INTERSECTIONS

Summer Poster Session

July 31, 2015

10am-Noon

Biomedical Research Building Atrium

2015 Intersections Poster Session
Case Western Reserve University

The 2015 Intersections Poster Session at Case Western Reserve University is comprised of eight research programs focused on the STEM fields that had approximately 50 undergraduate participants not only from Case Western Reserve University but also from colleges and universities around the country. Most of the programs began on May 26 and ended with the Intersections Cooperative Poster Session on July 31st. The Intersections Cooperative Poster Session is pleased to share the students' abstracts from the poster session.

The summer undergraduate research programs comprising the Cooperative are:

- Academic Careers in Engineering and Science (ACES+)
- Chemistry National Science Foundation – Research Experience for Undergraduates (NSF-REU)
- ENGAGE (**Cen**ter for Stem Cell and Re**g**ener**a**tive Medicine Under**g**raduate Stud**e**nt Summer Program)
- Heart, Lung, and Blood Summer Research Program (HLB)
- The Holden Arboretum Internship Program
- Independent Research with Faculty
- The Institute for the Science of Origins
- Provost Summer Undergraduate Research Grant (P-SURG)
- SOURCE Summer Research Program
- Summer Program in Undergraduate Research (SPUR) in Biology

2015 CWRU Summer Lunch Seminars
in the Biological and Social Sciences and Chemistry
Location Sears 480

Informal buffet lunch – 11:45am Student Presenter – 12:05pm Faculty Presenter – 12:15pm

May 27, Sears 480
Introductions

June 3, Sears 480
Sophia Senderak, Cognitive Science, 2017. *Radular Stalk Movement of the Aplysia Californica Buccal Mass.*
Jessica Fox, Assistant Professor, Biology. *Sensory information processing for fly behavior.*

June 10, Sears 480
Yuxi Zheng, Biochemistry, 2016. *The Role of Matricellular Proteins in Glaucoma.*
Paul Wille, Research Associate, Molecular Biology and Microbiology. *From pathogen to cure: viruses as scientific and therapeutic tools.*

June 17, Sears 480
Jenny Luppino, Biochemistry, 2016. *Delineating the Impact of DNA Variants on Genetic Predisposition to Colon Cancer.*
Cynthia Beall, Distinguish University Professor and S. Idell Pyle Professor of Anthropology. *Andean, Ethiopian, and Tibetan adaptations to high altitude: Three ways to live the high life.*

June 24, Sears 480
Laura Hertz, Biochemistry, 2017. *Functional Cationic Lipids for MiRNA Delivery.*
Rupa Mandala, Biology, Psychology, 2017, University of Illinois at Chicago. *The Effects Of Conducting A Poverty Simulation.*
Olga Nazarenko, Psychology, 2016. *Attention Bias and Electrocortical Activity in Response To Emotion Regulation Tasks In Youth: Associations Between Youth And Mother's Depressive Symptoms.*

July 1, Sears 480
Brian Ward, English, 2017. *Modeling Microcephaly of DNA-Repair Protein Disorders.*
Nicole Steinmetz, Assistant Professor, Biomedical Engineering. *The Nanoman – turn off the dark.*

July 8, Sears 480
Eben Alsberg, Associate Professor, Biomedical Engineering and Orthopaedic Surgery. *Guiding stem cell fate for tissue engineering applications via spatiotemporally controlled signal presentation.*

July 15, Sears 480
Ahmad Khalil, Assistant Professor of Genetics and Genome Sciences. *lincRNAs in human health and disease.*

July 22, Sears 480
Joseph Palmeri, Chemical Engineering, 2017. *Plasma Synthesis of Nanodiamonds.*
Jean Burns, Assistant Professor, Department of Biology. *Invasive species: how do they become a problem and what are their effects?*

July 29, Sears 480
Clarissa Kos, Biomedical Engineering, 2016. *A Platelet-Inspired Nanomedicine Approach for Targeted Thrombolytic Therapy.*
Dean Pontius, Chemical Engineering, 2016. *Using Cellular Metabolism as a Predictive tool for Studying Chondrogenesis in Mesenchymal Stem Cells.*
Ilakkiya Thanigaivelan, Biology 2017. *Haltere Role on Gaze Control in Drosophila.*

July 31, BRB Atrium, 10am-noon
POSTER SESSION

The CWRU Summer Lunch Seminars in the Biological and Social Sciences and Chemistry are coordinated by SOURCE (Support of Undergraduate Research & Creative Endeavors) the centralized office for undergraduate research and creative endeavors at Case Western Reserve University. Direct questions to Sheila Pedigo, SOURCE Director by email Sheila.pedigo@case.edu or by phone: 216-368-8508.

Table of Contents

Ford	Emma	ENGAGE	Development of an Organotypic Culture System for Studies of the Perivascular Niche in Glioblastoma	Jeremy Rich, CCF Stem Cell Biology and Regenerative Medicine	7
Frazier	Carleigh	ACES+	Investigating Synthesized Fatty Acids as Inhibitory Compounds for the Human Fatty Acid Binding Protein 5 (hFABP5)	Gregory Tochtrop, Chemistry	7
Graziano	Brendan	Chemistry NSF REU	Structure-Property Studies of Azadipyrromethene-Based Compounds for Organic Electronics	Geneviève Sauvé, Chemistry	8
Hammond	Rachel	Independent	Poly-Lactic Acid (PLA) Stents to Solve Tracheal Stenosis	Ozan Akkus, Mechanical and Aerospace Engineering	9
Hathcock	David	SOURCE	The Effects of Recreational Marijuana Legalization on Crime: A Spatial Analysis of Denver Dispensaries	Mariana Carrera, Economics	9
Hawley	Emma	Independent	A Comparison of Cell Source and Scaffold Geometries for Muscle Cell Powered Living Machines	Ozan Akkus, Mechanical and Aerospace Engineering	10
Holloway	Kristopher	HLB	The effects of GSNO on Tracheal and Bronchiole Dilation using Wire Myography Technique	Stephen Lewis, Pharmacology	10
Homere	Andrew	HLB	Correlations between Baseline Variables and their Influence on Goal Systolic Blood Pressure in SPRINT (Systolic Blood Pressure Intervention Trial)	Jackson Wright, Nephrology and Hypertension	11
Irizarry Nieves	Juan	HLB	Ephrin Receptor-SHIP2 SAM:SAM Heterodimer Binding Characteristics Through Targeted Mutagenesis	Matthias Buck, Physiology and Biophysics	11
Jett	Rashaad-Dreana	HLB	Glycosylation of Notch EGF Domain 9-13 Enhances Ligand Binding	Lan Zhou, Pathology	12
Jimenez	Alexandra	HLB	The Impact of S-Nitrosocysteamine on F508Del CFTR Protein Expression, Maturation, and Function to the Cell Surface	Benjamin Gaston, Pediatric Pulmonology	12

Johnson	Sean		HLB	Muscleblind-like 1 Regulates Transforming Growth Factor Beta During EMT	Andrea Ladd, CCF Cellular & Molecular Medicine	13
Kim	Hyung Chul		Capstone	Expression of lacZ reporter gene through endogenous and exogenous promoters in *Schistosoma mansoni*	Emmitt Jolly, Biology	13
Kraj	Pawel		Chemistry NSF REU	Hydrothermal Carbonization of Hybrid Magnetic Iron Oxide@Carbon Nanochains Using Simple Carbohydrates as Carbon Sources	Anna Samia, Chemistry	14
Larson	Jared		Department of Biology	Effects of Non-Lethal Injury on Predator-Prey Interaction in a Larval Dragonfly, *Pachydiplax longipennis*	Ryan Martin, Biology	14
Lee	Katelyn	Bedoyan, Sarah	ENGAGE	Differential Expression of Surface Markers on Breast Cancer Cell-Derived Exosomes	Huiping Liu, Pathology	2
Lopez	Alyssa		ACES+	Plant Viral Nanoparticles for Cancer Therapy Applications	Nicole Steinmetz, Biomedical Engineering	15
Luppino	Jennifer		P-SURG	Delineating the Impact of DNA Variants on Genetic Predisposition to Colon Cancer	Peter Scacheri, Genetics and Genome Sciences	15
Maalouf	Nicolas	Aryana, Rayan; Varghai, Kaveh; Monebi, Samuel	Independent	Efficacy of the bioreactor organ culture system as a physiological model of rat calvarial suture development and fusion.	Davood Varghai, Plastic Surgery	1
Maas	Zachary		HLB	Examining the Role of ARNT in Reconstitution of Hematopoiesis Following Myeloablative Therapy	Diana Ramirez-Bergeron, Case Cardiovasculary Institute (CVRI)	16
Mallipeddi	Nikhil		SOURCE	Determination of the mechanistic role of long non-coding RNAs in the development of β-catenin mediated fibrosis and dermal fibroblast identity	Radhika Atit, Biology	16
Mason	Maya		ACES+	Comparison of Collagen Extraction Techniques on the *Aplysia californica* Sea Hare	Ozan Akkus, Mechanical and Aerospace Engineering	17

McIntire	Rahne		Holden Arboretum Internship	The Effects of Climate Origin and Carbon Investment on Gas Exchange in Rhododendrons	Jean Burns, Biology; Juliana Medeiros, Holden Arboretum	17
Monebi	Samuel	Aryana, Rayan; Varghai, Kaveh; Maalouf, Nicolas	Independent	Efficacy of the bioreactor organ culture system as a physiological model of rat calvarial suture development and fusion.	Davood Varghai, Plastic Surgery	1
Nguyen	Tien		HLB	Effect of hyperoxia on bronchiolar walls and neutrophil count in lungs of neonatal mice	Richard Martin, Neonatology	18
Nkrumah	Ebenezer		ACES+	INTERFACING THE SPEX 1404 DOUBLE SPECTROMETER WITH AN ARDUINO	Kathleen Kash, Physics	18
Ortiz-Rodríguez	Luis		Chemistry NSF REU	Direct Measurement of the Singlet Oxygen Quantum Yield of 6-Thioguanosine	Carlos Crespo-Hernández, Chemistry	19
Osborne	Joshua	Draus, Francis	SOURCE; Independent	Cosmic Topology Search via Eigenspectrum Correlation	Glenn Starkman, Physics	6
Osigwe	Chinweoke		HLB	Phenotypic and genomic characterization of Glucose-6- Phosphate Dehydrogenase deficiency of the Malagasy population	Peter Zimmerman, Center for Global Health and Diseases	19
Palmeri	Joseph		P-SURG	Plasma Synthesis of Nanodiamonds	Mohan Sankaran, Chemical Engineering	20
Patel	Jill		Independent	Aplysia Californica Buccal Mass Muscle Extraction and Culture Techniques for Development of Biohybrid Devices	Ozan Akkus, Mechanical and Aerospace Engineering	20
Pérez-Ayala	Michelle		HLB	TRPV2 ion channel interacts with Rab7 to influence endosomal function	Vera Moiseenkova-Bell, Pharmacology	21
Pradhan	Pranoti		Independent	Drug discovery against infections of Acinetobacter baumannii	Menachem Shoham, Biochemistry	21

