, Ph.D., Department of Biology



Mapping Projections from the Medial Prefrontal Cortex to Serotonergic Neurons in a Mouse Model of RTT

Ashley Saunders, Department of Neurosciences; C. James Howell, Department of Neurosciences, Heping Yu, Department of Neurosciences

Rett Syndrome (RTT) is a neurodevelopmental disorder resulting from loss of function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2), a transcriptional regulatory protein. Some of the core symptoms of RTT include loss of speech, loss of acquired hand skills, social withdrawal, anxiety in response to new situations, respiratory abnormalities, and microcephaly. Previous studies in the Katz lab have shown that increasing activity in the medial prefrontal cortex (mPFC) can reverse some symptoms in a mouse model of RTT, specifically, abnormal breathing. My experiment is part of a larger project which aims to understand how the mPFC interacts with the respiratory network in the brain in RTT. The specific purpose of my study was to understand how the mPFC influences the caudal raphe nuclei in the brainstem, which are known to play an important role in the regulation of respiration. We wanted to know if there are projections to the raphe nuclei from those regions of the mPFC which, when activated, restored normal breathing in the RTT mice. Therefore, we used double immunocytochemical staining to look for colocalization of fibers from activated regions of the mPFC with raphe neurons identified by expression of the serotonin-synthesizing enzyme tryptophan-hydroxylase 2 (TPH2). Fiber projections were visualized by antibody staining for an anterogradely transported viral vector that had previously been injected into the mPFC. We hypothesized that virally-labeled mPFC neurons would project to the serotonergic neurons in the brainstem raphe nuclei.

Faculty Sponsor: Dr. David M. Katz, Department of Neurosciences

APPLICATIONS OF AZADIPYRROMETHENES IN ORGANIC FIELD-EFFECT TRANSISTORS

Justin Smith, Chemistry Major, Fisk University; Sandra Pejic, Department of Chemistry, Case Western Reserve University; Chunlai Wang, Department of Chemistry, Case Western Reserve University

A transistor is a semiconducting device that is used to amplify or switch electronic signals and electrical power. An organic field-effect transistor (OFET) uses an electric field applied by a terminal (the gate) to control the electric conductivity of a channel of one type of charge carrier in an organic semiconductor. In recent studies, azadipyrrromethene (ADP) and its derivatives have emerged as a class of compound with highly desirable and near-infrared photo-physical properties. Thus, this current study serves to investigate the application of an ADP as an organic semiconducting layers in a top-contact bottom-gate organic field effect transistor. The OFETs were fabricated on a highly doped silicon substrate coated with 250 nm silicon dioxide as the dielectric layer. The dielectric layer was cleaned and modified with octyltrichlorosilane. The ADP organic semiconductor was spin-coated and gold contacts were thermally evaporated atop the ADP to serve as the source and drain. Devices were tested under nitrogen using a probe station with varying gate voltages applied by a Keithley 2636B Source Meter. Field effect mobilities were calculated from transfer curves and compared to other organic semiconductors.

Faculty Mentor: Dr. Geneviève Sauvé, Department of Chemistry



TRP-ML (1,2,3) Ion Channel Potential Role in Cascade of NLRP3 Inflammasome Complex Activation in Macrophages

Alexander E. Sosa, Department of Physics, Case Western Reserve University; Dr. George Dubyak, Department of Physiology and Biophysics, Case Western Reserve University

Activation of the production of major cytokines, specifically IL-1 family, occurs through the innate immune system and is complex. Formation of the NLRP3 complex by recruitment of ASC and pro-caspase 1 to produce IL-18 and IL-1 β is canonically linked to a decrease in cytosolic potassium concentration, lysosomal destabilization, and oxidative stress in combination with NLRP3 re-localization. A novel role and broader understanding for TRP-ML2 in the innate immune response has recently been postulated by Lu Sun et al. (2015). Here, we have identified a channel that might play a role in NLRP3 activation through lysosomal destabilization. TRP-ML(1,2,3) are non-selective ion channels and are the primary candidates due to their pH- and thermo-sensitivity, their response to TLR activation and their presence in lysosomes. Using a calcium mobilization assay, a propidium iodide assay and a western blot protein identification technique, a clearer idea of these channel's role were established with respect to NLRP3 activation.

