



**SCIENCE 2009 UNPLUGGED**

**Inaugural  
Undergraduate Research  
Poster Reception Program  
October 15, 2009**

# **Welcome to the Inaugural Science2009 Undergraduate Research Poster Reception at the University of Pittsburgh**

We would like to take this opportunity to thank the undergraduate researchers and their faculty-mentors for sharing their scholarship with the University community at this event.

Undergraduates from the School of Arts and Sciences, the Swanson School of Engineering, the School of Health and Rehabilitation Sciences, the School of Nursing and the Honors College are presenting at this reception.

We hope you enjoy the presentations and take the opportunity to engage our undergraduate researchers in conversation about their work. Inside this program you will find research abstracts written by the undergraduates.

We wish to thank Science2009 for graciously including this undergraduate research event in its 2009 program.

Office of the Provost

University of Pittsburgh

For more information about Undergraduate Research at Pitt, <http://www.pitt.edu/~ugr>

**Omar M. Ayyash**

Department of Biological Sciences

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Faculty mentor: Dr. Lew Jacobson

## OPPOSING SIGNALING PATHWAYS REGULATE AUTOPHAGIC PROTEIN DEGRADATION IN *C. ELEGANS* MUSCLES

Protein degradation in *C. elegans* muscle cells is very tightly controlled by opposing signaling cascades. Excessive FGFR or low IGFR signaling, starvation or denervation all lead to muscle protein breakdown. Here we show that under conditions of activated FGFR, low IGFR activity or mutationally hyperactivated MAP kinase, soluble protein in muscle cytosol is degraded through autophagy. Degradation under these conditions is prevented by a reduction of function mutation in *unc-51* (encodes Atg1 homologue), by RNAi knockdown of *BEC-1* (beclin) or *ATG-7* (Atg7 homologue) or by treatment with N6,N6-dimethyladenosine, a presumed inhibitor of type III PtdIns-3-kinase. We infer that the MAP kinase cascade positively regulates autophagy. By contrast, protein degradation following starvation or disruption of cholinergic signaling (ACh-deficient *cha-1* mutant) is not prevented by interference with autophagy, consistent with the known sensitivity to proteasome inhibitors. The metabolic reserve of protein in muscle can be mobilized by catabolism in response to failure of any one of a variety of signals of 'healthy' conditions. To achieve versatility and flexibility of response, muscle cells evidently use multiple proteolytic systems and regulate them independently by integrating more than one regulatory input to each degradation system.

Funding: NSF MCB-0542355, HHMI, University Honors College

**William R. Barone**

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**VISCOELASTIC PROPERTIES OF THE RAT UTERINE CERVIX IN RESPONSE TO UNCONFINED COMPRESSION**

Preterm labor is the leading cause of neonatal mortality and accounts for 70% of the total cost of neonatal health care. One of the primary causes of preterm labor is premature softening of the cervix. The objective of this study was to understand the normal viscoelastic adaptations of Long-Evans rat cervix during pregnancy to assess its potential as a model for the study of preterm labor. Cervices of virgin and mid-pregnant (15 days) rats were isolated from the uterus and vagina, and transected into distal and proximal portions. A total of four groups were evaluated: Virgin/Distal (n=8), Virgin/Proximal (n=7), Pregnant/Distal (n=6), and Pregnant/Proximal (n=7). Specimens were placed between two custom steel plates and hydrated with a 0.9% saline solution at room temperature. The tissue was tested using an unconfined compression protocol (along the ventral-dorsal direction) that included a preload (0.15N) and 3 trials of a ramp and hold (4 min) test to 20% strain with 30 minutes of recovery between trials. Tissue responses from the 3rd trial were modeled using the quasi-linear viscoelastic (QLV) model. Parameter B, which governs the elastic response increased ~30% by mid-pregnancy when comparing the distal and proximal cervical portions independently ( $p < 0.05$ ). Parameter C, which governs the magnitude of the viscous response, increased by ~160% and ~400% by mid-pregnancy for distal and proximal portions respectively ( $p < 0.05$ ). Similar to humans, the changes in these parameters reveal that the rat cervix undergoes increased viscous behavior by mid-pregnancy most likely associated with remodeling of the fibrillar matrix.

Funding: NIH Grants-R01HD-045590 and K12HD-043441

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**EFFECT OF FIBER CONSUMPTION IN WEIGHT LOSS STUDY PARTICIPANTS**

**Background:** There is evidence to suggest that high fiber intake helps in reducing weight and possibly blood cholesterol.

**Methods:** We conducted a secondary analysis of baseline data from SMART, a behavioral weight loss study. After a 12-hour fast, participants' blood was drawn for the lipid profile. We assessed lipid-lowering medication use and conducted two 24-hour dietary recalls. We used the mean fiber intake value to examine its relationship with serum cholesterol and body mass index (BMI).

**Results:** Our sample (N=210) had a mean BMI  $34 \pm 4.5$ . The mean fiber consumption was 19.7 grams; those identified as having Stage II (BMI 30-34.9) and Stage III (BMI 35-39.9) obesity had the lowest fiber intake. The total serum cholesterol levels were  $<200$  mg/dL; however, these values were likely affected by medication usage since 29 participants (13.8%) reported taking  $\geq 1$  prescribed lipid-lowering medication. While we found no associations between fiber intake and serum cholesterol levels, we observed a negative association between mean fiber intake and BMI ( $r=-0.224$ ,  $p=0.001$ ).

**Conclusion:** Our data revealed no significant relationship between cholesterol and fiber intake which may be due to medications. As expected, participants with a high fiber intake were more likely to have a lower BMI. These results confirm that fiber intake can play a role in maintaining a healthy weight.

**Funding:** 3R01DK071817-04S1 - IMPROVING SELF-MONITORING IN WEIGHT LOSS WITH TECHNOLOGY, A Recovery Act Administrative Supplement Providing Summer Research Experiences for Students

**Rebecca Belan**

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**ENZYMATIC SENSORS FOR DETECTION OF NEUROTRANSMITTERS IN OXYGEN DEPRIVED TISSUE**

Electrochemical detection of neurotransmitters is an important method in understanding the brain. Single carbon fiber electrodes are widely used as their small size allows for detection of neurotransmitters without causing damage to the brain. In conjunction with enzyme trapping redox hydrogels, electrode arrays are especially useful for the detection of species that are not themselves electroactive. To date no array has been made of similar scale to a carbon fiber electrode.

We have been developing a triple-band platinum electrode array with aims of eventual in vivo use. The manufacture process utilizes commonly known lithography techniques in a novel manner where a polymer dagger shape is patterned as a base for the electrode array. To date, we have produced daggers with platinum bands with approximately 10x20um cross sections. Further research focuses on testing the electrodes and improving the design.

Funding: National Institute of Health Grant # MH075989

**Samantha L. Bell**

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**A YEAST-BASED SYSTEM TO CHARACTERIZE THE MALARIAL CHAPERONE, PFHSP70-1**

*Plasmodium falciparum*, the most virulent and deadly malarial parasite, has developed widespread drug resistance to the best antimalarial therapies, forcing researchers to identify new, more effective treatments. Therefore, the identification of new antimalarial targets is critical. There are a multitude of reasons to believe the parasite requires molecular chaperones for survival. Our research has focused on inhibiting one class of chaperones in the parasite, the 70 kDa heat shock protein (Hsp70). However, recent work has shown that putative Hsp70 inhibitors capable of preventing parasite growth have almost no inhibitory effect on the ATPase activity of PfHsp70-1, one of the parasite's six Hsp70s. To overcome this technical dilemma, we have created and transformed a vector carrying the gene for PfHsp70-1 into two different yeast strains, one containing a deletion of SSA1 and SSA2, two yeast Hsp70s, and one expressing a temperature sensitive form of Ssa1p. After ensuring these strains expressed PfHsp70-1, a variety of growth conditions were assayed to mimic stresses in the human host, including oxidative stress and elevated temperatures. Recent observations indicate that PfHsp70-1 complements the growth defects of these strains. To also study the ability of PfHsp70-1 to function in protein folding, a GFP-prion construct has been expressed in these strains. Fluorescent microscopy will show whether or not PfHsp70-1 is capable of preventing the aggregation of the fluorescently-labeled prion. Future plans include the utilization of additional in vitro and in vivo assays to further characterize how PfHsp70-1 allows survival under stress conditions.

Funding: Chancellor's Undergraduate Research Fellowship; HHMI Summer Undergraduate Research Fellowship

**Jessie L. Bobrzynski**

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## VISUALIZING CHANGES IN OAKLAND'S TOPOGRAPHY: A COMPARISON OF HISTORICAL AND MODERN DATA

Human activities have changed the topography of Oakland over the past century. Fortunately, much of the past topography has been preserved in a series of detailed topographic maps published between 1923 and 1937. Methods were developed to extract the elevation data from this historic series. This methodology allows direct comparison between these historical topographic maps to with recent LIDAR data, an emerging topographic mapping tool that captures unprecedented detail. This comparison allows visualization of changes in Oakland's landscape as a result of human activity and geologic processes over the past seventy two years. An accurate accounting of the human impacts allows improved understanding of landscape changes in urban systems and improves our ability to manage urban landscapes.

**Mark J. Brown**

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**CREATION OF STRAINS TO ASSESS THE ROLE OF DIFFERING O-ANTIGEN TYPES AND CHAIN LENGTHS OF SALMONELLA TO FITNESS AGAINST PREDATION BY AMOEBAE**

The O-antigen of Salmonella is composed of polysaccharide subunits that are polymerized to form chains. These chains can vary in types of sugars and linkages (different serovars), and they can also vary in chain length. Several genes control O-antigen chain length: *wzzB* allows for chains of 30-40 monomer chains while *fepE* allows for 100-120 monomer chains. Longer chain lengths allow Salmonella to evade the complement system, giving it a high fitness against a host; however, the shorter of the two classes of O-antigen is constitutively expressed while expression of the longer class is variable. It was therefore proposed that the host's immune system was not primarily responsible for selection of the varying O-antigen chain lengths. In this case, a trade-off in fitness may exist between expressing mainly longer chains to evade the host immune system versus expressing shorter chains to escape recognition by predatory amoebae. By using P1 mediated transduction, knockout mutations in *wzzB*, *fepE*, and both *wzzB* and *fepE* genes were created to vary chain lengths in seven different serovars. Fluorescent-tagging vectors were then electroporated into the mutant strains. The different serovars and chain lengths will then be subjected to predation by various amoeba populations. Flow cytometry will then be used to discriminate numbers of strains surviving predation. The results will determine which serovar and chain lengths were preferentially consumed by each type of amoeba population.

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**EFFECTS OF SEROTONIN TRANSPORTER GENOTYPE ON DEPRESSION IN  
TRAUMATIC BRAIN INJURY**

Genetic variations in the promoter region for the serotonin transporter (5-HTT) modify the rate of transcription, affecting the rate of serotonin uptake. This is associated with the development of depression. The single nucleotide polymorphism (SNP) rs25531 and a length variation within the polymorphic region of 5-HTT (5-HTTLPR) were studied in association with development of depression in 265 persons with severe traumatic brain injury (TBI). Functional and neuropsychological outcomes at 6 and 12 month time points and genotypes were collected. A subset of this population (N=66) was used to assess correlations between PHQ-9 scores, a measure of depression, and combined genotype for rs25531 and 5-HTTLPR, as well as with the presence of the variant allele. Preliminary analysis revealed trends showing the presence of the G allele in the rs25531 and persons homozygous for the short allele, independently, were both associated with greater depressive symptoms. Statistical analysis included chi-squared analyses for categorical data and non-parametric one-way ANOVAs for continuous variables. Further investigation of allelic variation on the development of depression is warranted. Future analysis will include examination of other 5-HTT variants such as the VNTR Intron 2, as well as associations with functional outcomes and other measures of depression. This study may provide a basis for further investigation of the role of genetic variation in 5-HTT or other serotonergic genes as a means for identifying those with a greater risk for the development of post-traumatic depression and treatment response to drugs that act on SERT, such as SSRIs.