Rector	Ashley		HLB	Dynamics of Cardio-Respiratory Coupling Assessed by Respiratory Sinus Arrhythmia and Cardio-Ventilatory Coupling Before and During Septicemia Endotoxemia in Rats; As Assessed by Respiratory Sinus Arrhythmia and Cardio-Ventilatory Coupling	Thomas Dick, Division of Pulmonary, Critical Care and Sleep Medicine	22
Sheth	Reena		Independent	Principles of Drug Design: Analysis of Substrate Specificity of Ribonucleotide Reductase	Michael Harris, Biochemistry	22
Sierra	Noémie		Undergraduate Research Capstone	Changes in maneuverability of *Manduca sexta* due to full or partial ablation of the hindwing.	Mark Willis and Jessica Fox, Biology	23
Snyder	Valerie		Chemistry NSF REU	Investigating Specific Binding of hnRNP H to RNA by Monitoring the Intrinsic Fluorescence of Tryptophan	Blanton Tolbert, Chemistry	23
Sosa	Alejandro		HLB	TRP-V3 candidate for non-selective Ion Channel in Cascade of NLRP3 Inflammasome Complex Activation in Macrophages	George Dubyak, Physiology and Biophysics	24
Steele	Miarasa		Independent	Characterizing inducible fibrosis via β-catenin responsive matrix genes	Radhika Atit, Biology	24
Thanigaivelan	Ilakkiya		SOURCE	Haltere Role on Gaze Control in Drosophila	Jessica Fox, Biology	25
Varghai	Kaveh	Aryana, Rayan; Monebi, Samuel; Maalouf, Nicolas	Independent	Efficacy of the bioreactor organ culture system as a physiological model of rat calvarial suture development and fusion.	Davood Varghai, Plastic Surgery	1
Wilkosz	Catherine		SOURCE	A Randomized Controlled Trial of Skin to Skin Contact (Kangaroo Care) Effects on Sleep in Premature Infants	Susan Ludington, Nursing	25
Yellets	Jonathan		HLB	Functional Characterization of a Prokaryotic Pentameric Ligand-Gated Ion Channel	Sudha Chakrapani, Physiology and Biophysics	26

Conductive Molybdenum Disulfide Composite Films

Laura Abelquist, Chemical Engineering
Project Contributor: Rachael Matthews, Department of Chemistry

A large amount of generated energy in industrialized countries is produced and lost in the form of heat. Current devices used to harvest this thermal energy are inefficient, costly, and heavy. Polymer-based composites are attractive alternatives, as they are inexpensive and scalable, the devices can be flexible, and the materials are readily available. Such thermoelectric (TE) materials must be electrically conductive, but thermally insulating. In this work, molybdenum disulfide (MoS_2) platelets are an n-type material with a direct band gap which facilitates transfer of electrons. Single platelets of MoS_2 can be accessed by mild sonication of the bulk material due to the weak van der Waals interactions between the MoS_2 sheets. In this way, MoS_2 is able to be exfoliated in select solvents, of which most are not easily removable afterward. Addition of polymer into solution expedites the exfoliation process. The polymer must also be soluble in the solvent. Complete conversion from bulk material into single layer platelets was confirmed with UV-visible spectroscopy. Composite films of MoS_2 and the organic polymers, polyvinylpyrrolidone and polymethylmethacrylate, were prepared in the vacuum oven or by drop casting, and electrical conductivity of the films was measured with four-point probe. Films with as little as 0.5% MoS_2 by weight and in varying polymer and solvent solutions were found to be conductive. This work lays the foundation for the development of n-type organic thermoelectric materials which, upon incorporation into devices, will facilitate energy interconversion and thermal energy management.

Project Mentor: Dr. Emily Pentzer, Department of Chemistry

***

Efficacy of the Bio-reactor Organ Culture System as a Physiological Model Of rat calvarial suture development and fusion

Rayan Aryana, Research Student at CWRU, (Orange high school**), Kaveh Varghai,** Biomedical engineering dept. **Samuel Monebi** BS, Physiology department, **Nicolas Maalouf** BS, Physiology department, Davood Varghai MD. Medical school, Plastic surgery dept**.**

Purpose: To demonstrate how well the bio-reactor organ culture system performs as a physiological model of rat calvarial suture development and fusion.
Background: *Craniosynostosis*, or the premature fusion of one or more cranial sutures, occurs one in 2000 live births. Despite the recent identification of genetic mutations and environmental parameters in numerous syndromes associated with craniosynostosis, the exact molecular events governing cranial suture fusion versus patency remain unknown. Study of the osseo-inductive growth factors pattern on the suture development can be done by in-vivo or in-vitro methods. Although in-vivo methods are better suited for observing the overall effects of an experiment on a living subject, in-vitro methods are less expensive, have higher throughput, faster and the controlled conditions present in the in-vitro system allow the researcher to discover effect of each parameter, step by step and with more detail. *Bio-reactor* may refer to any device or system that supports a biologically active environment.
Methods: Nine SD fetus rats' hemi-craniums were collected. Four calvarias were put into a bio-reactor and the other four samples were cultured in a conventional organ culture dish. The last sample was fixed for control. After 6 weeks all the samples were collected, fixed, stained with Alizarin, and every sample was evaluated with microscopic imaging and histology.
Result & Conclusion: Microscopic imaging showed bone plates overlapping in all of the sutures in conventional culture dish samples, and Posterior Frontal (PF) suture fusion was not completed in any of the samples. In the bio-reactor culturing system PF suture was completely fused in half of the samples and bone plates overlapping was significantly decreased. Histological review confirmed all of this information.

Project Mentor: Davood Varghai MD. Medical School, Plastic Surgery Dept.

Differential Expression of Surface Markers on Breast Cancer Cell-Derived Exosomes

Katelyn Lee*, Psychology Major; **Sarah Bedoyan***, Cellular and Molecular Biology and History Double Major; Dr. Golam Kibria, Department of Pathology

* These two summer undergraduate students contributed equally to the project and will present the poster together.

Breast cancer is the most common cancer among women in the United States. For these patients, it is not the primary tumor, but rather its distant metastases coupled with treatment failure, that ultimately leads to patient's death. Exosomes add an additional layer to our understanding about how tumor cells metastasize. Exosomes are small vesicles with a lipid bilayer membrane that enclose RNA, lipids, and proteins and have been found in different body fluids such as breast milk, saliva, urine and serum. Recently, they have been identified to regulate communication between cells by transporting contents such as proteins and miRNAs from one cell to other cells within the tumor microenvironment or to distant locations. Our hypothesis is that exosomes carry various membrane receptors and glycoproteins that play important cellular roles in regulating stemness, cell adhesion, proliferation and migration. Thus, exosomes may carry unique protein signatures mirroring their cells of origin. Exosomes derived from the plasma may correlate with clinicopathologic parameters of cancer patients, such as tumor grade, stage, and prognosis. This project focuses on characterizing exosomes isolated from various cancerous and normal epithelial cell lines. Using a combined approach of ultracentrifugation and size-exclusion chromatography to isolate exosomes, we then identified exosomal surface proteins using Flow Cytometry and Mass Spectrometry. Our goal was to find surface markers specific to breast cancer cell lines, which then eventually could be validated as potential biomarkers using breast cancer patient serum samples.

Project Mentor: Dr. Huiping Liu, Department of Pathology, Case Comprehensive Cancer Center, and National Center for Regenerative Medicine

***

Limited Proteolysis of hnRNP A1 Reveals Stable Domains Likely Involved in RNA Recognition

Anise K. Bowman, Department of Biochemistry, Jeffrey D. Levengood, Department of Chemistry, and Blanton S. Tolbert, Department of Chemistry

The heterogeneous nuclear ribonucleoprotein A1 (hnRNP-A1) protein is a multifunctional RNA binding protein with implications of alternative splice site selection regulation, the regulation of telomere biogenesis, and mRNA transport/trafficking. Mechanisms and hnRNP-A1 interactions have been inferred primarily from the structure of its UP1 domain. The UP1 domain is composed of two RNA recognition motifs (RRMs) and an inter-RRM linker. Though the structure of UP1 has been determined, the stable domains that take part in the hnRNP-A1-RNA recognition are unknown. Limited proteolysis is a technique of exposing proteins to specific proteases to pinpoint the partly folded states of the protein. These stable domains are likely the regions of hnRNP-A1 that interact with RNA. In this experiment cells with an over expression of the UP1 protein were grown, the protein was isolated, and then purified. Limited proteolysis experiments were performed using trypsin, papain, chymotrypsin, and proteinase K at different concentration ratios and time intervals. It was found that trypsin cleaved UP1 into 3 stable domains, papain cleaved 2 domains, and chymotrypsin cleaved 2 domains. Proteinase K almost completely degraded UP1. This information can be used to determine the interactions of these stable domains to RNA.

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

Armored Particles: Synthesizing and Characterizing Graphene Oxide Plated Polymer Spheres

Spencer Burton, Department of Polymer Science and Engineering; Bradley Rodier, Department of Chemistry

This project combines micro-emulsion polymerizations and the properties of graphene oxide (GO) in an effort to prepare graphene-polymer composite spheres. Graphene, an atom thick, flat sheet of carbon atoms, has garnered interest across many research fields owing to its chemical stability, conductivity, durability, flexibility, and low cost, but these sheets tend to stack together due to strong van der Waals interactions. GO, carbon sheets decorated with epoxide and alcohol oxygen functional groups, can be prepared from graphene by oxidation of bulk graphite which adds the oxygen functionalities and causes the platelets to crumple, and facilitates separating the sheets. GO is complimentary to pristine graphene, and has additional properties including acting as a surfactant for emulsions, and having reactive functional groups for covalent modification. We use GO platelets as the surfactant for micro-emulsion polymerizations, to prepare polymeric spheres whose surface is decorated with GO. In these emulsions, GO is assembled at the interface of an "oil" phase composed of a small molecule organic monomer and water; furthermore, GO is functionalized with acrylate groups that are covalently incorporated into the polymer particle upon polymerization of the oil phase using UV irradiation. The resulting "armored polymer particles" are characterized by dynamic light scattering and scanning electron microscopy (SEM) to observe their shape and size distribution. In future work, these materials will be incorporated into polymer composites and their surface will be covalently modified to change solubility and miscibility, en route to preparing mechanically robust, conductive materials.

Project Mentor: Professor Emily Pentzer, Department of Chemistry

***

Identification and Characterization of Forkhead box L1 Transcription Factor in Schistosome Parasites

Gregory C. Chang[1], Melissa Varrecchia[1], Emmitt R. Jolly[1]. [1]Department of Biology
Case Western Reserve University, Cleveland OH, 44106

Schistosome worms are responsible for the disease Schistosomiasis, which affects over 240 million people worldwide and is second only to malaria in terms of economic burden. Despite its global impact, limited research has been done to identify and characterize their early developmental genes expressed after they have entered their human host. Forkhead box (Fox) transcription factors play an important role during various developmental processes in a wide range of organisms. All members of the Fox family share a conserved DNA-binding domain (DBD) consisting of 110 amino acids and can be separated out into many subclasses based on sequence similarity. The FoxL1 subclass has been characterized in other organisms such as mice and zebra fish, however, our understanding of the molecular function of FoxL1, in particular with regard to any parasitic organism, is limited. With only one viable drug on the market and reports of resistance in labs and in the field, there is concern of the implications on global health. In addition, praziquantel is ineffective during the larval stages, and cannot be administered until adult worms have begun egg production in the host. By understanding key developmental genes in early schistosome development, we can identify new targets for more effective drugs in battling this parasite. Here, we aim to characterize *Schistosoma mansoni* FoxL1(SmFoxL1), a homolog to the human FoxL1 gene. The schistosome genome database is incomplete and only contains the sequence for the conserved DBD for SmFoxL1, with the remaining sequence of the transcript unknown. To identify the full SmFoxL1 sequence, we use 5' and 3' RACE (Rapid Amplification of cDNA Ends) to obtain the sequence in both the 5' and 3' direction. Since, FoxL1 is predicted to be a transcription factor, we will utilize a modified yeast one-hybrid system to test for positive transcriptional activity.

Project Mentor: Emmitt R. Jolly, Ph.D., Department of Biology

The Association of Vanin-1 with Oxidative Stress

Urieliz M. Cintron-Torres, Department of Biology; Xiaojing Di, Department of Physiology and Biophysics

Oxidative stress is the imbalance between the production of reactive oxygen species and antioxidant defenses. It has been hypothesized that oxidative stress is associated with high blood pressure. Vanin-1 is a membrane protein encoded by the gene VNN1, which has been shown to have a relationship to oxidative stress due to its patentheinase activity and the production of cysteamine, an antioxidant. Previous studies showed that the loss of function of this protein is related to a decrease in blood pressure. To investigate the association of vanin-1 with oxidative stress, we cultured kidney cells. Western blot analyses were performed using human embryonic kidney 293 (HEK 293) cells stably expressing vanin-1 variants and human proximal tubule cells. We observed a robust expression of vanin-1 in HEK 293 cells expressing the wild type vanin-1. Furthermore, we carried out a fluorescence assay to monitor oxidative stress using dihydroethidium (DHE) as a superoxide indicator to two different cell lines (HEK293 Empty Vector and HEK293 wild type-vanin-1) in three different conditions: blank, basal + DHE, and hydrogen peroxide (H2O2)-treated + DHE. H2O2 was used to induce oxidative stress to respective cells. We expect to find an association of vanin-1 with this oxidative stress marker. Our findings will shed light on vanin-1's role in oxidative stress regulation.