Project Mentor: Dr. George Dubyak, Case Western School of Medicine, Department of Physiology and Biophysics. Funded by the National Heart, Lung and Blood Institute, Grant # R25-HL03152 (Monica Montano, PI)

CCL 3 Enhances Immune Cell Trafficking to Lymph Nodes

Henry-Robert Thomas, Alice Wu, Frederick Allen, Dr. Agne Petrosiute, Dr. Alex Huang. Department of Pediatric Oncology

Tumors can suppress the antitumor immune response by upregulation of suppressing or modifying proteins. Augmentation of the immune cellular response is one way to overwhelm tumor suppressing mechanisms. Chemokines are cytokines that can help drive immunity through the recruitment and modification of immune cells and can be used to augment their responses. Our study investigates how the inflammatory chemokine, CCL3, influences the recruitment of T & B Cells in the lymph nodes (LNs). Using a CCL3 transfected colon tumor (L3TU), we analyze the trafficking differences between lymphocytes in the presence of a wildtype tumor alone (WTTU) or with L3TUs. Mice were inoculated subcutaneously with either WTTUs or L3TUs and their tumor draining (DLN) and non-draining LNs (NDLNs) were analyzed. Our first experiment looks at CD4, CD8 & CD19 cells trafficking into DLNs and NDLNs. Lymphocytes in the L3TU group showed enhanced accumulation within 24-hours post-tumor injection and peaked at day-7 where there was a sharp decline in both the L3TU and WTTU groups. This could be due to immune cell egression from the DLN to the tumor site. In addition to the DLN responses, the NDLNs also showed a spike in lymphocyte influx at day 5 that was enhanced by CCL3. Lastly, injection of CCL3 alone does not result in the same enhancement of lymphocytes in the DLN. We conclude that CCL3's response is similar to an adjuvant in that CCL3 effect in immune recruitment must be accompanied by some tumor associated factor.

Project Mentor: Dr. Agne Petrosiute, Frederick Allen, Department of Pediatric Oncology



Overexpression of SmMef2 Transcriptional Activator in COS Cells and Regulation of Potential Muscle-Specific Genes *in vivo*

Kyabeth Torres, Department of Biology, University of Puerto Rico at Humacao; **James Hagerty**, Department of Biology, Case Western Reserve University; and **Emmitt Jolly**, Department of Biology, General Medical Sciences and Center for Global Health and Disease, Case Western Reserve University

Myocyte enhancer factor 2 (Mef2) is a transcriptional activator that is expressed in muscle cells promoting myogenesis. Mef2 gene constitutes a part of the MADS box conserved sequence of genes that encodes for transcription factors controlling gene expression. In many organisms, Mef2 is critical for cellular differentiation, embryonic development and survival. Our lab identified and initially characterized schistosome Mef2 (SmMef2), however, the complex life cycle of schistosomes makes an in depth understanding of basic developmental mechanism challenging. Schistosomes are the disease-causing agents of Schistosomiasis, the most devastating parasitic disease after malaria. There are approximately 200 million people infected worldwide. Schistosomes penetrate mammalian skin to invade the circulation and other crucial organs in which they complete their life cycle. In the hope to expand our knowledge about schistosome early muscle developmental pathways and to circumvent the challenges of genetic manipulation of these previously intractable parasites, we will initially examine the expression profile of SmMef2 and potential SmMef2 target genes in Green Monkey Kidney cells (COS) cells. We will overexpress SmMef2 and determine if its target muscle genes are inappropriately express in kidney. The plasmid PKT01 was designed in order to introduce strong and stable Mef2 expression in COS cells. Mef2 was cloned under the control of a strong actin promoter and a stabilizing profilin 3' terminating untranslated region. We hypothesized that the evolutionary distant SmMef2 muscle-specific gene regulator from schistosomes will promote initial expression of myocyte cells genes. Further studies are needed to test our hypothesis using RT-PCR and Western Blot Analysis Techniques. Results will be discussed.

Project Mentor: Dr. Emmitt Jolly, Department of Biology

Structural Insights Into G-tracts Recognition by the hnRNP H Recognition Motif

Taylor A. Turley, Department of Chemistry; **Srinivasa R. Penumutchu**, Department of Chemistry; **Blanton S. Tolbert**, Department of Chemistry

The heterogeneous nuclear ribonucleoprotein H (hnRNP H) is a subfamily of hnRNP proteins, which eminently intricate with splicing RNA of cellular and viral mRNAs, particularly human immunodeficiency virus (HIV). The hnRNP H protein's structure contains three quasi RNA recognition motifs (qRRM) and two glycine rich domains. hnRNP H proteins bind to poly-G sequences (G-tracts) that are present in cellular and viral mRNA. To obtain a better understanding of the interaction of hnRNP H G-tract RNA recognition, we used recombinant DNA technology to insert the plasmid, containing the gene of interest (qRRM1, qRRM2, qRRM3), into BL21 cells. Later using the nickel affinity chromatography to purify each protein. We were then able to use the size exclusion chromatography (SEC) to collect the qRRM domains of hnRNP H for further analysis. To confirm the protein was collected, we ran a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Subsequently, we were able to analyze the interaction of RNA oligos sequence *AGGGU* against qRRM1, qRRM2, and qRRM3 by using isothermal titration calorimetry (ITC), examining the binding affinities. The purpose of this experiment is to further understand how the qRRM domains of hnRNP H bind to RNA as a whole, calculating the binding affinities of the qRRM domains of hnRNP H to G-tracts (AGGGU) that are present in cellular and viral mRNA.