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## MOLECULAR CIRCUIT CHARGES: PREDICTING ELECTROSTATICS OF TYPICAL ORGANIC MOLECULES

We have been developing an empirical charge model to predict atomic charges across a variety of molecules. We treat molecules as circuits which distribute charge across bonds based on atomic electronegativities and orbital overlap. We have been parameterizing and testing the fit of our model by comparing the calculated values with known electrostatic potential charges.

Funding: University of Pittsburgh

**Aaron S. Cantor**

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TESTING THE EFFECT OF ALLYL ISOTHIOCYANATE ON MYCORRHIZAL FUNGAL SPORE GERMINATION

Garlic mustard (*Alliaria petiolata*, Brassicaceae), hereafter GM, is a widespread noxious invader of N. American forest understories. Like other members of the mustard family, GM increases soil levels of allyl isothiocyanate (AITC) but at significantly higher concentrations than other mustards. AITC has powerful anti-fungal properties and has been shown to inhibit fungal spore germination. Since 80% of all plant species rely on mutualistic arbuscular mycorrhizal fungi (AMF) in their roots for critical mineral uptake from the soil, AITC has been proposed to be a novel weapon that facilitates the spread of GM within AMF-dependent plant communities. Our previous work demonstrated that AITC levels in GM-infested soil are between 0.005 and 0.009 mM/40 g soil. However, the bioactive (anti-fungal) AITC concentrations that arrest fungal spore germination have not been determined for any AMF species. Therefore, we quantified the allelopathic effect of AITC on the germination of *Glomus clarum*, an AMF species common to N. American understory soils. Our results show that even the lowest concentration of AITC that we detected in GM-invaded soils significantly inhibits the germination of in vitro *G. clarum* spores (ANOVA  $p < 0.0001$ ) and is sufficient to disrupt fungal spore viability in the AMF-plant mutualism.

Funding: Pennsylvania Space Grant Consortium

**Thomas W. Chase**

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**APPLICATION OF THE TRANSVERSELY ISOTROPIC BIPHASIC MODEL TO THE UNCONFINED COMPRESSION OF MANDIBULAR CONDYLAR CARTILAGE**

Tissue engineering of mandibular condylar cartilage (MCC) is a potential treatment for temporomandibular disorders. To characterize how well engineered and native MCC compare, compression tests must be utilized to compare their compressive properties. Because past MCC microscopic investigations determined that collagen fibers exist primarily in a transversely isotropic distribution, our objective was to model MCC behavior under compression using the transversely isotropic biphasic model (TIBM). We performed stress-relaxation tests using unconfined compression on six porcine MCC specimens. The TIBM utilizes five parameters: Young's moduli in the transverse and axial planes ( $E_1$ ,  $E_3$ ), Poisson's ratios in the transverse and axial planes ( $\nu_{21}$ ,  $\nu_{31}$ ), and permeability in the transverse plane ( $k$ ). The TIBM was first applied by setting  $\nu_{31}$  equal to zero and performing a three parameter curve fit using  $E_1$ ,  $\nu_{21}$ , and  $k$ . However, it is highly unlikely that  $\nu_{31}$  equals zero for the MCC since collagen exists in the axial plane. Thus we also used an alternate approach developed by Sergerie et al. involving a four parameter curve fit with  $E_1$ ,  $\nu_{21}$ ,  $\nu_{31}$ , and  $k$ . The two curve fits produced similar sum of squares errors and values for  $k$ , although differed in  $E_1$  and  $\nu_{21}$ . However, standard deviations for the parameters were fairly similar, suggesting that the three parameter fit is not necessarily more accurate than the four parameter fit. Therefore, because the four parameter fit includes  $\nu_{31}$  without sacrificing the accuracy of the curve fit, the four parameter fit seems more appropriate for modeling the MCC under unconfined compression.

Funding: University of Pittsburgh School of Dental Medicine

## **Christine Chen**

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### **NOD1 LIGANDS, BUT NOT NOD2 LIGANDS, POTENTIATE CYTOKINES TO STIMULATE NITRIC OXIDE PRODUCTION AND CHEMOKINE PRODUCTION IN HEPATOCYTES**

Little is known about hepatocyte (HC) responses to NOD ligands and the role of NOD receptors in the liver. In this study, we investigated the role of NOD1 and NOD2 in HC by assessing responses to specific NOD1 and NOD2 agonists.

Isolated primary mouse HC were treated up to 24h with 100ng/mL LPS (TLR4-ligand), 100ng/mL iE-DAP (NOD1 ligand), or 10ug/mL MDP (NOD2 ligand). Other cells were given cytokines IFN $\gamma$ ; (100U/ml), TNF $\alpha$ ; (500U/ml), IL-1 $\beta$ ; (100U/ml), alone and in combination with NOD ligands. Whole cell lysates were collected and immunoblotted for iNOS. Cell supernatants were analyzed by ELISA for chemokines and acute phase proteins and nitrite (NO) concentration determined by Greiss reaction.

iNOS was strongly upregulated in HC by cytokine mix (TNF $\alpha$ ;, IFN $\gamma$ ;, and IL-1 $\beta$ ;) . LPS, iEDAP or MDP alone did not induce NO production or upregulate iNOS. However, iEDAP (NOD1 ligand) significantly potentiated iNOS and NO production induced by cytokine mix (7.47mM $\pm$ 0.03 vs 34.72mM $\pm$ 0.2; p<0.0001). Treatment with iEDAP for 24h also significantly increased RANTES and KC levels compared with LPS (RANTES: 1189 $\pm$ 12pmol vs 508 $\pm$ 10pmol; p<0.05). MDP did not increase chemokine production in HC. Acute phase proteins (SAA and LBP) were also not significantly increased after treatment with MDP or iEDAP.

Our data show that HC respond to NOD1 ligand, iEDAP, to produce chemokines, but do not respond to MDP alone. iEDAP also potentiates cytokine effects, especially IFN $\gamma$ ;, to upregulate iNOS and release NO. These data suggest that HC are programmed to respond to NOD stimulation by attracting and activating leukocytes to a site of infection.

Funding:

**Danielle Chirdon**

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**INFRARED SPECTROSCOPIC ADSORPTION STUDIES- CARBON CAPTURE AND SEQUESTRATION**

In pursuit of a solution to climate changes arising from increased greenhouse gas emissions, materials that can aid in carbon dioxide capture as well as in carbon dioxide sequestration have been probed using infrared spectroscopy. The material related to capture is a nickel-based coordination polymer which shows an unusual dynamic structural response to CO<sub>2</sub> at elevated pressures. At low pressures, the material exists in a structural phase exhibiting low porosity with a low CO<sub>2</sub> adsorption potential. As the pressure of CO<sub>2</sub> is increased above a certain threshold pressure, the material undergoes a structural phase change which permits the rapid uptake of CO<sub>2</sub>. The resulting CO<sub>2</sub> adsorption versus pressure isotherms show a pronounced step-shape and large adsorption/desorption hysteresis. It is shown that the adsorption isotherms measured using attenuated total reflectance (ATR) FT-IR spectroscopy agree well with those measured separately on a volumetric instrument. The structural phases of the material and the effects of these structural phase changes on the CO<sub>2</sub> adsorption potential were monitored in situ by following the shifts in the sorbent and adsorbed CO<sub>2</sub> infrared adsorption spectra. On the subject of sequestration, carbon dioxide sequestration in coal seams has been targeted as a viable option for the mitigation of greenhouse gas emissions. An understanding of the mechanism by which CO<sub>2</sub> is stored in coals would provide a scientific foundation on which to base predictions of long-term CO<sub>2</sub> storage stability. Also of interest is the CO<sub>2</sub> coal seam storage capacity, which is typically estimated from adsorption isotherm measurements by volumetric or manometric techniques. Attenuated total reflectance infrared spectroscopy was used to observe CO<sub>2</sub> interaction with lignite coal from the Plains CO<sub>2</sub> Reduction (PCOR) partnership. The infrared data suggest that CO<sub>2</sub> is physically adsorbed on the coal. Correlation is shown between infrared carbon dioxide adsorption isotherm measurements onto PCOR coal and outside manometric isotherm measurements for the carbon dioxide storage capacity.

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**Kara T. Cohen**

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## AN EVIDENCE-BASED STUDY OF THE EVOLUTIONARY BEHAVIORAL SCIENCES

The evolutionary behavioral sciences are a relatively new subset of psychology that has largely been under heavy-scrutiny since its conception. This inter-disciplinary field, embracing such disciplines as psychology, biology, anthropology, and sociology, to name a few, has offered an especially important evolutionary perspective on the mind. Many critics, though, argue against the methodological approaches used to create hypotheses, claiming they have little to no empirical value and remain ignorant of modern evolutionary biology. In this project, in a novel methodological approach for philosophy, we used quantitative citation analysis, looking at three years of articles in the journal “Evolution and Human Behavior” (2000 through 2002) to see what era of evolutionary biology the authors were citing, along with what other fields the authors chose to reference. Our citation analysis argues against the claims that the evolutionary behavioral sciences are not knowledgeable of the biological sciences, or that they tend to cite out-dated theories, and instead urges philosophers of science to reevaluate the scientific worth of this field.

**Benjamin W. Cross**

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**DOES THE LENGTH OF THE SALMONELLA O-ANTIGEN AFFECT PREDATION BY AMOEBA?**

Serovars of *Salmonella* express different outer-membrane polysaccharides, termed O-antigens. Serovars of *Salmonella* show differential survival in the face of different amoeba predators. This difference is due, in part, to differences in the structure of O-antigen between different *Salmonella* serovars; amoebae more readily consume *Salmonella* with preferred O-antigens. For *Salmonella*, longer O-antigens increase virulence by providing protection against host complement, but the mechanisms that limit antigen length are unknown. We hypothesize that longer O-antigens may be more readily recognized by predators, thereby providing a disadvantage to ever-increasing O-antigen length. Directed gene knockouts were used to create strains of *Salmonella* that differed only in the length of their O-antigen. These mutants were competed in the presence of a protozoan predator. Preliminary results suggested that removing the longest class of O-antigen decreased risk of predation. What is not clear is how length affects predation risks for preferred versus non-preferred O-antigen structures, and how the relative contribution of O-antigen length and identity varies among different predators. Mutations affecting O-antigen length have been moved into seven *Salmonella* serovars via P1 transduction. To allow collection of robust data, fluorescent plasmids have been introduced into each mutant. These strains will allow use of flow cytometry to rapidly and precisely measure the abundance of different strains after competition tests. The feeding preferences of several amoeba species will be characterized based on the length and structure of their prey's O-antigen.

Funding: National Institutes of Health

**Christopher P. Davis**

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**GENETIC ANALYSIS OF THE HISTONE MODIFICATION DOMAIN OF THE PAF1 COMPLEX SUBUNIT RTF1**

Transcription is a crucial step in the production of functional proteins and regulatory RNAs encoded within the genome of an organism. Transcriptional regulators have been shown to cause alterations in chromatin structure through various modifications to the nucleosomal histone proteins, such as acetylation, methylation, and ubiquitylation. My project focuses on the Rtf1 subunit of the Paf1 complex, a group of transcription accessory proteins in *Saccharomyces cerevisiae* that play a role in the regulation of transcription elongation. Particularly, Rtf1 impacts histone H2B monoubiquitylation, which directs methylation of lysine residues in histone H3. Rtf1-dependent histone methylation leads to alterations in chromatin structure, thereby regulating transcription. I have investigated the function of Rtf1 by observing the effects of amino acid substitutions made within Rtf1. Particularly, these substitutions lie within a region known as the histone modification domain (HMD), which is responsible for several phenotypic effects. I have demonstrated that these substitutions in the Rtf1 HMD impair methylation of lysine residues in histone H3 and eliminate monoubiquitylation of histone H2B lysine 123. Strains containing these substitutions also show phenotypes associated with defects in transcription. These mutations do not impair acetylation of histone H3 lysine 14, indicating that Rtf1 does not play a role in this process. This project will provide insight on the mechanisms of histone modification and transcriptional regulation in yeast, leading to further application in biomedical research concerning transcriptional regulation pathways in humans.