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

***

EZH2 dependent lineage decisions in the cranial mesenchyme

Mahima Devarajan, Department of Biology; James Ferguson, Department of Biology; Gregg DiNuoscio, Department of Biology

Proper craniofacial development involves complex genetic and environmental interactions in the multipotential cranial neural crest cells (CNC) to allow for differentiation into nearly all the different tissues of the head and face. The supraorbital cranial mesenchyme is a population of CNC-derived stem cells located directly above the eye, and when properly differentiated, can give rise to the bones of the skull. It has been shown that in the absence of β-catenin, bone and dermis fails to form and is replaced by cartilage (Goodnough et al., 2012). This cartilage formation is due to the upregulation of multiple chondrogenic genes, such as *Sox9* and *Col2a1*, which are also known targets of the Polycomb Repressive Complex (PRC2). PRC2 is a gene-suppressing complex, providing a possible mechanism by which cartilage development is repressed during normal endochondral skull bone formation. We hypothesized that the interaction between B-catenin and PRC2 results in proper cranial bone development by repression of cartilage in the cranial mesenchyme. Specifically, deletion of EZH2, a key component of PRC2, should lead to changes in cell fate similar to B-catenin loss-of-function mutants. Understanding how Polycomb is recruited to specific genes in mammals will provide insight into how gene-specific epigenetic repression is achieved.

Project Mentor: Radhika Atit, Department of Biology

Relationship of leaf anatomy and function to climate tolerance differs among evergreen, deciduous, and semi-evergreen Rhododendrons

Callie Dowrey, Department of Biology, Jean H. Burns, Department of Ecology, and Juliana S. Medeiros, The Holden Arboretum, Charlotte R. Hewins, The Holden Arboretum

Rhododendrons are found in many temperature habitats, possibly facilitated by the evolution of diverse leaf habits. Subgenus Pontica is evergreen, while subgenus Pentanthera is deciduous; but those in subgenus Tsutsutsi are described as semi-evergreen or "dimorphic", with larger deciduous spring leaves and smaller evergreen summer leaves. We hypothesized evergreen and deciduous leaves would be anatomically different with dimorphic falling in between. We compared leaf and petiole anatomy of two species from differing mean annual temperature (MAT) in each subgenera. We found that leaf size and specific leaf area (SLA) decreased significantly as MAT increased, but there were differences among the subgenera in how leaf anatomy changed across MAT. In addition, net photosynthesis, SLA, spongy mesophyll and epidermal area were all higher for deciduous compared to evergreen. In contrast, leaf size and petiole Huber Value were lower for deciduous versus evergreens. When the species of Tsutsutsi were considered as a whole values tended to be intermediate compared to evergreen and deciduous, supporting our hypothesis. The dimorphic habit varied greatly, however. For *R.* indicum, spring and summer leaves followed patterns similar to deciduous and evergreen species. However, *R. yedoense* leaves resembled those of deciduous species, and spring and summer leaves showed few anatomical differences. We suggest differences in leaf and petiole anatomy contribute to habitat diversity. Deciduous leaf traits are associated with warm, sunny habitats. Evergreen traits are associated with cooler, low-light habitats. Finally, our data suggest dimorphic species may be able to acclimate to a variety of environments via trait plasticity.

Project Mentor: Juliana Medeiros, The Holden Arboretum
Co-Mentor: Jean Burns, Department of Ecology

***

Identification of Natural Product Modulators of Inflammation

Erika Drain, Department of Biochemistry; Gregory Tochtrop, Department of Chemistry; Mohsen Badiee, Department of Chemistry

Oxidative stress is defined as a chemical imbalance associated with either a high concentration of oxidizing free radicals or a low concentration of antioxidants in an organism, and plays a role in numerous pathological conditions, including inflammation, atherosclerosis, neurodegeneration, and cancer. Polyunsaturated fatty acids (PUFAs) are extremely prone to both enzymatic and non-enzymatic peroxidation – the oxidative degradation of lipids – due to the presence of a bis-allylic position, and easily break down into various lipid peroxidation products. Certain lipid peroxidation products are able to trigger peroxisome proliferator-activated receptor (PPAR) activity. PPAR is a family of transcription factors that control expression of a variety of genes responsible for lipid uptake, fatty acid storage and the regulation of carbohydrate and lipid metabolism. In addition, the lesser studied role played by PPARs in the regulation of inflammation and immunity has recently become of great interest. Because the metabolic products of oxidative stress on PUFAs can be very diverse in structure and function, this research is focused on the isolation and characterization of the specific lipid peroxidation products that bind with and activate PPAR. In this laboratory, we generated a mixture of lipid peroxidation products through the auto-oxidation of the Ω-6 PUFA arachidonic acid using ascorbic acid and $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. The lipid peroxidation products were later separated by analytical HPLC (high performance liquid chromatography) . Then several fractions were manually collected and organized based on the compound's retention time. PPAR activity of the individual fractions was measured using a luciferase assay on COS7 cells in modified high glucose media.

Project Mentor: Dr. Gregory Tochtrop, Department of Chemistry

Cosmic Topology Search via Eigenspectrum Correlation

Joshua Osborne[1], Francis Draus[1], J. Dulin1* , G. Starkman[123], C. Copi[1]
CWRU Dept. of Physics [1], CWRU Dept. of Astronomy[2], Director Institute of the Science of Origins[3]
*Alumnus

In this study, we attempt to calculate unique correlation spectra for conventional and oblique cosmic topologies with the aim of comparing them to the temperature fluctuations in the cosmic microwave background. We begin by calculating the specific series of eigenmodes that correspond to a periodic wave across a specific fundamental domain that we wish to examine. Individual fundamental domains of candidate topologies are categorized by a set of parameters used to describe its shape. The search will marginalize over possible domains using the resulting modes; we expand in terms of spherical harmonics in order to examine the modes from the perspective of an observer arbitrarily located and oriented within the topology. Once these harmonics are assembled into the power spectrum, we compute the correlation between individual pixels in the data set, and receive results in the form of an 837 by 837 correlation matrix, which corresponds to the number of spherical harmonics used to construct the data. Promising steps have already been made in this endeavor, and future expansions of the study are in development. Among them is the analysis of the computationally produced spectrum in comparison to the corresponding spectrum of the cosmic microwave background radiation recently observed via satellite by the Plank Collaboration.

Project Mentors: Dr. Glenn Starkman[123], Dr. Craig Copi[1]

***

Determining single-stranded DNA-binding specificities of POT1, POT1-TPP1, and RPA1

Jean C. Fernandez Gonzalez; Department of Molecular Biology (UPRRP), Maria de la Fuente; Department of Pharmacology, Malligarjunan Rajavel; Department of Pharmacology, and Derek Taylor; Department of Pharmacology

POT1 is a protein that binds to telomeric single-stranded DNA (ssDNA) and it is part of the telomere sheltering complex, along with five other proteins, which prevent telomeres from being recognized as DNA breaks and triggering DNA damage response. POT1 forms a stable complex, with another protein of the telomere sheltering complex, called TPP1. The binding of POT1 to ssDNA is enhanced by TPP1, which reduces POT1 dissociation constant. Replication Protein A (RPA) is an eukaryotic ssDNA binding protein. RPA is known to be essential for chromosomal DNA repair, replication and recombination. It is also involved in regulating DNA metabolism and transcription. RPA has 3 subunits (RPA1, RPA2, and RPA3) with different DNA binding domains (DBD) or OB-folds. In this project we analyzed HiTS-EQ data from POT1 and POT1-TTP1 to obtain binding affinities for 4096 distinct ssDNA sequences. The different affinities were used to determine ssDNA binding specificity of POT1 and POT1-TPP1. We intended to obtain HiTS-EQ data from RPA1 using 8N ssDNA oligos, to compare it with the POT1 and POT1-TPP1 data obtained. In order to do so, RPA1 was cloned, expressed and purified. Gel shifts were performed with different ssDNA sequences to test RPA1 binding. Our results showed that RPA was bound to every ssDNA sequence tested. Thus, to obtain accurate HiTS-EQ data of RPA1, an 8N loop oligo was designed and gel shifts were performed.

Project Mentor: Dr. Derek Taylor, Department of Pharmacology

Development of an Organotypic Culture System for Studies of the Perivascular Niche in Glioblastoma

Emma Ford (Ohio State University, Neuroscience), Chris Hubert (Cleveland Clinic Department of Stem Cell Biology and Regenerative Medicine), Jeremy Rich (Cleveland Clinic Department of Stem Cell Biology and Regenerative Medicine)

Glioblastoma (GBM) is the most prevalent and malignant primary brain tumor in adults, and it is predominantly drug and radiation resistant. Despite treatment with resection, radiation, and adjuvant chemotherapy, prognosis is dismal, with a median survival of only 14-16 months. This poor prognosis can be attributed to a set of self-renewing, tumorigenic glioblastoma stem cells (GSCs). GSCs have been found to reside in specific niches within the tumor, particularly near vasculature (perivascular niche) and in central, necrotic regions (hypoxic niche). To develop more effective treatments, it is critical to understand the basic biology driving these cells, including the interactions between GSCs and their microenvironment. While these tumor microenvironments can be recapitulated to an extent in vivo, there is a large disparity in methods to study them in vitro. Here, we describe the development of an organotypic microvascular culture system using endothelial cells and astrocytes. Following optimization, these cultures can be used to study the interactions between endothelial cells of the vascular system and GSCs residing in the perivascular niche.

Project Mentor: Dr. Jeremy Rich, Cleveland Clinic Department of Stem Cell Biology and Regenerative Medicine

***

Investigating Synthesized Fatty Acids as Inhibitory Compounds for the Human Fatty Acid Binding Protein 5 (hFABP5)

Carleigh Frazier, Department of Biology; Elizabeth Stewart, Chemistry Department

Retinoic Acid (RA) has been shown to cause cell arrest in certain types of cancers, while in other types, RA promotes cell growth and proliferation. These dual outcomes are due to two alternate receptor pathways in the cancerous cells. These pathways lead to nuclear receptors that control gene expression. RA is shuttled to the nuclear receptors by proteins. The cell growth pathway is facilitated by the human fatty acid binding protein 5 (hFABP5) which is an intracellular lipid binding protein. By inhibiting hFABP5 in cancerous cells, RA can be shuttled to the alternate cell arrest pathway, stopping tumor growth. The hFABP5 has high affinity to fatty acids and therefore, various synthesized fatty acids will be tested to discover the best inhibitory compound. After growing and purifying hFABP5, synthesized fatty acids are used in a competitive binding assay to quantify the affinity of the compound to the protein using the inhibitory constant, K_i. Using the data obtained, a viable compound can be identified. The compound with the most promise will have high affinity to hFABP5 (a low Ki value) and a low affinity to the nuclear receptor (a high Ki value). This research can be applied to enhance the use of RA as a cancer treatment in more cancer cell types through the inhibition of hFABP5.