Project Mentor: Dr. Blanton S. Tolbert, Department of Chemistry



Mimicking Physical and Biochemical Cues of the Heart to Direct Cell Phenotype

Analia Vazquez Cegla, General Engineering Major, Smith College; and Margaret Kilbane, Freshmen High School Student, Hathaway Brown School

Cell behavior can be directed by extracellular cues. The heart naturally adapts to topographical cues and to hemodynamic load by modifying cell orientation, and by adapting the growth rate of individual cells. In the scenario of maladaptive growth, excess hypertrophy, and tissue fibrosis, the energy demands of the heart can exceed the demands for functional output leading to heart failure. In this study, we investigate the role of mechanical forces and the extracellular matrix on cardiomyocyte growth. The extracellular matrix has proven to be a promising biological scaffold to be used in tissue engineering. We aim to optimize an isolation protocol for the extracellular matrix of the mammalian heart, which could potentially be used to enhance cardiac tissue regeneration approaches. Mouse and rat hearts were decellularized using different concentration of SDS and ionic buffers to determine the most effective detergent conditions for cell removal while retaining matrix proteins. DAPI nuclear stain analysis was used to confirm lysis of cells from the matrix. Western blot analysis was performed to survey the remaining proteins on the matrix. The dynamic function of the heart causes cardiac cells to experience mechanical forces *in vivo*. In this project we aim to gain a better understanding of the adaptive response of cardiac fibroblasts to mechanical stimuli. Fibroblast and myoblasts cells were placed under sinusoidal mechanical stress at 10% strain, 1Hz for 8 hours to model the adaptive features of tissue cells in the beating heart. The reorientation of cells according to the type of stimulus was documented and compared to previous related studies.

Project Mentor: Sam Senyo, Ph.D.; Department of Biomedical Engineering

Determination of the RNase P Mechanism for the Observed 3' to 5' Directional Processing of Polycistronic ptRNA

Melissa M. Yamsek, Medicinal-Biological Chemistry; Jing Zhao, Department of Biochemistry; Michael E. Harris, Department of Biochemistry

Ribonuclease P (RNase P) is a tRNA-processing enzyme that hydrolyzes the phosphodiester bond between the 5' leader sequence and the tRNA body to generate a mature tRNA, which can undergo further modifications by amino acids for use in downstream protein synthesis. Although tRNA processing is an important and ubiquitous cellular system, it is still not completely understood. The current model for tRNA processing includes initial cleavage of polycistronic ptRNA 1-2 nucleotides downstream of the CCA 3' terminus by RNase E; however, Kushner *et al.* discovered an RNase E-independent pathway for polycistronic ptRNA in *E. coli* where it was discovered that RNase P is responsible for the cleavage of the 3' *rho* terminator followed by the 3' to 5' directional cleavage of individual tRNAs. In a preliminary study performed by our group, the directional processing of polycistronic valVW RNA by RNase P was examined *in vitro* under single turnover conditions. The resulting data was consistent with the 3' to 5' ordered processing observed by the Kushner group; however, it was observed that the independent rates of the valVW construct were slow for the valV region and fast for the valW region, likely due to the formation of the stem loop secondary structure within the leader sequence of the valV. In this study, the reversed polycistronic construct, ValWV, was generated and examined under the same single turnover conditions to determine the mechanism of the RNase P enzyme.

Faculty Mentor: Michael Harris, Department of Biochemistry



Perturbations in energy metabolism in a rat liver model of propionic academia

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Propionic acidemia is an inborn disorder of metabolism characterized by the accumulation of propionate, 3-hydroxypropionate and ammonium in body fluids. It is caused by a defect in the enzyme propionyl-CoA carboxylase. The management of these patients is difficult and mortality is high. We reproduced the biochemical pathology of propionic acidemia in isolated rat livers kept alive by perfusion with oxygenated artificial plasma. To the plasma, we added the same toxic concentrations of propionate and 3-hydroxypropionate as observed in the human patients. Using enzymatic spectrophotometric assays, we measured the concentrations of a number of compounds involved in redox and energy homeostasis: lactate, pyruvate, β -hydroxybutyrate, acetoacetate, ATP, ADP, AMP. The [lactate]/[pyruvate] and [β -hydroxybutyrate]/[acetoacetate] ratio reflects the [NADH]/[NAD⁺] ratios in liver cytosol and mitochondria, respectively. The ratios [ATP]/[ADP] and [ATP]/[AMP] reflect the capacity of the cell to synthesize ATP, the energy currency of the cell. The data reveal major decreases in the [ATP]/[ADP] and [ATP]/[AMP] ratios. This reflects the inability of the liver cells to regenerate ATP at a rate that meets the energy requirements of the cells. The [lactate]/[pyruvate] and [β -hydroxybutyrate]/[acetoacetate] ratios were in the physiological range showing that the respiratory chain of the cell was not blocked. Other mass spectrometric assays revealed a blockage of the citric acid cycle (Krebs cycle) which generates the reducing equivalents that are oxidized in the respiratory chain. In conclusion the project has shed light on one of the important aspects of the metabolic perturbations observed in the liver of patients with propionic acidemia.

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