Funding: Beckman Foundation and National Institutes of Health

**Laura Dempsey**

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THE ROLE OF VARYING FIBRONECTIN CONCENTRATION ON CELL MIGRATION:  
THE RESPONSE OF ALPHA-ACTININ AND PAXILLIN IN GASTRULATING XENOPUS  
LAEVIS EPITHELIAL CELL SHEETS.

How and to what cells adhere can drastically affect their function and state. Cells either adhere to other cells or to an extracellular matrix (ECM). In doing so, they are capable of undergoing adhesion-driven morphogenesis or oncogenesis. Studying the spreading of an epithelial cell sheet on fibronectin, an ECM protein, can elucidate the reasons for substrate choice as well as factors influencing morphogenesis as a whole. *Xenopus laevis* was used as a model organism to study cell migration and cell-ECM adhesions during the embryo's early stages. Essential to the focal adhesions that allow cell movement are two specific molecules;  $\alpha$ -actinin, an anti-parallel homodimer protein that crosslinks and organizes actin filaments, and paxillin, a scaffold protein that aids in signal transduction necessary for cell movement. Varying the concentration of fibronectin and observing cell behavior can potentially elucidate the mechanism and role of  $\alpha$ -actinin and paxillin in cell motility. 1-cell stage embryos will be microinjected with fluorescently-tagged mRNA encoding for  $\alpha$ -actinin and paxillin. Using micromanipulation techniques, animal caps preceding gastrulation will be placed on a surface coated with a specific concentration of fibronectin. The behavior of the tissue during gastrulation will be observed via time-lapse movies using confocal and light microscopy. These movies will be analyzed using measurement and elastic registration tools in ImageJ for any trends arising from modification of the substrate concentration. Results will be interpreted using ANOVA and the Mann-Whitney U test. Strain, rate and force relationships will be the focus of an overall biomechanical analysis.

Funding: NSF CAREER Award to Lance Davidson

**Lora E. Deutch**

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**CASPASE ACTIVATION IN EXPERIMENTAL INTRAUTERINE INFLAMMATION IN MICE**

Intrauterine inflammation has been implicated in developmental brain injuries. In this process, oligodendrocytes are injured and undergo cell death. In rats, apoptotic cell death through activation of the extrinsic caspase pathway by Fas-receptor related mechanisms has been reported. To investigate the role of the extrinsic pathway in inducing apoptotic cell death, a mouse model of injury was developed and caspase activation was studied.

We hypothesize that caspase activation plays a role in cell death related to intrauterine inflammation in mice.

C57Bl6 mice (E15) were injected intracervically with LPS (0.1 mg/kg) or saline. Animals were sacrificed at 1, 4, 6, and 24 h. Fetal brains were removed and homogenates were made. Caspase-8 protein concentrations were determined by Western and caspase activity (Caspase-3, -8 and -9) was determined. Caspase activity after LPS was compared to shams and % change was calculated.

Pro-caspase-8 was cleaved in all LPS-treated pups at 1 and 24 h, but not in shams. Caspase-3 activity was increased at 1, 4 and 24 h ( $13\% \pm 0.5$ ,  $28\% \pm 7$ ,  $27\% \pm 2$  respectively). Caspase-9 activity increased at 4 and 24 h ( $49\pm 6\%$  and  $27\pm 4\%$ , respectively). Caspase-8 activity was increased at 1 and 4 h ( $21.3 \pm 0.5$  and  $19.5 \pm 1$  respectively).

We conclude that caspase activation occurs after experimental intrauterine inflammation and it appears that caspase-8 may play an important role in the pathogenesis. Since Fas activated cell death is caspase-8 dependent, it is possible that Fas knockout mice will be protected in this model.

Funding: United Cerebral Palsy Foundation

**Melissa B. Evans**

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Faculty mentor: Dr. Judith Erlen

**SELF REPORTED EXPERIENCE OF HIV PATIENTS IN A TELEPHONE-DELIVERED  
MEDICATION ADHERENCE INTERVENTION**

Important information is obtained by evaluating participants' experience in clinical trials. This information is useful for investigators to improve the quality of research and of interventions. This study examines the experience of 181 participants who completed a NIH funded clinical trial (R01 NR04749) who were randomized to receive either adherence counseling or usual care. All participants were monitored for adherence with MEMs electronic medication pill bottles, kept paper and pencil diaries, completed several questionnaire assessments, and concluded the study with an exit interview. Two raters evaluated an open-ended exit interviews to assess participants' satisfaction with the study, and their perception of study benefits. Responses were rated as positive, negative or neutral. Ratings were compared and disagreements between the two raters were reconciled by a third rater.

Results show that the majority (82%) of participants reported a positive experience with the overall study. Participants in the individualized intervention reported the most positive (90%) and negative (7%) feelings in response to overall experience in the study. Those in the individualized intervention reported the most emotional benefit (46%) from the study, while those in the structured group volunteered most often improved medication adherence (65%) as a study benefit. Notably, more usual care participants (17%) reported the diary as a benefit than any other group (7% individualized, 9% structured).

Limitations to the current study include the lack of assessment of study dropouts' perceptions, and the length of time from the intervention to the exit assessment.

Funding: National Institutes of Health

**Brandon D. Fields**

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Faculty mentor: Dr. Lew Jacobson

**DISRUPTION OF A MUSCLE ADHESION COMPLEX CAUSES MUSCLE PROTEIN DEGRADATION IN C. ELEGANS**

About one hundred “muscle” genes of *C. elegans* showed decreased expression levels after prolonged spaceflight. One of these genes encodes a member of an integrin-containing, transmembrane attachment complex that anchors the muscle contractile fibers to the hypodermis, and is highly homologous to human focal adhesion complexes. Knockdown via mutation (lowers protein function) or RNA interference (lowers amount of protein) of any one of eleven genes encoding members of this complex provokes protein degradation in muscle cytosol. This result was consistent for genes whose products reside inside the muscle cell, in the extracellular matrix, or traverse the muscle PM. Inhibition of either the proteasome or autophagy via drugs or RNAi failed to prevent protein degradation in attachment-disrupted mutants, suggesting that a novel protease or a combination of proteases is responsible. Partial inhibition of degradation resulted when amounts or functions of other cellular proteases such as calpains, cathepsins, or caspases were decreased individually or in combination, again suggesting the involvement of multiple proteases.

In addition to muscle protein degradation, which begins about 18-24 hours after acute disruption of the attachment complex, paralysis occurs in at least one attachment mutant within 24 hours of disruption. Starvation protects against paralysis in these mutants, but cannot rescue a pre-existing paralyzed phenotype. Starvation may be interfering with the activity of the protease(s) itself or with the signaling between the attachment complex and the protease(s). These observations may be useful in our future attempts to identify the protease(s) and how it functions.

Funding: NIH AR-054342; HHMI undergraduate fellowships

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**YKL-40 EXPRESSION IN TRAUMATIC BRAIN INJURY**

It is estimated that 1.4 million people/year in the United States suffer a traumatic brain injury (TBI). After TBI, there is acute inflammation with local cytokine release, reactive astrogliosis, infiltration of activated macrophages/microglia, and necrotic and apoptotic cells.

The controlled cortical impact (CCI) rat model of TBI was used to study expression of chitinase-like protein YKL-40, believed to be involved in neurodegeneration. Upregulation of YKL-40 has been reported in inflamed tissue, such as rheumatoid arthritis and several carcinomas. The biological function of YKL-40 is mostly unknown.

We hypothesized that YKL-40 is expressed by astrocytes and macrophages in the local injured region as part of the acute inflammatory response. In order to study the time-course of YKL-40 expression, tissues from 1-12 days post-injury were processed for immunohistochemistry or mRNA analysis. To determine which cells express YKL-40, coronal sections were dual-stained with antibodies to YKL-40 and either GFAP (astrocytes), CD68 (macrophages), or NeuN (neurons), respectively. Immunofluorescence and mRNA expression was quantified using laser confocal microscopy or RT-PCR, respectively. In human CSF from TBI patients, YKL-40 concentration was measured using enzyme-linked immunosorbent assay (ELISA).

Real-time RT-PCR results showed similar expression at days 1 and 2 post-TBI. YKL-40 immunofluorescent staining showed peak expression at 2-3 days post-CCI with diminished expression thereafter. Most YKL-40 staining was present in astrocytes. In humans, CSF YKL-40 is increased at least 5 days post-injury. In conclusion, the rat model shows acute YKL-40 expression localized to the injury site that rapidly diminishes, while humans exhibit longer duration of expression.

Funding: Midcareer Investigator Award (NIH K24-MH01717) and SIV Encephalitis and Disease Progression NIH (RO1 MH071151)

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**SIMULTANEOUS CONTROLLED RELEASE OF DOPAMINE AND DEXAMETHASONE  
VIA NANOPOROUS POLYPYRROLE**

Implantable micro-electrical devices that are directly placed in the brain can be used to record neural activity and deliver neurochemicals to modulate neural activity at high spatial and temporal resolution. Such devices offer great research and therapeutic potential, but the inflammatory host tissue response interferes with the device function in long-term. In this work, we report the development of an electrically controlled system for simultaneous release of dexamethasone (Dex) and dopamine. Dex, an anti-inflammatory drug, can be released to reduce the inflammatory response. Dopamine is a neural modulator and a potential therapeutic treatment for a variety of neurological disorders. Nanoporous polypyrrole (PPy) doped with Dex was electropolymerized on the electrode surface. Dopamine was then loaded into the nanopores and the PPy/Dex/Dopamine construct was capped with an additional PPy/Dex film. A variety of electrical release stimuli were applied, including cyclic voltammetry, constant negative potential, and pulse trains of positive and negative potentials. The drug release was quantified using UV spectroscopy. It was found that simultaneous release of both Dex and dopamine can be achieved across a range of stimuli and the ratio between two drug releases may be controlled by varying the stimulus type.

Funding: NSF Grant 0729869, NSF Career Award DMR-0748001, and the University Honors College of the University of Pittsburgh

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**ECORI ENDONUCLEASE MUTANT E111A DEMONSTRATES INCREASED BINDING AFFINITY, DEFECTIVE CLEAVAGE**

Recognition and modification of specific DNA sequences by proteins are central to most biological processes. Restriction endonucleases (REases) provide excellent models for determining the structural and energetic factors governing sequence specificity. Like other REases, EcoRI uses aspartic and glutamic residues to coordinate the catalytic cofactor, Mg<sup>2+</sup>, but the active site is only assembled upon insertion of the scissile phosphate into the negatively charged cluster. To probe the role of electrostatic repulsion in the active site and the energetics of specific binding by EcoRI, a mutation of a key residue in the active site was introduced. Mutant E111A was generated by site-directed mutagenesis, over-expressed and purified to 95% homogeneity. Nitrocellulose filter-binding assays revealed that E111A binds 40-50 fold better to the cognate site than wild-type protein. Thus removal of a negative charge in the active site results in a strong enhancement of binding by alleviating electrostatic strain. The repulsive interactions of the negatively charged cluster of the active site are unfavorable for EcoRI binding, and destabilize the ground-state of the protein-DNA complex, while strongly favoring coordination of metal cations and progression to the transition state. The first-order cleavage rate constant for E111A is approximately  $1 \times 10^5$  fold lower than that of wild type enzyme, highlighting the critical role of the negative charge of E111 in catalysis.