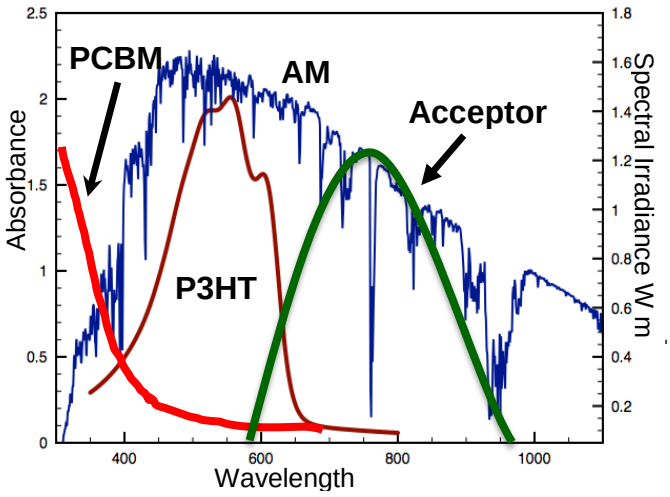
Project Mentor: Gregory Tochtrop, Chemistry Department

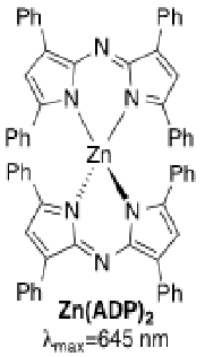
Structure-Property Studies of Azadipyrromethene-Based Compounds for Organic Electronics

Brendan Graziano[*]**,** Department of Chemistry; Sandra Pejic[†], Department of Chemistry; Geneviève Sauvé[†]**,** Department of Chemistry

[*]Ohio Northern University
[†]Case Western Reserve University

Organic photovoltaics (OPVs) provide a flexible, lightweight, and printable alternative to the commercially available crystalline silicon solar cells. Bulk-heterojunction (BHJ) OPVs contain a blended active layer consisting of donor and acceptor material. The donor:acceptor should have complementary light absorptions, high charge separation and nano-scale phase separation which will increase efficiencies. A well-studied donor:acceptor system is poly(3-hexylthiophene) (P3HT) and [6,6]-phenyl-C_{60} butyric acid methyl ester ([60]PCBM). Even though this optimized system has high efficiencies of 5%, PCBM exhibits drawbacks, including limited energy level tunability and poor light absorption in the near-IR region, lowering efficiency. While P3HT strongly absorbs light between 400-650 nm, there is a need for new non-fullerene acceptors, such as azadipyrromethene (ADP). ADP shows promising characteristics as fullerene alternatives due to light absorbing properties in the near-IR region and tunability. Zinc-chelated ADP with side arms in the pyrrolic position have shown promise in increasing efficiencies in OPVs as a potential rival for PCBM. When blended with P3HT, the blend absorbs light between 400-800 nm, the bulk of the visible to near IR region of the solar spectrum. However, even though the ADP-modified blend absorbs more light than the P3HT:PCBM blend, it still exhibits lower current density, limiting efficiencies. Through structural-property relationship studies of modified-ADP chelates, we examine surface morphology and free carrier mobilities to improve device efficiencies through OPV fabrication.





Project Mentor: Professor Geneviève Sauvé, Department of Chemistry

Poly-Lactic Acid (PLA) Stents to Solve Tracheal Stenosis

Rachel Hammond, Department of Macromolecular Engineering; Dr. Ozan Akkus, Department of Mechanical and Aerospace Engineering

Tracheal stenosis is the narrowing of the trachea, commonly referred to as the windpipe, which can be caused by birth defects, trauma, or disease. The current gold standard of treatment is surgical reconstruction although placement of stents is a popular and sometimes necessary option for patients whose narrowing may be inoperable.[1] Currently there are various designs of stents made out of metal, silicon and bioabsorbable materials. Metal stents are not biodegradable, making them susceptible to fracture and fatigue while being difficult to remove. Silicone stents tend to have a high migration rate, necessitating further procedures and generally causing problems for the patient later on. As such, research is moving toward bioabsorbable materials because of their superior integration capabilities.

The goal of this project was to prototype a bioabsorbable tracheal stent. Poly-lactic acid (PLA) was chosen as it is readily available, has the desired degradation profile and mechanical properties. Filament winding is a technique used in various industries which creates tubular structures by winding filaments of material in a controlled pattern onto a mandrel. This technique was applied in this study to create a porous tubular structure, where control of the mandrel was obtained using a computerized numerical control (CNC) system, and control of filament extrusion was achieved using the 3Doodler 3D printing pen. Several prototype "stents" were created with various dimensions and porosities in order to determine the best design to move forward. Heat and chemical techniques for welding the joints of the stents were also compared to determine the optimal treatment for creating stents. Future work for this project will include testing mechanical properties of the ideal stent designs and acquiring medical grade PLA to ultimately prepare the stent for in vivo studies.

Project Mentor: Dr. Ozan Akkus, Department of Mechanical and Aerospace Engineering

References:

[1]Sarper, A., Ayten, A., Eser, I., Ozbudak, O., & Demircan, A. (2005). Tracheal Stenosis after Tracheostomy or Intubation: Review with Special Regard to Cause and Management. *Texas Heart Institute Journal*, *32*(2), 154–158.

***

The Effects of Recreational Marijuana Legalization on Crime: A Spatial Analysis of Denver Dispensaries

David Hathcock, Department of Economics

Over the past few years, the legalization of recreational marijuana has become a divisive issue, appearing as referendum in many states and rising to the forefront of national public policy debates. While the potential advantages of legalization are well documented, it is unknown whether increased recreational marijuana use will lead to an increase in social harms, such as crime. The 2012 legalization of recreational marijuana in Colorado provides the first opportunity to study whether such negative effects exist. With geo-coded crime reports from the Denver Police Department, I use an event study framework to analyze how monthly crime rates changed in areas nearby recreational marijuana dispensaries as they began to open in 2014. This approach also helps to identify whether dispensaries cause new crime or whether they simply cause a spatial redistribution of already existing crime. Additionally, I consider other crime-affecting factors, such as increases in tourism and police policy changes, to determine the extent to which changes in crime can be explained by dispensary openings. With this model, I investigate the impact of marijuana dispensary openings on several types of crime including property crime, violent crime, marijuana crime, and other drug crime. I find that crime related to marijuana and other drugs increases following a dispensary opening, while other crimes generally tend to decrease or remain unaffected. Overall, these results suggest a significant causal impact of the legalization and dispensary openings on each of the various types of crime.

Project Mentor: Professor Mariana Carrera, Department of Economics

A Comparison of Cell Source and Scaffold Geometries for Muscle Cell Powered Living Machines

Emma Hawley, Dept. of Biomedical Engineering

Biohybrid devices are part of an "up and coming" field of research, with potential medical applications. They provide novel approaches to device actuation and locomotion. Key components to these devices are biocompatible materials and a reliable source of cells to provide actuation for extended periods of time. Collagen proves to be biocompatible, and can be compacted into three-dimensional scaffolds, making it a desirable material to use in device fabrication. Two potential sources of cells are the heart and skeletal muscles which have been isolated from day 13 chick embryos.

In past experiments, cardiomyocytes have been effective actuators when seeded on electrocompacted aligned collagen (ELAC) scaffolds. In more recent experiments, myotubules formed from mechanically dissociated skeletal muscle cells seeded on ELAC scaffolds have resulted in improved locomotion. The cells from skeletal muscles are stronger, more robust and more accessible upon chick embryo dissection. These characteristics qualitatively show the skeletal muscle as a superior cell source when compared to cardiomyocytes.

In addition to comparing cell sources, different geometries and cross-linking ratios of ELAC scaffolds have been explored. The variables in scaffold geometry can be categorized into leg length and leg number. The basic shape of the scaffold is an elongated rectangular body with four or six legs protruding at uniform lengths. Increasing the number or length of legs complicates the fabrication process because the scaffolds become more fragile, but these methods also present potential for increased surface contact and therefore improving locomotion and stability.

Victoria Webster[1], Ozan Akkus[1], Hillel J. Chiel[2], Roger D. Quinn[1]
1) Dept. of Mechanical and Aerospace Engineering, 2) Dept. of Biology

***

The effects of GSNO on Tracheal and Bronchiole Dilation using Wire Myography Technique

Kristopher Holloway, Department of Biology, Helly Einisman, Department of Pediatrics, Nitish Rana, Department of Physiology and Biophysics, Paulina Getsy, Department of Pediatrics and Department of Physiology and Biophysics, and Stephen J. Lewis, Department of Pediatrics and Department of Pharmacology

Nitric Oxide (NO) is a well-known smooth muscle relaxant in pulmonary airways. In cells, NO can react with glutathione (GSH) to form S-Nitrosoglutathione (GSNO), an active bronchodilator due to its role in signaling pathways involved in airway smooth muscle relaxation. Our study aims to discover the mechanism(s) that GSNO uses to dilate lung bronchia. We hypothesize that GSNO is released from vesicles packed within epithelial cells in order to decrease upper airway resistance. GSNO will bind to receptor(s) on smooth muscle cells triggering relaxation. To test our hypothesis, we will dissect and mount live-mouse trachea and bronchiole samples on a wire myograph system. Wire myography allows us to measure the tension of the trachea and bronchiole samples at baseline and after dose dependent administration of GSNO. Our results show that the bronchiole (N=2) and trachea (N=2) samples significantly reduce tension as increasing doses of GSNO are added. We conclude that GSNO, when administered exogenously, can significantly dilate bronchioles and trachea. Further experiments would look at individual epithelial cells to identify if GSNO is packed in secretory vesicles. This novel finding can hopefully provide a means for clinicians to increase airflow to the lungs of patients suffering from obstructive sleep apnea and asthma.

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

Correlations between Baseline Variables and their Influence on Goal Systolic Blood Pressure in SPRINT (Systolic Blood Pressure Intervention Trial)

Andrew Homere (Biology), Carolyn Harmon Still PhD, RN, ARNP-BC, Alberta Bee PhD, RN, ARNP-BC
Department of Medicine-Nephrology and Hypertension, School of Nursing

Hypertension is a chronic medical condition that affects about 1 in every 3 American adults. Elevated blood pressure poses increased risks such as stroke, heart failure, heart attacks, kidney disease, and cognitive declines. The Systolic Blood Pressure Intervention Trial (SPRINT) is a 2-arm, multicenter, randomized clinical trial that aims to test whether controlling systolic blood pressure (SBP) to under 120mmHg (Intensive Group) is more effective at reducing cardiovascular disease risk than the standard control to under 140mmHg (Standard Group). Additionally, a SPRINT MIND portion will examine cognitive function in participants via memory tests. Responses were collected via self-reporting surveys and tests, and were analyzed using correlations/regressions. We looked at three categories of variables that may influence each other and SBP control at 12 months at UH Case Medical Center: sociodemographic information, participant satisfaction, and chronic conditions. Using data from the 102 recruited participants; we were able to demonstrate that satisfaction of medical care appears to positively correlate with a participant's satisfaction with the treatment regimen, and satisfaction with the treatment regimen appears to positively correlate with adherence to prescribed medications. Additionally, it seems that younger participants, participants with more chronic diseases, and participants with normal cognitive abilities are more likely to reach their SBP at 12 months. Overall, understanding the influences of different variables on lowering SBP may help us improve practices for optimal results.

Mentor: Jackson T. Wright, Jr. MD, PhD, Department of Medicine-Nephrology and Hypertension

***

Ephrin Receptor-SHIP2 SAM:SAM Heterodimer Binding Characteristics Through Targeted Mutagenesis

Juan Irizarry Nieves, 1st Year Medical Student, Linus Lee, Undergraduate, Jeannine Muller-Grever, Post Doctoral, Soon Kim, Post Doctoral

Ephrin receptors are tyrosine kinases that are activated through cell to cell interactions and mediate processes such as cell motility. Little is known of the protein-protein interactions that occur between signal proteins such as Ephrin receptors. The C terminal of Eph receptors contains a SAM domain and was found to interact with SHIP2, an important phosphatase that regulates PI3K insulin pathways and actin cytoskeleton remodeling. This project consists of modeling the interaction of the SAM domain of EphA1 receptors with that of the SHIP2 enzyme. The Pymol molecular visualization program was used along with previous studies to identify a probable binding sites for SHIP2 with EphA1. The following cancer associated mutant residues were taken from the COSMIC databank and used for the mutagenesis study: R916Q, R926C, R926G and R966C. Microscale Thermophoresis and Isothermal Calorimetry were performed on the wild type EphA1 Receptor and each mutant, and will allow us to determine their affinities towards SHIP2.