Funding: HHMI Summer Undergraduate Research Fellowship

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**PRESSURE ULCER PREVENTION: EXAMINING THE RELATIVE EFFECTS OF PRESSURE, SHEAR, AND SKIN TEMPERATURE**

Pressure ulcers are prevalent in people with limited mobility. Increased pressure, shear force, and skin temperatures are extrinsic causative factors for pressure ulcers. Previous animal studies discovered that shear force combined with pressure caused skin breakdown quicker than with pressure alone, and local cooling of the skin could reduce severity of tissue damage. The objective of this pilot study was to estimate the combination effect of pressure, shear force and temperature to healthy human skin. One subject was recruited, and the study used a repeated measures design with 18 combinations consisting of pressure (60 and 100 mmHg), shear forces (0, .98, and 1.96 N), and temperature (28, 32, and 36 °C). Reactive hyperemia is a normal blood flow response that occurs after tissue ischemia and was the main outcome of this study. Laser Doppler flowmetry was used to measure this response non-invasively, and short-time Fourier transform was used to decompose the blood flow signal to investigate the underlying mechanisms. We found that the combination of largest pressure, shear force, and temperature induced the biggest reactive hyperemia. This indicated that the largest pressure, in combination with shear force, under highest temperature caused maximum vasodilation upon pressure relief. This may be due to the increased accumulation of metabolic vasodilation substances during tissue ischemia and the increased smooth muscle relaxation following pressure relief. More subjects and trials will be performed in the future to fully understand the effect of pressure in combination with shear force and skin temperature.

Funding: National Science Foundation, Project EEC 0552351

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**COMPARATIVE MAPPING OF SEX CHROMOSOMES IN TWO SPECIES OF WILD STRAWBERRY**

The majority of flowering plant species are hermaphroditic- their flowers have both male and female reproductive structures. However, dioecy (having separate male and female individuals) has evolved many times among angiosperms. *Fragaria virginiana*, a species of strawberry, is a compelling model for studying the early steps in the evolution of dioecy due to its intermediate, subdioecious sexual system: females, males, hermaphrodites and occasional neuters coexist in wild populations. This sexual system is accounted for by a genetic map of *F. virginiana*, which has revealed a proto-sex chromosome that possesses two closely linked but still recombining loci that control male and female function, respectively. *Fragaria chiloensis*, a close relative of *F. virginiana*, has well-defined male and female individuals. We are interested in whether the sex chromosome of *F. chiloensis* shares an evolutionary history with that of *F. virginiana*. To examine this possibility, we have created a mapping population of *F. chiloensis* individuals that we are screening with microsatellite markers from the existing *F. virginiana* genetic map. In a preliminary PCR assay, 19 out of 26 primer pairs that produced mappable markers in *F. virginiana* also produced products in the parents of the *F. chiloensis* mapping population, indicating a high degree of marker transferability between species. We will also assess sex phenotype and map male and female function as phenotypic traits. Significant homology between the sex chromosomes of *F. virginiana* and *F. chiloensis* would indicate that the two species are at different points on the same continuum of evolving separate sexes and provide a novel system for studying the molecular evolution of sex chromosomes.

Funding: Howard Hughes Medical Institute

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**BIOREACTOR FOR VISUALIZING TUMOR CELL INVASION**

Bioreactors are useful experimental systems for reconstructing tissue from cells to perform research on disease. Current bioreactor designs made for visualizing chemotactic invasion of tumor cells are characterized by transwell chambers. These models suffer from irreproducibility, non-uniform invasion, and are confounded by deformability property of cells. Based on the inadequacies of existing bioreactors, a new model has been developed to allow for invasion of multiple cell types and ability to collect cell migration rates.

The new bioreactor design is made from two optical surfaces on a support with a set of nutrient perfusion fibers running between them. Ports for introduction of cells are placed in the corners of the top optical surface. Care is taken to assure that the nutrient perfusion fibers are not under the cell introduction ports. A central port is provided for addition of chemoattractant if desired. This bioreactor design can be used for any cell migration study.

A typical experiment begins by inoculating the bioreactor with a biomatrix of collagen and placing it in a sterile flow circuit in an incubator. About .05 mL of each cell type expressing fluorescent marker are seeded in the cell ports. Simultaneous fluorescence microscope pictures are taken every 6 hours for 48 hours to identify the invading front of tumor cells. Initial results show cell movement towards nutrient perfusion fibers containing chemoattractant media after 24 hours and 48 hours. Based on initial experimentation, this bioreactor can be used to evaluate four different cancer cell lines at one time.

Funding: Department of Bioengineering, University of Pittsburgh

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**DOES THE SEX CHROMOSOME INFLUENCE MALE QUALITY IN WILD STRAWBERRY?**

Accompanied by the development of sex chromosomes, one of the major evolutionary transitions in plants is dioecy, the separation of sexes, from hermaphroditism. While most flowering plants possess both male and female reproductive functions, only seven percent have separate sexes, suggesting an advantage to such a separation under certain conditions. As specific traits beneficial to only one sex evolve to have sex-limited expression, sexual dimorphism results. In the sexually dimorphic strawberry, *Fragaria virginiana*, females coexist with hermaphroditic pollen-bearing morphs, which vary in their ability to set fruit. A linkage group housing one locus that codes for male sterility and one for female fertility has been identified. It is hypothesized that the region determining pollen quality is closely associated with the regions linked to sex determination. A set of pollen-producing plants with known differences at the putative female fertility locus were screened for differences in three measurable features of pollen quality: pollen germination, pollen tube growth rate, and fertilization success. Our work demonstrated dramatically that pollen-producing plants differing at the putative female fertility locus differed in pollen quality. Under equivalent pollen application rates, pollen from 'gg' plants germinated at a 17% greater rate, grew faster, and thus a greater proportion (35%) reached the bottom of the style than 'GG' plants. Overall, 'gg' plants had 13% higher realized fertilization success than 'GG' plants. This data provides compelling evidence for a selective disadvantage to pollen carrying the female fertility 'allele' relative to that carrying the female sterility allele.

Funding: Howard Hughes Medical Institute

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## SILICA-BOUND NITROXIDES FOR ALCOHOL OXIDATIONS

Amino TMIO (5-Amino-1,1,3,3-tetramethylisoindolin-2-yloxy) and 4-Amino TEMPO (4-Amino-2,2,6,6-tetramethylpiperidine-1-oxyl) were attached to Silica through an isocyanate linker. The major motivations for this modification were the ease of recycling of these catalysts. The silica-conjugates were tested for the oxidation of 4-nitrobenzyl alcohol to 4-nitrobenzaldehyde. A future goal is the use of similarly modified nanoparticles as a free-radical scavengers.

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**Structure-Function Studies of Mucolipins**

Ion channels are membrane proteins found in all biological cells. These proteins form a pore which controls ion flow, affects membrane potential, and modulates local ion concentration. Recently, ion channels were shown to regulate membrane traffic. Our lab studies a subfamily of Transient Receptor Potential (TRP) ion channels, TRPML. This channel is non-selectively permeable to cations and inactivation of TRPML1 results in membrane trafficking defects and utilization, resulting in a neurodegenerative disease, Mucopolipidosis Type IV. In order to understand these trafficking defects, we must first understand the structure and function of this channel. Using mutagenesis and patch clamp analysis, we have identified key pore and voltage sensing residues in both TRPML1 and TRPML3. We have also confirmed a constitutively active mutant, A419P, which unlike wildtype TRPML, is insensitive to copper inhibition, therefore this mutation causes a change in the structural regulation of this channel. With these data we have begun to establish a relationship between the structure and function of the TRPML channels.

Funding: NIH, NSF, HHMI

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**MINI-STRUCTURAL GENOMICS USING MYCOBACTERIOPHAGES**

Every year 8 million people contract tuberculosis, 2 million of whom die. In most parts of the world there are strains of *Mycobacterium tuberculosis*, the causative agent of TB, that have developed resistance to some, or even all drugs currently used to treat the disease. There are Mycobacteriophages that specifically infect and kill this pathogen, and we have discovered, isolated, and sequenced the entire genome of sixty of these phages. We hypothesize that somewhere within each of these genomes there is at least one protein that confers the toxicity of the phage to the Mycobacterium. Such proteins could be useful targets for new anti-TB drugs. In an attempt to find and further study these targets we have isolated and purified proteins for crystallization and structural analysis by X-ray diffraction. One particular protein is from a phage named Pukovnik and is named gene product 37 (gp37). This protein has been purified, crystallized, and has diffracted x-rays to a resolution of 2.2 angstroms. Seleno-methionine-labeled protein will next be used for a method called anomalous dispersion to determine the structure of gp37. We have also identified and cloned two proteins from a phage called L5 that have been shown to be toxic to mycobacteria. Purification and crystallization will soon be attempted for these proteins. Additionally, ~85% of these proteins have no detectable sequence homology to proteins of known structure, which can make this collection of proteins a valuable reservoir of new protein folds. The identification of novel folds would benefit the structural, as well as the bioinformatic, and protein data base communities.

Funding: Howard Hughes Medical Institute

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**INTERSPECIFIC CO-FLOWERING PRAIRIE PLANTS COMPETE FOR POLLINATORS**

Habitat fragmentation of native prairies and the introduction of non-native species threaten populations of native plant and animal species by reducing mating possibilities and increasing interspecific competition. In this study, our objective was to measure the effect that the species flowering simultaneously with purple coneflower (*Echinacea angustifolia*) have on pollinator visitation in 10 various sized prairie remnants. We honed in on the most abundant native and exotic co-flowering species within the floral neighborhoods of purple coneflower: leadplant (*Amorpha canescens*), a common native prairie legume, and alfalfa (*Medicago sativa*), a common exotic legume introduced for use as animal feed. Our results show that the presence of alfalfa and purple coneflower within purple coneflower's floral neighborhood both had positive effects on pollinator visitation, while the presence of leadplant had a negative effect. Still, there is no evidence that either alfalfa and purple coneflower or leadplant and purple coneflower interact in their effect on pollinator visits.

Funding: National Science Foundation, Chicago Botanic Garden REU program

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**HAPTOGLOBIN GENOTYPE AND MORTALITY AFTER SUBARACHNOID  
HEMORRHAGE**

Background: Haptoglobin binds hemoglobin, thereby inhibiting free radical production. It is presumed that haptoglobin  $\alpha_2$ - $\alpha_2$  genotype is associated with worse functional outcome after aSAH related to its isoform's weaker affinity for hemoglobin binding.

Objective: The objective is to describe the relationship between haptoglobin genotype and functional outcome 3 months after aSAH.

Methods: A total of 192 subjects age 18-75 with a diagnosis of aSAH, Fisher Grade  $\geq 2$ , DNA and outcome data available and without pre-existing chronic neurologic disease/deficit were enrolled into an ongoing study (NR004339). Demographic and medical condition variables were extracted from medical records. Modified Rankin Score (MRS) was assessed at 3 months after hemorrhage. Data analysis included univariate analysis as well as multivariate logistic regression, controlling for covariates including age, sex, and severity of hemorrhage (Fisher grade).

Results: The sample was primarily female (n=137; 71.4%) with a mean age of 54.44 years old. The study was limited to Caucasians due to significant allele frequency differences noted between Caucasians and non-Caucasians and the insufficient sample size in non-Caucasians. Haptoglobin genotype was not significantly correlated with mortality, as defined by a MRS of 6 neither with univariate analysis (p=.40) nor after controlling for covariates (p=.50).

Conclusions: Haptoglobin genotype is not a significant predictor of mortality after aSAH. Further work should explore genetic variations in haptoglobin alleles related to recovery from aSAH.