Project Mentor: Dr. Matthias Buck, Department of Physiology and Biophysics

Glycosylation of Notch EGF Domain 9-13 enhances ligand binding

Rashaad-Dreana Jett, Shuiliang Yu PhD, Weihuan Wang MD PhD, Yiwei Wang, Lan Zhou MD PhD, Department of Pathology, Case Western Reserve University School of Medicine

Hematopoietic stem cell (HSC) transplantation is currently the only treatment for many malignant diseases, such as certain leukemia's, some lymphomas, and multiple myeloma. HSC transplants reconstitute the bone marrow after it is destroyed by either chemotherapy or radiation. HSCs reside in the marrow niches, specifically the osteoblastic and perivascular niches. . All current methods fail to mobilize sufficient HSCs from the niche to the periphery in 5-30% of patients; therefore, more efficient mobilization methods are needed. Notch signaling is an evolutionarily conserved pathway in multicellular organisms that regulates cell-fate determination during development and maintains adult tissue homeostasis. Post-translational modifications to Notch can affect its stability, degradation, signal diversity and ligand binding affinity. Previous work in the lab has shown that HSCs with defective Notch-ligand interaction display less efficient adhesion to marrow osteoblasts and stromal cells expressing endogenous or exogenous Notch ligand, compared to wild type HSCs, and show distal occupancy to the osteoblastic niche. Furthermore, removing specific Notch-Ligand pairing and inhibiting Notch-2 (N2) signaling is a nontoxic way to promote HSC mobilization. Our lab is working towards developing and testing an N2 decoy molecule that will block N2 ligand engagement and activation to induce HSC and progenitor mobilization. My work focuses on one of the candidates that contains 9-13 Epidermal Growth Factor (EGF)-like repeats of N2 Receptors. The coding sequence of N2 9-13 EGF repeat was cloned and expressed in CHO-CL17 cells. By co-expressing L-fringe glycosyltransferase and supplemental fucose in the cells, the resulting peptide was modified by glycosylation. We propose that glycosylation enhances the binding affinity of N2 EGF 9-13 to Notch ligands, and we hope the modified N2 peptide can be effectively used in the future mobilization regimen to mobilize HSCs.

Project Mentor: Shuiliang Yu, PhD, Lan Zhou, MD. PhD. Department of Pathology

***

The Impact of S-Nitrosocysteamine on F508Del CFTR Protein Expression, Maturation, and Function to the Cell Surface

Alexandra Jimenez, Candidate for Bachelors in Biology, Whittier College, Heart Lung and Blood Research Program, Laura Smith[2], Benjamin Gaston, M.D.[1,2,3,4]

Case Western Reserve University School of Medicine[1], Department of Pediatric Pulmonology[2], UH Case Medical Center[3], UH Rainbow Babies and Children's Hospital[4]

Cystic fibrosis can be a devastating disease having the ability to shorten the lifespan of a patient. There are 30,000 people in the United States that suffer from cystic fibrosis today. F508del is the most common Cystic Fibrosis Transmembrane Regulator (CFTR) defect. Though there have been advances in discovering pharmaceutical targets for Cystic Fibrosis, there are no ideal treatments for correcting the F508Del CFTR protein to the cell surface. The existing drugs and methods used to treat F508Del CFTR still leave patients at risk of advancing to severe lung disease and death. S-Nitrosoglutathione (GSNO) is currently being tested for its potential to treat F508Del CFTR in patients. S-nitrosocysteamine (CA-SNO), S-nitroso-Coenzyme A (CoA-SNO), and S-nitroso-L-cysteine (L-CSNO) have also been found to be F508Del correctors in a previous study. These s-nitrosothiol signaling molecules increase the expression, maturation, and function of the F508Del CFTR. The goal of the current study is to get a better understanding of the mechanism of action that the molecules use to correct F508Del CFTR. Biochemistry, pharmacology, catabolism, transport, and synthesis of CA-SNO, COA-SNO, L-CSNO, and GSNO in the airway epithelium are also to be investigated. Western blot was used to observe the effect of different concentrations of CA-SNO and Tropine (LAT inhibitor) on the CFTR maturation for primary airway cells from F508Del CFTR homozygous CF patients and CFBE-410 CF cells.

Project Mentors: Laura Smith, Benjamin Gaston, M.D. Department of Pediatric Pulmonology

Muscleblind-like 1 Regulates Transforming Growth Factor Beta During EMT

Sean Johnson[1], Ryan J Coram[1], Andrea N Ladd[1]

[1]*Department of Cellular & Molecular Medicine, Cleveland Clinic*

Epithelial-Mesenchymal Transition (EMT) is the process by which stationary epithelial cells are converted to motile mesenchymal cells through certain cell signaling pathways. EMT plays a role in several developmental processes such as morphogenesis and in diseases such as cancer. When the post transcriptional regulator muscleblind-like 1 (MBNL1) is knocked down, EMT has been found to be enhanced. The levels of transforming growth factor beta (TGFβ), which induces EMT, was found to increase as well when MBNL1 is knocked down. TGFβ exists in two basic forms: the latent form and the active form. The latent form is exported from the cells that produce it, into the extracellular matrix where it is usually proteolytically cleaved into the active form. Preliminary experiments have shown that the active TGFβ protein levels rise when MBNL1 is knocked down but the TGFβ transcripts remained constant in atrioventricular heart cells. We hypothesize that active TGFβ is being up-regulated when MBNL1 is knocked down. Adopting Normal Murine Mammary Gland (NMuMG) epithelial cells as a model, TGFβ3 levels were assessed in the cells, total protein, and the extracellular matrix, using western blots and ELISAs. Future directions for this study might include a closer look at the mechanism by which MBNL1 affects the activation of TGFβ.

Project Mentor: Dr. Andrea N Ladd, Department of Cellular & Molecular Medicine, Cleveland Clinic

***

Expression of lacZ Reporter Gene Through Endogenous and Exogenous Promoters in *Schistosoma mansoni*

Hyung Chul Kim, Department of Biology; Kenji Ishida, Department of Biology; Dr. Emmitt Jolly, Department of Biology

Schistosome worms are causative agents of schistosomiasis, a neglected parasitic disease that affects more than 240 million people worldwide. Schistosomes have complex life cycle that contributes to dearth of genetic tools for research. Furthermore, the lack of established reporter gene for visualizing gene expression in schistosomes adds to the challenge of genetic manipulation. This research is concerned with exploring usage of lacZ, a commonly used reporter gene, in schistosomes to identify expression of introduced genes. lacZ gene encodes beta-galactosidase, an enzyme that catalyzes lactose into glucose and galactose, and it allows evaluation of promoter activity and transfection efficiency. Moreover, we will also investigate the difference in gene expression induced through exogenous and endogenous promoters in schistosomula. By using pCI-neo plasmids with cytomegalovirus (CMV) promoter or SmActin1 promoter, we will determine the suitability of lacZ as a reporter gene. Developing this new reporter gene for schistosomes would resolve challenges of assessing promoter activity and transfection efficiency in schistosomes.

Project Mentor: Dr. Emmitt Jolly, Department of Biology
Kenji Ishida, Department of Biology

Hydrothermal Carbonization of Hybrid Magnetic Iron Oxide@Carbon Nanochains Using Simple Carbohydrates as Carbon Sources

Pawel Kraj, Department of Chemistry; Shu F. Situ, Department of Chemistry; Anna Cristina S. Samia, Department of Chemistry

This study focuses on the examination of the effect of various simple carbohydrates as carbon sources on the morphology of iron oxide@carbon nanochains during their fabrication using the hydrothermal carbonization method. Core-shell magnetic carbon nanochains were fabricated hydrothermally in the presence of iron oxide nanoparticles, which serve as templates for the formation of chain-like structures and provide unique magnetic properties for the resulting composites. The iron oxide nanoparticles were synthesized from an iron oleate precursor complex, a key reagent which affects nanoparticle size, shape, and monodispersity. The procedure for making the precursor complex was optimized to produce particles with uniform morphology, which could then form well-defined nanochains. Moreover, the structural, chemical, and physical properties of the magnetic carbon nanochains were evaluated by transmission electron microscopy (TEM), FT-IR, Raman spectroscopy, and powder x-ray diffractometry. Such multifunctional magnetic nanochain material has high potential to be developed into an effective and recyclable antibacterial agent.

Mentor: Dr. Anna Cristina Samia, Department of Chemistry

***

Effects of Non-Lethal Injury on Predator-Prey Interactions in a Larval Dragonfly, *Pachydiplax longipennis*

Jared Larson, Department of Biology; Michael P. Moore, Department of Biology; Ryan A. Martin, Department of Biology

Predation often strongly shapes the traits of organisms. In some predation events, the prey may incur non-lethal injuries, which may themselves mediate individuals' subsequent traits and fitness. We studied how non-lethal injuries affect the interactions of a larval dragonfly (*Pachydiplax longipennis*) with a common predator (*Aeshnidae* sp.) by removing individuals' legs and comparing survivorship and orienting behavior to non-wounded larvae. We predicted that leg removal would decrease survivorship and alter behavior, but, as the middle legs are less important for mobility, the removal of these legs would have the weakest effect. We introduced two larvae (one wounded, one non-wounded control) to an arena with one predator, and observed individual's positions in the arena for 5 hours and survivorship through 24 hours. We found lower survivorship among wounded larvae (χ^2=4.88, df=1, P = 0.027), and while survivorship increased with body size in the controls (z=4.64, P=0.025), size did not improve survivorship in the wounded larvae (z=0.106, P=0.901). Moreover, contrary to our prediction, the survivorship of the wounded larvae did not vary among leg removal treatments (χ^2=8.72, df=5, P = 0.121). However, we observed that perching behavior varied among treatments ($F_{6,55}$=4.37, P=0.001), with individuals missing their rear-right leg spending the most time perching. Overall, our findings indicate that non-lethal injuries strongly decrease future fitness, which may favor the evolution of leg regeneration in dragonfly larvae. Furthermore, although the fitness costs did not vary among leg removal treatments, behavioral differences may result in additional fitness variation in natural populations, and merit future consideration.

Project Mentor: Professor Ryan Martin, Department of Biology

Plant Viral Nanoparticles for Cancer Therapy Applications

Alyssa Lopez, Department of Bioengineering; Anna E. Czapar, Department of Pathology; Nicole F. Steinmetz, Department of Biomedical Engineering, Radiology, Material Science and Engineering, Macromolecular Science and Engineering

Nanotechnology is an emerging field with many important applications for drug delivery, imaging, and immune modulation. While many synthetic nanoparticles have been developed, viral nanoparticles (VNPs) offer unique advantages. VNPs are noteworthy because they are naturally monodisperse, exceptionally stable, and biocompatible.[1] Further, use of VNPs derived from plant viruses ensures that these nanoparticles will not be infectious to humans or other animals. This project examines the use of two such plant viruses: tobacco mosaic virus (TMV) and cowpea mosaic virus (CPMV). Both viruses have distinct characteristics and advantages. Among them include their size and shape. TMV is rod-shaped and measures to be 300 x 18 nm while CPMV is icosahedral and measures to be 30 nm. Current work focuses on using TMV as a carrier for a newly developed cisplatin derivative that has been shown to be effective against cancer cell lines which are resistant to standard-of-care platinum chemotherapeutics. Through in vitro experiments using ovarian cancer cells, potency of this drug has been compared to traditional platinum anticancer drugs. Free drug as well as TMV-encapsulated drug delivery was studied. In addition to using TMV as a nanocarrier, we have also begun the development of CPMV carriers for drug delivery. Toward this goal, we investigated ways to remove RNA from CPMV. This process is important, because the removal of the RNA increases the total internal volume of CPMV and reduces non-desired RNA-drug interactions. With these results, we hope to move forward with in vivo experimentation with a final goal of human cancer therapy.

Project Mentor: Anna Czapar, Department of Pathology
Faculty Sponsor: Nicole F. Steinmetz, Department of Biomedical Engineering, Radiology, Material Science and Engineering, Macromolecular Science and Engineering

[1] Steinmetz, N. (2010). Viral Nanoparticles As Platforms For Next-generation Therapeutics And Imaging Devices. *Nanomedicine: Nanotechnology, Biology and Medicine,* 634-641.