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## THE RATIONAL DESIGN OF POLYMER MICROSPHERES FOR CONTROLLED DRUG RELEASE

For many chronically administered therapeutic drugs, optimal performance can be achieved with a consistent and sustained dosing. Such dosing would maintain serum drug concentrations within the therapeutic window, high enough concentrations to be effective, yet low enough to avoid toxicity. To achieve this constant bioavailability, daily or regular oral-administration is often employed. However, macromolecule therapeutics are not adsorbed through the intestinal lining, necessitating regular injections instead of ingestion, which drastically reduces patient compliance and quality of life. Our research focusing on the rational development of biodegradable polymer microspheres for the extended release of macromolecules aims to increase patient compliance and quality of life through reducing the frequency of injections.

We have explored several strategies for sustaining a constant rate of macromolecule delivery. Initial mathematical investigation of surface eroding polyanhydrides indicated unsustainable release, particularly with particles suitable for injection. Following this, PLGA polymer blend microparticles, which showed great potential for calculable sustained release, were examined, but partial miscibility between polymers proved to make predictions extremely difficult, and thus impractical. This second approach led to a simpler utilization of polymer microspheres, mixing microparticles made of different types of polymer rather than attempting to make particles each composed of multiple polymers. This simpler process requires minor mathematical modeling in order to tailor the mix of microparticles for a specific release profile. The product is an injectable vehicle for controlled drug release using FDA approved biodegradable materials. The strategy has been tested with a formulation designed for constant drug delivery over the course of a month with promising results, providing a working strategy for a macromolecule release platform.

**Matthew Keddie**

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**TAILORING THE VOLUMETRIC PARAMETERS OF NITROGEN-DOPED CARBON NANOTUBE CUPS**

The synthesis of nitrogen-doped carbon nanotube cups through chemical vapor deposition (CVD) has created an intriguing form of carbon nanostructures with potentially useful applications in energy and medicine. By performing CVD with iron catalyst, ethanol, and a nitrogen source of acetonitrile, graphitic multiwalled structures are synthesized in a “stacked-cup” conformation, capable of separation through mechanical grinding. Additionally, the presence of a nitrogen precursor during growth results in numerous nitrogen functionalities as an intrinsic characteristic of nanocups. We have observed through scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) that the diameter of such nanostructures is contingent upon a proportional catalytic nanoparticle diameter. Furthermore, segment length appears to be heavily influenced by nitrogen concentration. Based upon these principles, we have tailored the physical parameters of individual segments through the controlled synthesis of monodispersed diameter distributions of iron nanoparticles. Additional work will be performed to increase the control of volumetric parameters by nitrogen doping. This work serves as a foundation for understanding and controlling the growth mechanism of a novel nanomaterial for engineered systems, such as storage media and biomedical applications.

Funding: The National Energy Technology Laboratory

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**INTERACTION ANALYSIS OF FBXW7, A LIGASE POTENTIALLY INVOLVED IN THE REGULATION OF TBX6**

T-box transcription factors are a family of proteins expressed at specific times and places during the development of various vertebrates. One family member, Tbx6, is expressed in the primitive streak and in cells migrating out, which will later form the mesodermal somites. Tbx6 mRNA and protein are rapidly down-regulated as the somites form. The suggested mechanism for the down regulation of Tbx6 is ubiquitin-mediated proteolysis. Previous work suggested that Tbx6 interacts with the E3 ubiquitin ligase, Fbxw7 that targets substrates for ubiquitination. We used the GAL4-Yeast Two Hybrid (Y2H) screening system to verify whether or not Tbx6 and Fbxw7 interact. The Y2H system is a stringent test to identify potential protein interactions using the eukaryotic yeast model organism. The system uses the GAL4 promoter fused to a reporter gene, adenine, which is only transcribed if there is a protein interaction. To use the Y2H test, we fused the Gal4 DNA-binding domain to the Tbx6 protein and fused the Gal4 DNA-activating domain to the human Fbxw7 protein. Our results indicated no adenine production when Tbx6 and Fbxw7 were transformed and thus, refuted the potential interaction. Future work will study other proteins that may down-regulate Tbx6. Only when we understand how Tbx6 is controlled can we analyze its effects on proper development.

Funding: Howard Hughes Medical Institute

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**POLYMER BASED SILICA COATINGS**

Coatings are usually made of a solution, water in most cases, and particles such as silica. When this coating dries and solution leaves, the particles left behind are caught on the surface of the substrate with binder. Properties of the coating are thus directly related to how the particles are positioned and how they got there. The main regions or pathways that they take are evaporation, sedimentation, and diffusion. In this project, a new experiment is done where the coating is made of mostly a polyvinyl alcohol and water solution rather than pure water. The polymer chains complicate the process because they do not evaporate like water does when the coating dries but rather are entangled with the particles in the dry coating. The project looks at how the polymer behaves in the above three pathways, how the particles interact with it, and the properties of this new polymer based coating. To begin, the polymer solution is made and its viscosity is measured for various polymer concentrations. This is used to find important relationships like the Peclet and Sedimentation numbers. Through previous work, a drying map has been developed that predicts the drying behavior of a monodispersed particulate system containing no soluble polymer based on these Peclet and sedimentation numbers. The regions in which the particles could lay on the drying map are evaporation, sedimentation, and diffusion. Experiments are run to see this in actuality at different regions of drying in the coating through different pathways and conditions. To image this, the sample can be frozen in liquid nitrogen and broken to be analyzed microscopically at the cross section with a Scanning Electron Microscope (SEM). The silica made was around 200 nm, 500 nm, and 1  $\mu\text{m}$  in diameter and the polymer will have a certain viscosity based on the concentration of the coating. Standards such as these are needed in order to have only the desired effect changing so that an accurate analysis can be made and the behavior predicted. Also with these standards it will be possible to see when samples move between the regions or pathways such as from evaporation to diffusion.

Funding: U of MN Materials Research Science and Engineering Center (MRSEC)

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**IDENTIFICATION AND ANALYSIS OF A NOVEL TRPM8 SPLICE VARIANT USING A RAT NEUROPATHIC PAIN MODEL**

Neuropathic pain is a type of chronic pain that afflicts nearly four million in the United States alone. Our studies and others' suggest that transient receptor potential (TRP) splice variants may contribute to pain hypersensitivity associated with neuropathic pain conditions. TRP genes code for calcium channels that act as an interface between the environment and nervous system. Through alternative splicing, functionally distinct protein variants are produced from a single TRP gene. Alternative splicing is a major source of protein variation in humans, and splice variants have been linked to various diseases. This study utilized a rat neuropathic pain model to mimic a human chronic pain condition. By performing quantitative polymerase chain reaction (qPCR) and 5' rapid amplification of cDNA ends (5' RACE), we have demonstrated the presence of a novel TRPM8 splice variant in rat. This discovery may help to elucidate the role of TRP splice variants in neuropathic pain.

Funding: Grants to John Pollock from the Samuel and Emma Winters Foundation and the Hunkele Dreaded Disease Award

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**POSH PROTEIN SCAFFOLD MUTANT ANALYSIS**

During the development of a simple, single-celled embryo into an elaborate, multi-cellular organism many processes, like migration, programmed cell death, and morphogenesis, are regulated by multiprotein signaling pathways. To keep the components of these pathways organized and properly functioning, cells often utilize scaffolding proteins. Scaffolding proteins aid in assembling the elements of signal transduction pathways, localizing components of the signaling complex to certain regions within the cell, or even eliminating interactions from other pathways that may alter the specificity or integrity of the signal. For example, POSH (Plenty Of SH3 domains) serves as a platform for the assembly of the Jun N-terminal Kinase (JNK) signaling components and is proposed to stimulate JNK signaling and direct it selectively towards cell death. Proper regulation of this pathway by POSH is thought to be necessary for apoptosis in response to death cues, while improper regulation gives rise to inappropriate cell death, like that observed in neurodegenerative diseases such as Parkinson's and Alzheimer's. We have observed that POSH misexpression in *Drosophila* results in epithelial defects associated with an increase in programmed cell death. We hypothesize that a loss-of-function POSH mutant would cause a reduction of programmed cell death, resulting in either delayed apoptosis or the presence of extra cells. In tissues normally fated to die during development, like the larval salivary glands and inter-ommatidial cells of the pupal retina, we will look for the presence of extra cells or the delay of tissue degradation to signify a decrease in programmed cell death.

Funding: National Institutes of Health

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NOVEL SYNTHESIS OF PYRROLODIAZEPINE SCAFFOLDS BY A SPONTANEOUS  
RETRO-MANNICH DOMINO REACTION

Microwave irradiation facilitated the synthesis of 4-arylthio-3-oxazolin-5-ones from ethyl cyanoformate, thiophenol, and ketones. Subsequent decarboxylation and in situ [3+2] cycloaddition provided novel 2,3,4,5-tetrahydro-1H-pyrrolo[1,2-c][1,3]diazepine scaffolds after a spontaneous retro-Mannich domino reaction.

Funding: National Institutes of Health (P50-GM067082)

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INVESTIGATION OF METHANE HYDRATES: TAKING STRIDES TOWARDS THE FUTURE

With the depletion of fuel sources, countries are turning to research in order to discover a novel and efficient fuel source. One leader in alternative fuel sources currently being investigated at the National Energy Technology Laboratory (NETL) is methane hydrates.

Methane hydrates are a type of clathrate, a chemical compound consisting of a lattice of one molecule trapping and containing a second molecule. The structure of the hydrates consists of one methane molecule that is trapped by a crystalline structure similar to ice. One can find natural deposits of methane hydrate in two very different environments in the world: the Arctic permafrost and the deep ocean sediment, both of which are suitable for natural hydrate formation. In addition to methane, methane hydrate creation relies on three other components- low temperature, high pressure, and water. All four work in unison to build an adequate hydrate, such that 1 m<sup>3</sup> of methane hydrate is able to contain up to 170 m<sup>3</sup> of methane at STP. Methane hydrate has the appearance of ordinary ice and burns readily in the solid form.

One area of methane hydrate research that is being performed at NETL is attempting to produce methane hydrates within a fraction of the time that other laboratories currently need to prepare methane hydrates. Included in this area of research is investigating the effect of agitation on the uptake of methane while preparing the synthetic hydrates for eventual storage and transportation capabilities.

Funding: National Energy Technology Lab (NETL)

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**BEHAVIORAL WEIGHT LOSS STUDY PARTICIPANTS' ADHERENCE TO ENERGY AND FAT GRAM GOALS AT 6 MONTHS**

**Background:** Adherence to dietary goals is a challenge for most individuals. We examined participants' adherence to energy and fat gram goals at 6 months in a behavioral weight loss study.

**Method:** We performed a secondary analysis of data from the SMART weight loss trial. Each participant was assigned an energy and fat gram goal based on gender and baseline weight. For participants weighing <200lbs the calorie goal was 1200 (females) or 1500 (males) kcal/day; for those  $\geq$  200lbs, 1500 (females) or 1800 (males) kcal/day. The fat gram goal was  $\leq$  25% of the total calorie intake. Adherence was defined as consuming at least 1000 kcal and not exceeding the goals. To measure adherence, we conducted two 24-hour dietary recalls at 6 months.

**Results:** The sample (N=210) was 78.1% White, 84.8% female with a mean BMI of  $34.0 \pm 4.5$ . Of female participants <200lbs, 21.4% were adherent to the energy goal; while 33.8% of those  $\geq$  200lbs were adherent. None of the males <200lbs were adherent, while 27.6% of males  $\geq$  200lbs were adherent. Overall, 39% were adherent to the fat gram goals.

**Conclusion:** These findings reveal that a minority of weight loss study participants are adherent to their dietary goals; however, slightly more meet the fat gram goal than the calorie goal. Researchers need to examine how realistic the dietary goals are for participants in the first phase of a weight loss study and also develop new strategies to improve adherence.

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**DEVELOPING SMALL MOLECULE GROWTH INHIBITORS OF THE HUMAN  
PATHOGEN TOXOPLASMA GONDII**

*Toxoplasma gondii* is an intracellular parasite and a human pathogen that can cause severe illness. A key virulence factor in this organism is ROP18, a secreted protein kinase. To facilitate studies on this protein, we screened seventeen computationally defined inhibitors on ROP18-expressing *Toxoplasma* parasites. One particular inhibitor, F17920016, was found to inhibit parasite growth by 97% at a concentration of 10  $\mu$ M but is not fully soluble. Therefore, dose curves, with strains of *Toxoplasma* that do and do not express a virulent allele of ROP18, were performed to determine the concentration that would 1) inhibit parasite growth and 2) not precipitate out of solution. Initial results show that the compound still inhibits growth by 80% at 1  $\mu$ M and is fully soluble, with an  $IC_{50}$  of  $\sim$ 1 nM. These experiments also suggest that this compound may not be exclusively binding to ROP18, but rather may interact with two distinct binding sites to inhibit growth of the parasites *in vitro*. Initial results suggest that the effects of the compound are partially reversible and that the compound is lethal to the parasites after three days of treatment. Future studies will be carried out to determine if the compound is toxic to host cells. We also plan to use N-ethyl-N-nitrosourea (ENU) mutagenesis to develop parasites that are resistant to this compound as a way to identify F17920016 target genes using genetic complementation. To assess its potential effectiveness as an anti-*Toxoplasma* drug, the inhibitor will also be used on parasites *in vivo* during mouse infections.

Funding: Howard Hughes Medical Institute and the Boyle lab

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**ISOLATION AND CHARACTERIZATION OF NOVEL STREPTOMYCES LIVIDANS BACTERIOPHAGES**

Streptomyces are Gram-positive, non-pathogenic, soil-dwelling bacteria with a complicated life cycle. Bacteriophages, viruses of bacteria, are able to infect their host using either a virulent or temperate approach. It has been reported that there are  $10^{31}$  phage in the environment (Hendrix, 1999), making it relatively easy to find them in soil. The variety of phages that infect Streptomyces, however, is not yet known. Several bacteriophages were isolated from environmental soil samples using *S. lividans*. Each phage forms a unique area of infection, or plaque. The morphology of each phage varies in size and proportion, as analyzed using electron microscopy. One isolated phage appears to be temperate, indicating that lysogenic bacteria are formed upon its infection. DNA from each isolated phage was extracted using a small-scale preparation, and has been submitted for sequencing.

Funding: Howard Hughes Medical Institute

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**COGNITIVE FUNCTION FOLLOWING TREADMILL EXERCISE IN THERMAL PROTECTIVE CLOTHING**

Background: Firefighters regularly perform physical exertion in extreme heat and injuries are common. The thermal protective clothing (TPC) worn during fire suppression inhibits thermoregulation and places firefighters at risk of hypohydration and hyperthermia that may lead to mildly altered cognition. We hypothesized that exertion in TPC would result in immediate measurable changes in cognitive performance.

Methods: Ten healthy volunteers aged  $28.1 \pm 5.3$  years completed this study. A battery of validated neurocognitive tests evaluating short term memory, sustained and divided attention, reaction time, visuomotor tracking, and coordination was administered before and after performing treadmill exercise while wearing TPC in a heated room. Data were analyzed by ANOVA with significance set at  $p \leq 0.05$ .

Results: Subjects performed  $46.4 \pm 3.6$  minutes of exercise and achieved a heart rate of  $167 \pm 20$  beats per minute. At the end of exercise, core temperature was  $39.0 \pm 0.5^\circ\text{C}$  and body mass was reduced by  $1.34 \pm 0.50$  kg, indicating significant hyperthermia and hypohydration. No changes in cognitive function were observed following exertion in TPC.

Conclusions: The results of this study suggest that the aspects of cognition tested with this battery are not affected by hyperthermia or hypohydration. Additional studies are needed to confirm these findings and to examine other aspects of decision making.

Funding: Pittsburgh Emergency Medicine Foundation

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**COMPARISON OF ADENO-ASSOCIATED VIRAL (AAV) VECTOR SEROTYPES FOR TRANSDUCTION AFTER INTRACISTERNAL INJECTION**

A large problem facing gene therapy today is the sufficient and selective delivery of a therapeutic gene to target cells or tissue. The use of adeno-associated viral (AAV) vectors as a gene delivery vehicle is appealing because the AAV is safe and its effects last for a relatively long period of time. In the current study, we compared transduction of AAV serotypes (1,2 and 4-9) *in vivo* by injecting an adeno-associated viral vector expressing green fluorescent protein (AAVGFP) into the left lateral ventricle of Sprague-Dawley rats. A week after injection, the brain and spinal cord tissue was collected, sectioned, and examined under a fluorescent microscope for GFP expression. Although GFP was expressed in almost all of the animals, only AAV serotype 6 exhibited the desired results. GFP was observed surrounding the entire left lateral ventricle, in the choroid plexus, in the 3rd and 4th ventricles, and along the outer surface of the cerebellum in both animals injected with AAV6. The fact that the choroid plexus in these animals had a high GFP expression proposes an additional benefit—as the choroid plexus produces CSF, secreted therapeutic genes may be more robustly produced if delivered here. Since many of the injections were not successful because the needle missed hitting the ventricle, further testing must be completed in order to improve the procedure. Once the most efficient AAV serotype(s) are identified, the GFP gene can be replaced with a therapeutic gene for testing in models of neurodegenerative diseases such as Parkinson's or Alzheimer's.

Funding: National Institutes of Health

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THE EFFECTS OF LIFESTYLE REGULARITY AND MASTERY EVENTS ON PRE-LOSS  
COMPLICATED GRIEF IN ELDERLY CAREGIVERS

Few studies have focused on grief symptomatology and its relationship to elderly caregiver health during and after the care giving experience. There is extensive research showing that caring for a disabled spouse can make caregivers vulnerable to mental and physical illness, and, in some cases, death. Complicated grief has been identified as a risk factor for mental and physical morbidity in elderly bereaved spouses. It is possible that a pre-loss manifestation of complicated grief could also be predictive of poor mental and physical health while the spouse is in the care giving role. The well being of the caregiver, such as getting sufficient sleep, may be affected by the perception that they are able to manage new tasks successfully. New tasks, in addition to the tasks associated with the care of the spouse, will require time that, prior to the illness, may have been allotted to other activities (leisure, social, or recreational in nature). Research findings suggest that regularity of lifestyle and increased activity in elderly bereaved spouses is predictive of low levels of depression after the death of a spouse. In this study, we examine the phenomenology of this pre-loss manifestation of the complicated grief reaction. Specifically, we examine the effects of mastery events and lifestyle regularity on pre-loss CG and its relation to various demographic, clinical, psychosocial, and functional status variables.

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**MOLECULARLY IMPRINTED POLYMERS - NEW TOOLS IN THE DRUG DISCOVERY PROCESS**

Molecularly imprinted polymers (MIPs) are highly cross-linked materials that are prepared by polymerizing functional monomers with cross-linkers in the presence of a “template” molecule. Upon extraction of the template, the MIP contains empty binding sites that retain the shape and functional complementarity of the original template molecule. Due to their low cost and ease of creation, MIPs have been studied for wide range of applications including use as biosensors, nanoreactors, and stationary phases for chromatography. Our investigation focuses on whether the specificity of these binding sites can be used to measure the diversity within a chemical library. Using hydrocortisone and estrone, we employed a combinatorial approach to develop and optimize a set of MIPs. Using these MIPs as stationary phases for high pressure liquid chromatography columns, we intend to measure the diversity of a small library of estrone derivatives based on the retention time of each compound. The ultimate goal of this investigation is to develop an experimentally repeatable substitute or complement to computational methods of measuring diversity.

Funding: Eli Lilly and Company; NIH (P50-GM067082)

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**COMPUTER SIMULATIONS OF A RECEPTOR-LIGAND COMPLEX WITH EXTREMELY WEAK BINDING AFFINITY**

Forkhead-associated (FHA) domains mediate phospho-peptide interactions and have been found in various functionally diverse proteins. Here, we examine the complex of one of these domains, the FHA domain of antigen Ki67, with a heptapeptide fragment (residues 260 to 266) of the human nucleolar protein, hNIFK. Although this receptor-ligand complex has extremely weak affinity ( $K_D$  of  $45 \pm 5$  mM), the binding appears to be specific based on NMR titration experiments. The hNIFK heptapeptide, which is intrinsically unstructured in its unbound state, extends the anti-parallel beta sheet of Ki67FHA upon binding Ki67FHA. We have investigated the specificity of binding by shifting the beta sheet register upwards as well as downwards by two backbone hydrogen bonds to obtain different starting conformations of the complex and running a large ensemble of short molecular dynamics simulations. These simulations were run on a distributed computing resource from the starting conformations as well as from the final conformation. Results provide insight about the nature of receptor-binding by an intrinsically unstructured peptide as well as the specificity of beta sheets.

Funding: NSF Career Award

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**IMPROVING LIMB-STATE DECODING USING A LIQUID STATE MACHINE**

Functional electrical stimulation systems require online feedback for stable control. The spiking activities of primary afferent neurons are a natural source of useful information including muscle length and velocity. Simple linear regression (SLR) models have been used to estimate limb-state from this activity, but are limited by dependence among neurons within the afferent ensemble. We adopted the liquid state machine (LSM) as a model for limb-state decoding. Using primary afferent spike train data from the L7 dorsal root ganglion and kinematics recorded from a walking cat we trained linear readouts of the LSM to estimate limb angular position and velocity and compared this to SLR estimates for the hip, knee, ankle, and metatarsal-phalangeal joints. The results show the LSM to be a better model for limb-state decoding than SLR. The LSM decreased root-mean-squared error of kinematic predictions by as much as 31% for position, 28% for velocity, and 44% for acceleration.

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**ROSCOVITINE PROLONGS MEAN OPEN TIME OF UNITARY MAMMALIAN N-TYPE CALCIUM CURRENTS**

Calcium influx through presynaptic calcium channels triggers transmitter release, and alterations in the gating of these calcium channels results in changes in the magnitude of transmitter released. Roscovitine is an inhibitor of cyclin-dependent kinases (cdks). Roscovitine exists as (R)- and (S)-enantiomers, and both are effective cdk inhibitors, but only (R)-roscovitine demonstrates cdk-independent effects directly on some types of voltage-gated calcium channels (Yan et al., *J. Physiol.* 2002; Buraei et al., *Biophys. J.* 2005; Cho and Meriney, *Eur. J. Neurosci.* 2006). (R)-roscovitine appears to act directly on N- and P/Q-type Ca<sup>2+</sup> channels (1) altering whole cell current deactivation kinetics, and enhancing Ca<sup>2+</sup> tail currents following step depolarizations, and (2) decreasing current amplitude (Buraei and Elmslie, *J. Neurochem.* 2008). We have used whole-cell and cell-attached patch clamp methods to study the agonist effects of (R)-roscovitine on mammalian N-type calcium channels expressed in TSA-201 cells. In whole cell recordings we confirmed the prolongation in deactivation kinetics of calcium current. Decay time constants were fit by two exponentials, and roscovitine increased both the longer time constant and the proportion of the decay represented by this longer time constant. Cell-attached recordings at the single channel level revealed no effect on single channel conductance (~17 pS), but a significant roscovitine-mediated prolongation of single channel mean open time. In control patches, mean open time could be fit by two exponentials (~0.3 and ~2.0 msec). Roscovitine had no effect on the fast time constant (0.3), but increased the slow time constant to ~3.0 msec. Furthermore, the proportion of channels that exhibited this slow gating behavior was increased after roscovitine exposure.