***

Delineating the Impact of DNA Variants on Genetic Predisposition to Colon Cancer

Jennifer Luppino[1]; Andrea Cohen[2]; Dr. Olivia Corradin[2]; Alina Saiakhova[2]; and Dr. Peter Scacheri[2]
[1]Department of Biochemistry, [2]Department of Genetics and Genome Sciences

Recent genome wide association studies (GWAS) have identified dozens of loci associated with genetic predisposition to colorectal cancer (CRC). Most of the loci are located outside protein coding genes, complicating their interpretation. Using epigenomic profiling, we mapped enhancers in twenty-nine CRC cell lines as well as primary tumors. We then compared the risk-associated loci to the active enhancer landscape. We noted about 75% of the colon cancer risk-associated variants co-localize to active enhancers in colon cells. Further, we interpreted the loci in the context of gained variant enhancer loci (VELs), defined by the acquisition of enhancer signal in cancer cells compared to normal tissue. Of the risk-associated variants overlapping enhancers, ~50% map to commonly gained VELs (enhancers acquired in at least 8/29 of the profiled colon cancer patients). We observe a similar trend across many different cancer types: over 30% of all variants associated with increased risk to bladder, breast, prostate, and ovarian cancers map to CRC common gained VELs. Enhancers function as regulators of gene expression, and the presence of a VEL has been shown to result in differential expression of the genes it regulates in cancerous versus normal cells. The convergence between the cancer risk variants and the recurrent epigenetic alterations in CRC strongly implicate these enhancer regions and their associated genes in the pathogenesis of CRC and possibility even other common epithelial cancers.

Project Mentor: Dr. Peter Scacheri, Department of Genetics and Genome Sciences

Examining the Role of ARNT in Reconstitution of Hematopoiesis Following Myeloablative Therapy

Zachary P Maas (B.S. Biological Sciences '13, B.A. Social Science/Economics '13), VCU School of Medicine, CWRU NHLBI Summer Research Program; Dr. Diana Ramirez-Bergeron, PhD, Case Cardiovascular Research Institute (CVRI)

Myeloablative therapy is the accepted standard of care for bone marrow malignancies, as well as bone marrow transplant preparative regimes. The heterodimeric Hypoxia Inducible Factor (HIF) is widely known as the end modulator of the hypoxia-angiogenic response pathway, but studies have shown that its role extends into bone marrow vascular niche homeostasis. Our lab has observed impaired reconstitution of hematopoiesis in ARNT (HIF-1β subunit) endothelial specific conditional-knockout mice, after either irradiation or 5-fluorouracil (5FU) myeloablative treatment. To further elucidate HIF's role in this process, we examined the impact of 5FU on endothelial cell cultures in vitro. Normoxic (21% O_2) vs. hypoxic (2% O_2) conditions, ARNT deletion, and immortalized vs. primary endothelial cell lines were all examined. Secondly, we studied the early impact of 5FU on endothelial-HIF compromised mice in vivo. Blood samples were collected and analyzed to monitor Complete Blood Count (CBC) values. Bone marrow was collected and analyzed for proangiogenic transcripts. Finally, murine femurs were fixed and analyzed for architectural integrity. Clarifying the role of ARNT at the vascular bone marrow stem cell niche is critical in that it will expand the range of potential tools available for chemotherapeutic adjuvant therapy, and improve outcomes in hematopoietic stem cell transplantation. This research has also gained traction due to recent insights into the role of HIF1-α and ARNT in the context of chronic disease. These patients may respond differently to myeloablative therapy, and could stand to uniquely benefit from the translational impact of this research.

Project Mentor: Dr. Diana Ramirez-Bergeron, PhD, Case Cardiovascular Research Institute (CVRI)

***

Determination of the mechanistic role of long non-coding RNAs in the development of β-catenin mediated fibrosis and dermal fibroblast identity

Nikhil Mallipeddi, Department of Biology, B.S. Biology, Nathaniel Mullin, Department of Biology, Ahmad Khalil, Department of Genetics, Radhika Atit, Department of Biology, Genetics, and Dermatology

Expression of the Wnt/β-catenin pathway is necessary in order to induce dermal fibroblast stem cell identity and fibrosis, and published literature documents the presence of lncRNA transcripts in regulating the identity of various categories of stem cells. Long non-coding RNAs (LncRNAs) are RNA molecules present within the cell that, while transcribed off the genome, are not specifically translated into proteins via ribosomal regulation. LncRNA transcripts are readily present within the cytosol and nucleus of the cell, and the lack of ribosomal translation implies alternate roles for lncRNAs within the confines of cellular and extracellular regulation. This project focuses on studying the function of Wnt/β-catenin responsive lncRNA transcripts in fibrosis and embryonic skin. We hypothesize that these dynamic, Wnt/β-catenin responsive lncRNA transcripts are necessary for promoting β-catenin induced fibrosis and embryonic dermal fibroblast specification. We have analyzed expression levels of our candidate lncRNAs in β-catenin gain-of-function and loss-of-function adult models through quantitative PCR (q-PCR), and we focused on two robust lncRNA candidates for further experimentation, Gm12603 (Knight Rider) and Gm12606 (Mighty Mouse). We assayed for lncRNA candidates present within wildtype embryonic tissue, and we observed increased levels of our lncRNA candidate expression in both embryonic head and dorsal skin in comparison to the liver, gut, brain, and heart. We are developing lentiviral particles to induce the overexpression of our lncRNA candidates within wildtype fibroblast cells. After viral transfection, we will assay for molecular markers of fibroblast identity, morphological changes in cellular shape, and quantify the proliferation of collagen matrix proteins analogous to the fibrotic phenotype. Finally, we are currently in the process of identifying the spatial expression of our lncRNA candidates through in situ hybridization. We seek to gain insight into characterizing our lncRNA's function through the visualization of our lncRNAs presence within embryonic head dermis tissue. We are on track to identify new lncRNAs that can modulate the role of Wnt/β-catenin signaling in embryonic dermal identity and skin fibrosis.

Project Mentor & Faculty Sponsor: Radhika Atit, Department of Biology/Genetics/Dermatology

Comparison of Collagen Extraction Techniques on the *Aplysia californica* Sea Hare

Maya Mason, Biomedical Engineering; Katherine Chapin, Department of Mechanical and Aerospace Engineering; and Vickie Webster, Department of Mechanical and Aerospace Engineering

Collagen is a fibrous protein found abundantly in skin, bone, and as such, can be readily extracted from any of these structures from any species. This study compared several methods of collagen extraction on several organs of the *Aplysia californica*; specifically, the mantle, gizzard, and skin. Although collagen is insoluble under normal physiological conditions, fractions of collagen can be extracted using acid and pepsin, an enzyme found in stomach acid. The extraction methods tested were acid-only, pepsin-only, and a combination of acid and pepsin. The acid-only procedure used acetic acid to extract acid-soluble collagen. The pepsin-only procedure extracted pepsin-soluble collagen. The combination procedure extracted both the acid-soluble and pepsin-soluble collagen. The study also investigated the purity and compactibility of the extracted collagen. Purity was tested by performing a dimethyl methylene blue (DMMB) assay that measured the amount of extracellular sugars present in the collagen. Compactibility was determined using the electrocompaction method, in which a voltage is applied to a collagen gel in order to align the molecules within it to make a compact, strong structure. Compacted collagen contains fibers that can be aligned via stretching mechanisms. These aligned fibers allow for various types of cell differentiation. For example, skeletal muscles seeded onto aligned collagen differentiate into myotubes. Future directions for this project include cultivating myotubes by seeding *Aplysia californica* skeletal muscle cells on sheets made from the extracted collagen, and then stimulating the myotubes with cultured neurons.

Faculty Sponsor: Dr. Ozan Akkus, Department of Mechanical and Aerospace Engineering

***

The Effects of Climate Origin and Carbon Investment on Gas Exchange in Rhododendrons

Rahne McIntire, Biology at Iowa State University; Katiuska Hernandez, Biology at College of Mount Saint Vincent; Dr. Jean Burns, Department of Biology; Dr. Juliana Medeiros, The Holden Arboretum

The plant genus Rhododendron is found in a variety of ecological niches, and bear three types of leaf habits that help them survive in these climates: evergreen, deciduous, and semi-evergreen. These potentially differ in their carbon investment in growth rate versus cold-hardiness, which is suggested by difference in specific leaf area among niches and leaf habits. Concerning photosynthesis, evergreens invest more carbon into leaves for a given photosynthetic return compared to deciduous species. However, evergreen leaves are more stress tolerant. We addressed the question, how does climate origin and carbon investment strategy affect gas exchange? We collected gas exchange data using a LI-COR 6400XT portable photosynthesis system in the Layer Garden, at The Holden Arboretum, a cold shady environment. Species from colder climates presented significantly higher rates of photosynthesis than warm climate species. Stomatal conductance and transpiration showed a significant negative relationship to light requirements. In addition, species from drier climates had significantly higher stomatal conductance and transpiration than those from wetter environments. Transpiration and stomatal conductance increased significantly with specific leaf area. These data suggest climate of origin and carbon investment strategy are important in determining gas exchange rates even when grown in a common environment. Species that come from climates more similar to The Holden Arboretum tended to have higher rates of photosynthesis but were more conservative with their water use. Carbon investment did not show a clear relationship with photosynthesis, however, species that invested more carbon per leaf area were more conservative with their water use.

Project Mentors: Dr. Jean Burns, Department of Biology; Dr. Juliana Medeiros, The Holden Arboretum

Effects of hyperoxia on bronchiolar walls and neutrophil count in lungs of neonatal mice

Tien Nguyen[1], Anjum Jafri[2], Richard Martin[2]

[1]Department of Biology, Georgetown University, Washington, D.C., 20057
[2]Division of Neonatology, Rainbow Babies & Children's Hospital and Department of Pediatrics, Case Western Reserve University, Cleveland, OH 44106

Preterm infants often require supplemental oxygen for respiratory support in the early postnatal period. However, the infants may have an increased risk of developing long-term lung disorders. Wheezing and asthma are prominent in infants born prematurely with and without bronchopulmonary dysplasia (BPD). The effects of hyperoxia on developing conducting airways are poorly understood, but are significant in determining lung function. We hypothesized that exposure to hyperoxia would decrease the number of bronchiolar-alveolar attachments and increase the number of neutrophils. We used a neonatal mouse model to investigate the consequences of neonatal inhalation of hyperoxic gas on the bronchiolar walls. Neonatal mice (C57BL/6J) born at term were exposed to 7 days of room air, 50% O_2, CPAP with room air, and CPAP with hyperoxia. Bronchiolar walls were analyzed 14 days after the oxygen exposure ended. We analyzed the number of neutrophils as well as bronchiolar-alveolar attachments. Neonatal hyperoxia resulted in significantly fewer bronchiolar-alveolar attachments at P21 ($p<0.05$). There was no significant difference between the number of neutrophils in the mice exposed to hyperoxia and the mice exposed to room air. These data suggest that hyperoxia may affect structural changes in the developing small conducting airways that contribute to longer-term respiratory diseases. Exposure to hyperoxia during the neonatal period leads to reconstruction of bronchiolar walls from the decrease in bronchiolar-alveolar attachments in adulthood, which could also develop into poor lung function.

Project Mentors: Richard Martin MD, Division of Neonatology and Department of Pediatrics; Dr. Peter MacFarlane, Department of Pediatrics

***

INTERFACING THE SPEX 1404 DOUBLE SPECTROMETER WITH AN ARDUINO

Ebenezer Nkrumah, Physics Department; Glynis Schumacher, Physics Department; and Kathleen Kash, Physics Department

Raman spectroscopy is a technique used to observe vibrational, rotational, and other low-frequency modes in a system. Raman spectroscopy is commonly used in chemistry to provide a fingerprint by which molecules can be identified and it is commonly used by research Physicists to identify energies of phonon modes of crystals. Our lab uses the Spex 1404 double spectrometer to do Raman spectroscopy. Unfortunately, the original interface of the Spex 1404 is outdated and not functional for our lab needs, being around 3 decades old. The goal of this project is to interface the spectrometer with computer control via an Arduino and take readings using a Matlab script. We use the Arduino Uno to send digital signals to the stepper motor which is connected to the spectrometer, and receive analogue signals on our computer from the Arduino Uno as feedback. A prototype model which simulates the actual procedure was built using an LED and a photocell. The success of this project will allow us once again to use the highly sensitive and precise SPEX 1404 spectrometer with easier, interactive and modern experimental procedures.

Project Mentor: Professor Kathleen Kash; Physics Department

Direct Measurement of the Singlet Oxygen Quantum Yield of 6-Thioguanosine

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

Thiopurines have been prescribed as a treatment for cancers, chronic inflammatory diseases, and autoimmune diseases for over five decades. These drugs are metabolized to the nucleoside analogue, 6-thioguanosine (6tGuo), which is subsequently incorporated into the DNA of proliferating cells. While this metabolic activity makes thiopurines useful as pharmaceuticals, recently the long-term use of these drugs has been associated with up to a 200-fold increase in the incidence of skin cancer. In contrast to the natural DNA bases, thiopurines and their metabolites have the ability to absorb UVA radiation (320 to 400 nm). This is the major type of UV radiation reaching the Earth's surface and is able to penetrate deep into the replicating dermal layers of the skin. Earlier works have shown that 6tGuo acts as a dangerous photosensitizer, causing DNA damage upon UVA exposure and eventually leading to mutations and skin cancer formation. Surprisingly, the mechanism by which thiopurines induce these mutagenic responses remains unclear. In an effort to better understand the photosensitization mechanism of 6tGuo, we report a detailed kinetic investigation of this metabolite based on advanced photophysical and photochemical techniques. In particular, previous reports suggest that the mutagenicity of 6tGuo may be mediated by its ability to efficiently form the reactive oxygen species, singlet oxygen. Significantly, our re-evaluation of 6tGuo has revealed a singlet oxygen quantum yield more than fourfold lower than that previously reported under similar conditions (13% vs 55%). The potential implications of this new finding will be discussed.

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

***

Phenotypic and genomic characterization of Glucose-6- Phosphate Dehydrogenase deficiency in a Malagasy population

Chinweoke Osigwe, Department of Biochemistry, Case Western Reserve University, College of Arts and Sciences; Seth Schulte[1,2], Julie Rosenjack[2], Melinda Zikursh[2], Rosalind Howe[2], Rajeev Mehlotra[2], Peter A. Zimmerman[2]

Department of Biology[1], Case Western School of Medicine Center for Global Health and Diseases[2]

Glucose-6-Phosphate Dehydrogenase deficiency (*G6PD*d) is caused by specific point mutations and small deletions, which leave the red blood cells (RBCs) vulnerable to oxidative stress. Oxidative stress eventually leads to acute hemolytic anemia (AHA), and can be initiated by consumption of certain foods or drugs. It has been discovered that the administration of the anti-malarial drug Primaquine (PQ) causes AHA in *G6PD*d individuals. About 2.5 and 2 billion people are exposed to *Plasmodium vivax* and *falciparum* respectively, and PQ is important in treating the hypnozoite stage of vivax malaria infection, and eradicating the gametocyte stages of falciparum malaria infection. Thus, *G6PD*d presents an important health concern regarding safe administration of PQ when treating infected individuals in countries such as Madagascar. The purpose of this study is to correlate *G6PD* phenotypes, using the Fluorescent Spot Test (FST), with *G6PD* genomic data using *Illumina Next-Generation* sequencing.

Eighty-five Malagasy blood samples were phenotyped using FST (*Trinity Biotech G6PD* spot test kit). These samples were compared to control samples, which were classified as *G6PD* normal, intermediate, or deficient (*Trinity Biotech*). PCR products from genomic DNA preparations from these samples were subjected to *Illumina* sequencing.

In a subset of 25 samples, FST was able to distinguish the three different phenotypes. The number of normal, intermediate, and deficient individuals were reported to be 13, 6, and 6 respectively. This illustrates FST's ability to characterize *G6PD*d in the Malagasy population. Further experiments are in progress on these samples which involve FST phenotyping and *G6PD* sequencing using *Illumina*.

Project Mentor: Dr. .Peter A. Zimmerman, Center for Global Health and Disease, Case Western School of Medicine

Plasma Synthesis of Nanodiamonds

Joseph Palmeri, Department of Chemical Engineering; Dr. Mohan Sankaran, Department of Chemical Engineering

Nanodiamonds are nanoscale forms of carbon exhibiting structure and properties similar to diamond, one of the hardest and most chemically inert materials on Earth. However, there are significant challenges to synthesizing nanodiamonds. Much like diamond, current methods rely on high pressures and high temperatures to synthesize nanodiamonds. For example, detonation nanodiamonds, as they are known, are produced when a carbon source, usually TNT, is detonated inside of a closed chamber. This process yields a mixture of products, including amorphous carbon soot and nanodiamonds that must be purified to recover the nanodiamonds. In addition to being time consuming and costly, the steps result in significant contamination of the product. Our research aims to design a steady state, continuous, high-yield plasma process where nanodiamonds with controllable properties can be homogeneously nucleated in the gas phase. Preliminary results show that we are able to synthesize material from ethanol vapor at a rate of 0.6mg/hr-0.8mh/hr. Experiments are currently underway to optimize the reactor parameters to control particle size, morphology, and the ratio of diamond to non-diamond products. When fully developed, this novel production method could replace both HPHT and detonation diamond due to the low energy consumption and high control over nanodiamond properties.

Mentor: Dr. Mohan Sankaran, Department of Chemical Engineering

***

Aplysia Californica Buccal Mass Muscle Extraction and Culture Techniques for Development of Biohybrid Devices

Jill Patel, Department of Biochemistry and Psychology; Vickie Webster, Department of Mechanical and Aerospace Engineering; Emma Hawley, Department of Biomedical Engineering; Ozan Akkus, Department of Mechanical and Aerospace Engineering; Hillel J. Chiel, Department of Biology; Roger D. Quinn, Department of Mechanical and Aerospace Engineering

The Aplysia Californica has been studied for years in the field of neuroscience as a concise yet effective model system. The Aplysia's nervous system proves to be an ideal choice with approximately 10,000 large and relatively simple connected neurons. This allows researchers to study physiology, learning, memory, variability, and much more in invertebrates. One benefit of such a nervous system is that the connections between sensory cells, neurons, and muscles, can be more readily explored. In the case of the Aplysia's feeding apparatus, the buccal mass, a simple circuit of neurons protraction and retraction enables the animal to grab and ingest food. Due to the robust nature of these animals, and the level of detail in understanding of the nervous system, Aplysia Californica are a potential source of material for the development of biohybrid robots. In order to develop such biohybrid devices, sterile muscle isolation techniques and culture conditions have been developed. Muscle attachments and actuation has been investigated on untreated culture plates, poly-d-lysine coated culture plates, and Electrochemically Compacted and Aligned Collagen (from bovine source). Wells coated in poly-d-lysine showed the best results, with muscle attaching, contracting, and projecting further into the well. These techniques form the foundation for further development of Aplysia derived biohybrid devices.

Project Mentor: Professor Ozan Akkus, Department of Mechanical and Aerospace Engineering

TRPV2 ion channel interacts with Rab7 to influence endosomal function

Michelle C. Pérez-Ayala, Department of Biology; Jennifer Pilat, Department of Pharmacology; and Matthew Cohen, Department of Pharmacology

Transient receptor potential vanilloid type 2 (TRPV2) is a calcium-permeable intercellular membrane channel linked to spinal cord injury and it plays an important role in neuron, endocrine, immune and cancer cell physiology. Our lab recently discovered that TRPV2 is upregulated by nerve growth factor, a neurotrophin involved in neuronal development, to enhance neurite outgrowth. Furthermore, we found that TRPV2 localizes to Rab7-positive late endosomes in developing neurons. Rab7 is a GTPase that regulates late endosomal formation and facilitates neuronal development. This led us to hypothesize that TRPV2 and Rab7 may form a complex to influence endosomal function. To test this, I performed co-immunoprecipitation experiments to determine if TRPV2 and Rab7 interact in cells. I found that TRPV2 and Rab7 form a weak interaction, suggesting that the interaction may depend on the enzymatic state of Rab7. In the future, I will test if TRPV2 interacts with GDP- or GTP-bound Rab7 using Rab7 mutants.

Project Mentor: Professor Vera Moiseenkova-Bell, Department of Pharmacology

***

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 regulator 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 set small molecule library from The Developmental Therapeutics Program of the National Cancer Institute consisting of 1500 compounds. Five purine based compounds exhibited significant inhibition of PLD at concentrations of 100 ug/mL and 10 ug/mL. Future plans include testing the compounds at lower concentrations and screening for similar purine based compounds.

Project Mentor: Dr. Menachem Shoham, Department of Biochemistry

Dynamics of Cardio-Respiratory Coupling Assessed by Respiratory Sinus Arrhythmia and Cardio-Ventilatory Coupling Before and During Septicemia Endotoxemia in Rats; As Assessed by Respiratory Sinus Arrhythmia and Cardio-Ventilatory Coupling

Ashley Rector, Plant Sustainable Plant Systems/ Medieval and Renaissance Studies; Yee-Hsee HseihHsieh, Division of Pulmonary, Critical Care and Sleep Medicine, Deparartment ofment of Medicineicine, Division of Pulmonary and Critical Care; Frank J. Jacono, Division of Pulmonary, Critical Care & Sleep Medicine, Department of Medicine; and Thomas E Dick, Division of Pulmonary, Critical Care & Sleep Medicine, Department of Medicine.

Current biomarkers of septicemia do not identify a tipping point where a patient may be at risk of sudden deterioration and multiple organ dysfunction. The change in cardiorespiratory coupling during septicemia could be a biomarkers in identifying the transition into this at-risk-state. Respiratory sinus arrhythmia (RSA) a measure of Heart rate variability associated with respiration and cardio-ventilatory coupling (CVC) a measure of the blood pressure influence on the respiratory pattern are independently controlled variables reflecting cardiorespiratory coupling (CRC). RSA decreases during endotoxemia; the change in CVC has not been reported. The goal of this project is to define the dynamics of these measures in endotoxemic conditions. We hypothesized that the RSA and CVC would both decrease simultaneous; specifically both would lose these markers of brainstem function. Blood pressure and ventilatory patterns were simultaneously recorded in chronically instrumented rats (n=6) on Day 0 (Baseline) and on the following 3 days after receiving an intraperitoneal (ip) injection of lipopolysaccharide (24 mg/kg). An additional three rats received saline ip. Cardiorespiratory variables: heart and respiratory rates increased relative to baseline, Surprising, the high-frequency component (0.8-2.5 Hz) of the power spectral analysis of heart rate variability, which reflects RSA varied. It even increased in one rat. Similarly, changes in CVC varied within a rat and between rats in the endotoxemic group. Further analyses need to be completed; in particular, correlating the change in these variables with the presence of inflammatory markers in the brainstem. In summary, the magnitude of RSA and CVC vary in health, these variables can decrease dramatically during endotoxemia. However, we have observed dramatic increases as well. We anticipate that more experiments will reveal different cardiorespiratory control networks during septicemia.