Funding: NIH, Office of Experiential Learning

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**MOBILE INTERNET VULNERABILITY ASSESSMENT--PROCESSING SMART PHONE  
SENSOR DATA IN SEMANTIC WEB**

Semantic web—a data framework in which the semantics of information are used to create an ontology of concepts, terms, and relationships within a given knowledge domain—has traditionally been used to aid in the analysis of semi-structured text data sources. Our Mobile Internet Vulnerability Assessment (MIVA) was a proof of concept development effort to determine whether semantic technology is conducive to the processing of sensor data, which unlike text data, is generally sizable, numeric/calculable, and conducive to visualization. If semantic web could process sensor data, it would demonstrate that the technology is not only useful for linking data sets, but also for outputting a reduction of an individual data set as a human-readable graphical representation of the data.

For this study, we created a program in the Google smart phone platform Android that collects wireless access point security information within a set radius of a notional cyber attack target. We converted the raw sensor data to a network graph using a semantic data framework and employed a custom algorithm to process the data and return a much smaller “results object”. Our research then implemented a novel approach for encoding pre-processed data within semantic web entities using the JavaScript Object Notation (JSON). Using JSON, we overlaid wireless vulnerability data on a three dimensional globe aligned over notional cyber attack targets. Our studies demonstrated that server-side pre-computing is an effective tool for processing sensor data stored in semantic web provided that data has context through visualization and can undergo batch processing.

Funding: Johns Hopkins Applied Physics Laboratory

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## A NOVEL MATHEMATICAL MODEL OF HUMAN LACTATION

Lactation in human mothers is controlled by the interactions of several hormonal factors, including prolactin, which stimulates milk component synthesis, and oxytocin, which stimulates milk ejection. The details of prolactin control of milk synthesis during established lactation remain a mystery. This work analyzes alternative hypotheses concerning the effects of suckling on prolactin receptor regulation through a novel mathematical model of the cascade of interactions governing milk synthesis and ejection during established lactation.

Funding:

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**TRANSGENERATIONAL INDUCTION OF DEFENSE IN ARABIDOPSIS THALIANA**

After exposure to insect herbivory, some plants have shown the ability to induce a higher level of resistance in their offspring, a process known as “transgenerational induction”. This pattern has been observed in *Drosophila* as well, where the mother can influence the development of her progeny through the production of small interfering RNA (siRNA) molecules. The likely benefit of transgenerational induction is that offspring growing near their mother will be better defended against local enemies.

Our previous research asked whether a bacterial infection of *Pseudomonas syringae* in *Arabidopsis thaliana* can cause induction of defense in the next generation. Our results from several trials using two wild-type backgrounds show that offspring from parental generations exposed to pathogens have significantly stronger bacterial defenses than offspring from unexposed parents. *P. syringae* grew to dramatically smaller concentrations in progeny from threatened parents. We have also found significantly higher concentrations of tetraguaiacol peroxidase in the leaf tissues of offspring from bacterially-exposed parents as compared to control progeny. This enzyme belongs to a category of peroxidases that strengthen cell walls and create reactive oxidative species to impede bacterial infection. Bacterial growth tests were also performed on mutant lines of *Arabidopsis* that are defective in either miRNA or siRNA production. Our data suggest that these mutants are unable to produce a transgenerational induction response.

These responses are novel for both *Arabidopsis thaliana* and plant bacterial infection.

Funding: Howard Hughes Medical Institute, University of Pittsburgh

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**BXB1 GP47, A LYSOGENY PROTEIN, ALSO HAS A LYTIC-CYCLE ROLE**

DNA recombination has many diverse and essential roles in organisms from all three domains of life. Temperate mycobacteriophage Bxb1 provides an excellent system for studying site-specific DNA recombination. Bxb1 encodes two proteins involved in recombination: Integrase catalyzes site-specific integration of the phage genome into the host chromosome, and Gp47 is a recombination directionality factor (RDF) which alters Integrase specificity to effect excision of the phage DNA.

Intriguingly, Bxb1 Gp47 is not homologous to other known RDFs but is closely homologous to uncharacterized proteins of fifteen other mycobacteriophages, including L5 (Gp54). The lysogeny system of L5 has been characterized and does not involve Gp54. Bxb1 gp47 and homologues are found alongside DNA replication proteins in their respective genomes and contain a phosphoesterase domain associated with DNA polymerases. Together these observations suggest that Bxb1 Gp47 has an additional role, putatively in phage DNA replication.

To investigate this possibility, we have generated several Bxb1 mutants. We found gp47-deletion phage to be inviable in lytic propagation, while a Bxb1 gp47 lysogeny-knockout mutant undergoes normal lytic growth. These results suggest an important lytic-cycle role for Gp47. We also found Bxb1 gp47-strepII phage designed for pull-down experiments to be inviable. In current work, we are producing phosphoesterase domain motif knockout mutants; it will be interesting to see whether there is a correlation between motif functionality and phage viability. A single-motif knockout mutant undergoing purification appears fully viable in replication, but knocking out conserved residues from more than one domain motif might be necessary to impair functionality.

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**IDENTIFICATION AND CHARACTERIZATION OF NOVEL NITRATED FATTY ACIDS  
IN HUMAN URINE**

Nitrated fatty acids (NO<sub>2</sub>-FA) comprise a novel clan of endogenous lipid signaling molecules that in general mediate the resolution of inflammation. These molecules are believed to be formed through NO-dependent oxidative reactions with unsaturated fatty acids. During formation, the nitro group binds to the unsaturated fatty acid at the double bond, forming a nitroalkene (i.e. nitro olefin) which is highly electrophilic. Recently, free NO<sub>2</sub>-FA have been detected in human urine. Covalently bound NO<sub>2</sub>-FA adducts have also been detected. Most recent data has shown that the average concentration of nitrated linoleic acid (LNO<sub>2</sub>) in urine from a healthy man is  $46.75 \pm 11.52$  nM and is  $54.57 \pm 9.59$  nM in urine from a healthy woman. The average creatinine concentration in urine from a healthy man is  $77.64 \pm 23.29$  pmol/mg and is  $94.91 \pm 19.06$  pmol/mg from a healthy woman. Current work is being done to analyze the concentrations of LNO<sub>2</sub> and creatinine in sepsis patients with and without acute kidney infection (AKI). Due to their electrophilic nature and their ability to react with biological thiols such as protein cystine residues and glutathione, it is hypothesized that NO<sub>2</sub>-FA form in urine to act as antimicrobials that protect the urogenital tract against infection. This project has two aims: to identify and characterize the different NO<sub>2</sub>-FA molecular species present in urine and to evaluate their antimicrobial activity.

Funding: American Diabetes Association

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**SENSITIZATION OF RESPONSES OF UROTHELIAL CELLS TO PURINERGIC AND TRPV4 RECEPTORS AGONISTS BY CYCLOPHOSPHAMIDE AND BRADYKININ**

Purinergic and TRPV4 receptors are located on the bladder urothelium and have been implicated in bladder sensation and pain, through the release of ATP and other agents that act on afferent nerves. This study examined changes in the responses to urothelial purinergic and TRPV4 receptor agonists induced by bradykinin (BK), an inflammatory mediator, and by treatment of rats with cyclophosphamide (CYP), a model of cystitis in rats.

Experiments were performed in adult rat urothelial cell cultures using Ca<sup>2+</sup> imaging. Increases in the intracellular Ca<sup>2+</sup> concentration induced by consecutive applications of ATP (100 μM), a purinergic agonist, and of 4a-phorbol 12,13-didecanoate (4aPDD 1 μM), a TRPV4 agonist, were analyzed in cells from control rats, from CYP-treated rats (125 mg/kg, i.p.) and in cells pretreated with BK (2 μM).

In cells from control rats, three consecutive applications of 4aPDD led to substantial desensitization with 21.4%, 5.4%, and 3.6% of cells responding, respectively (n=56 cells). In cells pretreated with BK, three consecutive applications of 4aPDD elicited Ca<sup>2+</sup> responses in 27.9%, 23.3%, and 69.8% of cells, respectively (n=86 cells). In cells from rats treated with CYP consecutive applications of 4aPDD produced responses in 57.5%, 12.8%, and 17.0% of cells, respectively (n=47 cells).

Consecutive applications of ATP elicited Ca<sup>2+</sup> responses with amplitudes 190.6%, 179.1%, and 176.2% above baseline (n=203 cells). In cells treated with BK, three consecutive ATP applications elicited 187.4%, 159.6%, and 194.1% increases from baseline (n=249 cells). These results suggest that changes in TRPV4 and purinergic receptor properties might occur during cystitis and contribute to bladder pain.

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**DESCRIPTIVE CHARACTERIZATION OF LEPTIN LEVELS AFTER SEVERE TBI**

Previous studies have shown the homeostatic and satiety signaling hormone leptin to be associated with neuroprotection in response to NMDA toxicity, lesions induced by ibotenate-mediated excitotoxicity, and other destructive treatments. To further explore this phenomenon a preliminary study was undertaken to study the neuroprotective and pro-inflammatory effects of leptin in traumatic brain injury (TBI) patients post-acutely up to 144 hours after injury. A total of 88 cerebrospinal fluid samples collected from 18 patients (8 males, 10 females) were assayed for leptin concentration up to 5 days post-TBI using an enzyme immunoassay (Assay Designs, sensitivity). Concentrations from TBI patients were compared with 10 healthy control subjects (5 males, 5 females). Leptin concentrations were also compared by age (greater than 45 years of age versus 45 years of age and younger). Preliminary findings suggest that females, especially those under or equal to 45 years of age (N=7), exhibit significantly higher concentrations of leptin. While young females showed a mean leptin concentration of  $2366.7 \pm 752.65$  pg/mL on day 1 post-TBI, males showed a significantly lower concentration within the control range of  $62.05 \pm 22.2$  pg/mL. Such a finding may implicate the idea that females under the age of 46 show better Glasgow Outcome Scale (GOS) scores. Further analysis on a larger cohort must be conducted to confirm this hypothesis. This preliminary data has also generated an interest to investigate the mechanistic interplay between leptin and other biomarkers of TBI, such as pro-inflammatory markers (IL-1, IL-6, and TGF $\alpha$ ), neurotrophins and growth factors (BDNF, NGF, IGF, PDGF, VEGF, and GH) using microsphere based multiplexing system from a previous study.

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**MUSCLE LOADING PROTOCOLS AFFECTS THE FIBER TYPE EXPRESSION OF  
MOUSE STEM CELLS TRANSPLANTED INTO DYSTROPHIC MUSCLE**

**Background:** The purpose of this study was to examine the effect of two different loading protocols, treadmill running (TM) and neuromuscular electrical stimulation (NMES), on the fiber type differentiation of MDSCs transplanted into dystrophic muscle. **Methods:** Dystrophic immunodeficient mice (12-weeks-old) were divided into 3 groups: MDSC (n=2), MDSC+NMES (n=2), and MDSC+TM (n=3). The tibialis anterior (TA) was injected with 100,000 MDSCs suspended in 20 $\mu$ l of saline solution. TM and NMES interventions were applied 5 times/week for 4 weeks. The TM group was trained to run for 30 min at 10mpm. Speed and duration gradually increased throughout the protocol reaching 60 min at 13mpm with gradually increasing velocity until 16 mpm. The NMES consisted of 20 TA contractions, NMES intensity gradually increased throughout the training. All muscles were evaluated using immunohistochemistry for the number of slow and fast myosin heavy chain fibers. **Results:** MDSC+NMES and MDSC+TM showed a decrease of slow-twitch fibers when compared to MDSC group ( $141\pm 1.41$ ;  $96\pm 16.97$ ;  $23\pm 2.65$ , respectively). However, “intermediate” fiber types subjected to TM increased 2-fold compared to only MDSC group ( $229\pm 52.83$ ;  $111\pm 41.01$ ) while “intermediate” fibers in the NMES group stayed very similar to MDSC ( $113.5\pm 53.03$ ;  $111\pm 41.01$ ). **Conclusion:** In contrast to what we expected, the results may indicate that TM running is more effective to enhance fiber type transition. We suggest muscle loading protocols are viable methods to dictate the fiber type phenotypic expression of MDSCs after transplantation. Further studies are necessary to better understand whether terminal differentiation of transplanted cells is influenced by host muscle type or environmental stimuli.