Project Mentor: Professor Thomas E. Dick, Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine. Supported by NS069220

***

Principles of Drug Design: Analysis of Substrate Specificity of Ribonucleotide Reductase

Reena Sheth, Department of Biochemistry; Junye Wang, Department of Biochemistry; Andrew Knappenberger, Department of Biochemistry; Chris Dealwis, Department of Pharmacology; Michael Harris, Department of Biochemistry

Ribonucleotide reductase (RNR) is the enzyme responsible for catalyzing the conversion of ribonucleotides to deoxyribonucleotide triphosphates (dNTPs) for DNA synthesis. Because of its importance in *de novo* DNA synthesis, RNR is a common chemotherapeutic target. However, this chemotherapy is highly toxic and inefficient. In RNR, nucleoside triphosphate effectors allosterically regulate RNR's preference for nucleotide diphosphate substrates. Normal cellular function is reliant on this substrate specificity, because it allows for the enzyme to adjust production of dNTPs in response to intracellular conditions. This project focuses on generating RNR bearing mutations in loop 2 to test the molecular interactions involved between the effector-binding and substrate-binding sites. Loop 2 is a highly conserved region of RNR amongst eukaryotes. However, two differences exist between trypanosome and human loop 2 in the 291 and 294 positions. In order to test how these changes affect substrate interaction, we are generating the mutations N291G and P294K in human RNR protein. Size-exclusion chromatography of initial wild-type RNR protein preparations revealed nucleic acid contamination. When treated with RNase, nucleic acid contamination decreased significantly, but was still present in non-negligible amounts. It was found that performing a streptomycin sulfate cut and an ammonium sulfate cut during preparation yielded optimal purity. Future experiments will utilize these methods to purify RNR bearing the N291G-P294K mutations. The specificity of the mutant will be tested using anion exchange high-performance liquid chromatography (HPLC) and boronate chromatography. By better understanding the physical interactions involved in RNR substrate recognition, we can advance the state of cancer chemotherapy.

Faculty Mentor: Dr. Michael Harris, Department of Biochemistry

Changes in maneuverability of *Manduca sexta* due to full or partial ablation of the hindwing.

Noémie Sierra, B.S. Biology, B.A. Evolutionary Biology, Department of Biology; Jacob Lockey, Department of Biology; Sean Copley, Department of Biology; and Dr. Mark Willis, Department of Biology.

In many insects, the front and hindwings are mechanically coupled to form a single lift generating blade for increased thrust. While not indispensable, the hindwings appear to affect maneuverability of the animal and the ability to execute rapid turning maneuvers necessary for predator evasion. Complete ablation of the hindwing of model species *Manduca* sexta results in an inability to maintain flight during pheromone tracking behaviour. By performing ablations of the hindwing at several levels distal to the body, this study aimed to determine whether the resulting loss of maneuverability was due to a reduction in thrust from the decreased surface area of the wing or to the loss of sensory information transmitted by mechanoreceptors (campaniform sensilla) clustered at the base of the wing and dispersed along the wing veins. We analysed flight trajectories during pheromone tracking in three dimensions using 3D-DLT calibration, and found that individuals with partial ablation of the wing (which reduced lift but left the mechanoreceptors at the base of the wing intact) showed more erratic flight patterns and slower turning maneuvers, while individuals with total ablation of the hindwing resulted in an inability to remain airborne. We conclude that the hindwing of Manduca sexta serves primarily to transmit information about changes in inertial forces experienced during flight to aid in course correction, and changes in information transmitted due to a total loss of these mechanoreceptors may leave the animal unable to make changes in body dynamics necessary to sustain flight.

Project Mentors: Dr. Mark Willis, Department of Biology, Dr. Jessica Fox, Department of Biology.

***

Investigating Specific Binding of hnRNP H to RNA by Monitoring the Intrinsic Fluorescence of Tryptophan

Valerie L. Snyder, Chemistry Department, Edinboro University of Pennsylvania; Srinivas R. Penumutchu, Department of Chemistry, Case Western Reserve University; Blanton S. Tolbert, Department of Chemistry, Case Western Reserve University

Heterogeneous nuclear ribonuclearprotein H (hnRNP H) is involved in alternative splicing. hnRNP H contains three quasi RNA recognition motifs (qRRMs) that have been shown to recognize the G-tracts of both human and viral RNA, including HIV. Fluorescence experiments were conducted to determine the affinity of qRRMs to the G-tract of RNA. The first two domains of hnRNP H were isolated and purified. The plasmid containing the desired proteins were inserted into competent *E. coli* cells, these cells were cultured, grown, and then induced to express the proteins. The protein was purified through a two-step affinity chromatography method and, finally, size exclusion chromatography. SDS-PAGE was run and the protein was shown to be >95% pure. hnRNP H contains two tryptophan (W) residues, the first W residue is located on the first domain, amino acid 14, and the second W residue is located at the linker boundary qRRM1 and qRRM2, amino acid 89. The intrinsic W fluorescence was used to investigate the qRRM12 interactions with different sequences and lengths of RNA. The fluorescence method was set up to excite only the W residues and monitor W photon emission. The purified qRRM12 protein was titrated with six different RNA sequences and binding affinity was calculated from the data. Comparing the sequence AGGGU and GGAGG, hnRNP H has a stronger binding affinity to AGGGU. The sequence AGGGU$(C)_x$AGGGU, as x increases, the binding constant decreases, showing that hnRNP H has a stronger affinity to the longer sequences. The data agree that hnRNP H has a strong binding affinity to the G-tracts of RNA.

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

TRP-V3 candidate for non-selective Ion Channel in Cascade of NLRP3 Inflammasome Complex Activation in Macrophages

Alejandro Sosa, Department of Physiology and Biophysics

Activation of the production of major cytokines, specifically IL-1 family occurs through the innate immune system and is multi-faceted. Formation of the NLRP3 complex to produce Il-18 and IL-1β had canonically been linked to a decrease in calcium concentration in the cell however it was later shown that under particular circumstances there could be an increase in calcium in the cell. This increase in the calcium concentration could be a non-selective ion channel. TRP-V3, a non-selective ion channel, traditionally expressed in keratinocytes was a candidate due to its pH and thermo-sensitivity. Using calcium mobilization assays and western blot protein identification technique, there is suggestive evidence that this channel could be expressed in macrophages and activated by drugs such as Camphor, Drophenine and 2-APB. There are still experiments that need to be performed to further verify that this is the responsible channel in this cascade of NLRP3 formation in macrophages.

Project Mentor: Professor George Dubyak, Department of Physiology and Biophysics and Michael Katsnelson, Department of Physiology and Biophysics

***

Characterizing inducible fibrosis via β-catenin responsive matrix genes

Miarasa Steele, Emily Hamburg-Shields, Gregg DiNuoscio, Dr. Radhika Atit, Department of Biology.
Case Western Reserve University

During skin homeostasis, dermal fibroblasts produce connective tissue of the skin and contribute to scar formation during healing. In fibrosis, fibroblasts produce excess connective matrix proteins, compromising the function of tissues or organs, resulting in disease and death. Previous studies have shown that sustained β-catenin activity in postnatal hypodermal fibroblasts is sufficient for inducing skin fibrosis. Our current research has focused on characterizing our inducible model of fibrosis. In this model, triple transgenic mice are given doxycycline, which triggers the production of stabilized β-catenin that accumulates in the nucleus and results in dermal fibrosis. Analysis of histology, cell death, proliferation, and marker gene expression will reveal stabilized β-catenin activity-dependent fibrosis phenotypes. In addition we will determine which matrix deposition regulating genes and other secreted proteins are direct targets of β-catenin. The genes in question will not only act as biomarkers, but also potentially serve as new targets for future antifibrotic treatments.

Supported by CWRU SDRC Pilot and Feasibility Grant
Project Mentor: Dr. Radhika Atit, Department of Biology

Haltere Role on Gaze Control in Drosophila

Ilakkiya Thanigaivelan, Department of Biology; Shwetha Mureli, Department of Biology; and Dr. Jessica Fox, Department of Biology

Animal behavior requires the integration of multiple sensory information streams. Humans integrate visual and vestibular information to control their gaze. Flies also control gaze using the visual system. In addition, they control gaze using external organs of equilibrium, their halteres. Fruit flies are a model organism for studying sensory integration because halteres are external unlike the vestibular organs of vertebrates. Previous studies have shown that halteres are not are not strictly necessary for visually guided wing steering responses, but their influence over wing-steering behavior can change depending on visual context. In this study, we observed head orientation in tethered flies with complete and partial haltere ablation. Partial haltere ablation involves removing the mass at the edge of the halteres, decreasing the force on the sensory cells at the haltere base without damaging them. Experiments were conducted in open and closed loop conditions. Open loop experiments involve presenting lights to the fly in the form narrow and/or wide bars with different combinations of static and moving patterns. Closed loop experiments are similar to open loop experiments, but in these experiments the fly is allowed to control the narrow bar, the figure, or the wide bar, the ground. The results show that haltere ablation decreases head movement responses to moving figures, but does not affect responses to wide-field motion.

Project Mentor: Dr. Jessica Fox, Department of Biology

***

A Randomized Controlled Trial of Skin to Skin Contact (Kangaroo Care) Effects on Sleep in Premature Infants

Catherine Wilkosz, FPB School of Nursing; Susan M. Ludington-Hoe Department of Nursing; Mark Johnson, University Hospitals; Kathy Morgan, University Hospitals; Judy Gutman, University Hospitals; Mark S. Scher, University Hospitals.

The neonatal intensive care unit (NICU) environment is not conducive to sleep, and infant sleep in incubators is fragmented because sleep contributes to brain maturation, interventions to foster sleep are needed. During skin-to-skin contact (Kangaroo Care) behavioral indicators of Quiet Sleep have been observed, but not confirmed by objective electroencephalographic (EEG) analysis. The study concluded in 2006 but the results have not been published. Publication of the results is the research activity with which I assisted.
Objectives. My purpose was to interpret, analyze, and formulate a manuscript for final publication. The purpose of the study was to determine the effects of skin-to-skin contact (SSC) on EEG-based sleep and cardiorespiratory patterns by comparing SSC sleep to incubator sleep.
Method. A randomized controlled study with 90 preterms (SSC = 50; control = 40) in which SSC infants received 2-3 hours of SSC between feeds after a comparable pretest period in an incubator and control infants remained in an incubator during the 2-3 hours pretest and test periods was completed. In the incubator infants were inclined, prone, and nested; in SSC infants were inclined, prone, and chest-to-chest underneath a blanket.
Results. Quality of Quiet and Active Sleep improved during SSC as shown by a decrease in arousals between the incubator and SSC periods. During SSC, heart and respiratory rates increased as did stability of these measures. The completed manuscript has now been submitted for publication.
Discussion. Sleep during one 2-3 hour session of SSC is better than sleep in the incubator. Practice of undisturbed SSC improves preterm sleep by reducing arousals and promotes cardiorespiratory stability. The data-based manuscript is under review.

Project Mentor: Dr. Susan Ludington, Frances Payne Bolton School of Nursing

Functional Characterization of a Prokaryotic Pentameric Ligand-Gated Ion Channel

Jonathan Yellets, Department of Physiology and Biophysics; and Nicolaus Schmandt Department of Physiology and Biophysics

Erwinia ligand-gated ion channel (ELIC), is a bacterial pentameric ion channel cys-loop receptor, which is activated by primary amines. The membrane protein is a homolog of the eukaryotic nicotinic acetylcholine receptor (nAChR), that responds to the binding of the acetylcholine neurotransmitter. Because of the mechanistic similarity between ELIC and its homolog, there are several structural and pharmacological advancements that can be gained by examining the receptor's functionality. This study explored the functional properties of ELIC by investigating its activation by gamma aminobutyric acid (GABA) and stimulation by a change in pH (pH5). Xenopus oocytes injected with ELIC mRNA were subject to two electrode voltage clamping and titrated with GABA, pH5, and GABA + ph5 buffer solutions to measure modulations in membrane current. Currents were not observed when titrated with pH 5 alone, but currents were observed under GABA, and pH5 + GABA parameters. This suggests that ELIC is not activated by pH, but is activated by GABA, and is furthermore cation selective once activated.

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

Please note:

Research project titles, student names, mentor names and abstracts were submitted by the student researcher. The SOURCE Office cannot ensure the accuracy or omission of information for publication.

The Intersections Poster Session is coordinated by SOURCE (Support of Undergraduate Research & Creative Endeavors) the centralized office for undergraduate research and creative endeavors at Case Western Reserve University. Information on SOURCE can be found at www.case.edu/source. Information on Intersections can be found at http://case.edu/provost/source/intersections/intersections.html. Direct questions to Sheila Pedigo, SOURCE Director by email sheila.pedigo@case.edu or by phone: 216-368-8508.