**Funding:** Physical Therapy Foundation Grant

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PLASMA VITAMIN D LEVELS ARE POSITIVELY ASSOCIATED WITH BETA CELL  
FUNCTION

The role of vitamin D is recognized in the pathogenesis of type 2 diabetes and insulin resistance. While a positive correlation between 25-hydroxyvitamin D [25(OH)D] and insulin sensitivity has been established, a correlation between 25(OH)D and beta cell function needs to be confirmed.

This study included 150 glucose tolerant subjects [59% female, mean age: 26+6 y.o., BMI: 24.53+4.15 kg/m<sup>2</sup>]. A hyperglycemic clamp was used to determine acute insulin response (AIR) and insulin sensitivity index (ISI). Adjusted beta cell function (ABCF) was defined as the product of AIR and ISI. Serum 25(OH)D measurement was assayed from fasting samples using a LC/MS assay.

AIR was closely and inversely correlated to ISI ( $r=-0.5517$ ,  $P<0.0001$ ). 25(OH)D was positively correlated with both ISI ( $r=0.4349$ ,  $P<0.0001$ ) and ABCF ( $r=0.2903$ ,  $P=0.0003$ ). The positive correlation remained significant after adjustment for age, gender, BMI, ethnicity, physical activity and season of study (ISI:  $r=0.2622$ ,  $P=0.005$ ; ABCF:  $r=0.1883$ ,  $P=0.04$ ).

This study confirms the positive correlation of 25(OH)D and insulin sensitivity and establishes the positive correlation between 25(OH)D and beta cell function. Therefore, vitamin deficiency may lead to beta cell dysfunction and insulin resistance, both contributing factors to the development of type 2 diabetes.

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EXAMINING THE ROLE OF MECHANICAL FORCES IN ALK1 REGULATION

Zebrafish violet beaugarde (*vbg*) mutants harbor a mutation in the gene encoding the TGF-beta family type I receptor, *alk1*, and thus serve as a model for the arteriovenous malformations and abnormal vasculature that are characteristic of the human genetic disorder, Hereditary Hemorrhagic Telangiectasia type 2 (HHT2). We have previously demonstrated that blood flow and heart beat are required for the activation of *alk1*, implicating a role for *alk1* in the stabilization of vessels upon the onset of flow. We hypothesize that the mechanical forces, or shear stress, that arise from blood flow are important for proper *alk1* regulation. A literature search was performed to determine genes that are reported to potentially be regulated by both *alk1* and shear stress, typically in cultured endothelial cells. Two flow-responsive genes, nitric oxide synthase-1 (*nos1*) and heme oxygenase-1 (*hmox1*) were selected for further investigation. cDNA clones were generated and gene expression was assayed via in situ hybridization. Preliminary data suggest that *nos1* is expressed in the vasculature, while *hmox1* is expressed diffusely throughout the head. Further work will continue to investigate expression patterns in *vbg* and other mutants with altered flow and determine epistatic relationships between *alk1* and flow-responsive genes. These results will help us to better understand the role of Alk1 within the endothelium.

Funding: Howard Hughes Medical Institute

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**EFFECTS OF HYPERGLYCEMIA AND DEXAMETHASONE IN A MODEL OF NEURODEVELOPMENT**

Background: Neuroblastoma (NB) is a common solid pediatric tumor originating from embryonic neuroblasts of the developing sympathetic nervous system. NB exhibits heterogeneity in presentation, with high-risk NB attributed with significant mortality. NB is known to undergo spontaneous regression as well as chemically-induced differentiation, which are associated with lower rates of tumor progression. Dexamethasone, a glucocorticoid, has been reported to have a differentiating effect in NB. Hyperglycemia induces elevated serum glucocorticoid levels, and glucocorticoid therapies induce hyperglycemia in both diabetic and non-diabetic patients. Goals of this project: Cultures of undifferentiated and differentiated cells of the NB line SH-SY5Y were used as a model system to determine the impact of hyperglycemia and glucocorticoids on neuronal function during in vitro differentiation. Results: Undifferentiated SH-SY5Y cells treated with 60 mM, 90 mM, 120 mM, and 150 mM of glucose exhibited lower viability at increasingly hyperglycemic concentrations over two days of treatment. However, such detrimental effects of hyperglycemia were not observed in differentiated SH-SY5Y cells exposed to high glucose concentrations, as they formed more numerous and elongated processes and persisted over longer times periods with lower rates of cell death. Future Directions: The effects of dexamethasone, a synthetic glucocorticoid, will be tested on the undifferentiated cells, and the combined effects of dexamethasone and glucose on the differentiated cells will be examined. The increased sensitivity of these cells to oxidative stress will also be analyzed, as both hyperglycemia and glucocorticoids have been shown to impact cellular defense against oxidative stress in various models.

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POSTURAL ADAPTATIONS TO REPEATED TRIPS

Proactive postural adjustments, generated in anticipation of a known balance perturbation, play an important role in falls prevention [1]. Balance-challenging environments can include slipping and tripping hazards. It has been shown that older adults can exhibit proactive postural adjustments comparable to young adults during repeated exposure to slipping [2]. However, postural adjustments adopted by young and older adults in anticipation of a trip remain to be understood. The purpose of this study was to investigate proactive postural adjustments when repeatedly challenged by tripping hazards. Adaptations to the estimated center of mass position with respect to the stance foot pre-trip were compared for six young and four older adults during exposure to multiple trip perturbations. Subjects were informed that in the next set of trials, at some point they would experience a trip. No knowledge regarding the number of trips or the exact timing at which they would occur was provided. Three trips were randomly inserted into 5 unperturbed trials and trips were induced to catch the left foot mid-swing. The difference between the estimated center of mass position relative to the ankle of the stance foot was calculated in the medial-lateral, anterior-posterior and vertical directions. Young and older adults behaved in the same manner with exposure to multiple trips. However, older adults walked with a reduced anterior-posterior distance between the estimated center of mass and ankle. This suggests older adults adopted a more cautious gait than did young adults.

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**BME PROCESS MAPS: A LOOK AT TEAM DYNAMICS**

We are studying how team dynamics affects bio-engineering product design processes and artifacts using 16 student “inventor” senior design team cohorts from the University of Pittsburgh and Rose-Hulman Institute of Technology. We propose that positive team dynamics during the process leads to higher-quality design and innovative projects.

During the multi-term design project, twice a week each team member provided the primary tasks he/she worked on and a self reflection of the team’s progress. This information was then distilled into an overall narrative of the team throughout the project, which included a review of the team members’ activities, a summary of all breakthrough moments, and specifics of the team dynamics from week to week. At the end of the project, the students were given a final team dynamic survey, which measured three dimensions of teamwork: Communication, Collaboration, and Decision Making. Each member individually rated his/her team members, as well as themselves, on a series of items in order to produce a final team development reflection. Using the combined data sets, we evaluated how team dynamics relates to the final product score enabling us to address the following questions: 1) Do in process team dynamics affect design artifact scores? 2) Are such process dynamics correlated with final team development ratings? 3) Are there noticeable trends in team dynamics?

Funding: National Science Foundation

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**SYMPTOM DISTRESS, QUALITY OF LIFE, AND CHALLENGES OF ILLNESS AMONG WOMEN WITH BREAST CANCER ACCORDING TO RACE, INCOME, AND DISEASE STAGE**

**Purpose:** The purpose of this study is to measure symptom distress, quality of life and challenges of illness among women with breast cancer (BC) and to determine whether they differ according to race, income and disease stage.

**Methods:** The data were collected from the out-patient clinic at an urban National Cancer Institute (NCI) designated cancer center for women with breast cancer for at least one month. This was a 2X2 mixed methods prospective design. Women with BC were categorized into four groups based on race and income: white low income (WL), white higher income (WH), black higher income (BH) and black low income (BL) and further classified according to early stage or metastatic disease. Symptom distress, quality of life, and classification of women according to race and income were determined from 1) Socio-demographic questionnaire, 2) Symptom Distress Scale, 3) Functional Assessment of Cancer Therapy, and 4) Semi structured interview assessing the BC experience.

**Results:** Preliminary results are presented for 141 women for whom full data are available. The mean age of the sample is 54.1 years. Prevalent themes among all racial and economic groups were of hope, faith, and progressive loss. Important distinctions were evident among low income women. BL women spoke of physical, social distress and uncertainty regarding treatment goals while WL women verbalized an overall optimism, minimization of self and symptoms while describing themselves as "lucky".

**Conclusions:** The findings support the universality of the BC experience, yet there are unique emerging racial and economic influences, particularly among low income women that should be considered in better tailoring of care.

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**SMALL MOLECULE INHIBITORS OF THE MCM2-7 COMPLEX BLOCK GROWTH IN SACCHAROMYCES CEREVISIAE**

Small Molecule Inhibitors of the Mcm2-7 Complex Block Growth of *S. cerevisiae*

Rebecca Theophanous, Nick Simon, Anthony Schwacha

The Mcm2-7 complex is the highly conserved eukaryotic replicative helicase that unwinds duplex DNA at the replication fork. It is an essential regulatory component in DNA replication and if misregulated can lead to a predisposition for cancer. The helicase is a ring-shaped complex formed from six functionally distinct and essential subunits, each containing a unique ATPase active site. To study the function of each ATPase active site, small molecule inhibitors were previously identified that blocked the *in vitro* DNA unwinding activity of Mcm2-7. Many of these inhibitors represent a class of compounds called fluoroquinolones, drugs that are extensively used as antibacterial agents. To determine their potential *in vivo* use against the Mcm complex, I have tested seven fluoroquinolone-derived inhibitors for toxicity against yeast cells. Yeast growth in varying inhibitor concentrations was measured by optical density, or light absorbance, and a 50% inhibitory concentration (IC<sub>50</sub>) value was determined for each drug. Our best compound has an IC<sub>50</sub> value of 25  $\mu$ M. To determine if these inhibitors cause lethality by blocking DNA replication, drug-treated cells were examined by microscopy to determine if they accumulate with an arrest in S-phase. Results indicate that inhibitor addition causes S-phase accumulation, consistent with a block in DNA replication. To test if the Mcm complex is the main target of these chemicals, we are currently attempting to isolate and characterize drug-resistant mutants. Our results suggest that particular fluoroquinolones may serve as potential inhibitors of *in vivo* Mcm function.

Funding: Howard Hughes Medical Institute

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**SITAGLIPTIN ENCHANCES ANGIOTENSIN II-INDUCED HYPERTENSION AND CARDIAC HYPERTROPHY IN SPONTANEOUSLY HYPERTENSIVE RATS**

Our recent studies indicate that in kidneys of spontaneously hypertensive rats (SHR), but not in normotensive controls, activation of renovascular Y1 receptors by neuropeptide Y1-36 markedly enhances renovascular responses to angiotensin II (Ang II). Furthermore, sitagliptin a dipeptidyl peptidase IV (DPP IV) inhibitor and antidiabetic drug, by inhibiting DPP IV blocks the metabolism of neuropeptide Y1-36 and augments neuropeptide Y1-36-induced enhancement of the renovascular effects of Ang II. In the present study, we investigated the chronic effects of sitagliptin on Ang II-induced changes in arterial BP and cardiac and renal hypertrophy in SHR. Adult SHR were treated for the duration of the study (5 weeks) with enalapril (60 mg/kg/day) to block the endogenous renin-angiotensin system. Two weeks into enalapril treatment, a subsets of SHRs were treated for 3 weeks with sitagliptin (40 mg/kg/day; SITAG group), a slow-pressor” dose of Ang II [200 ng/kg/min via osmotic minipump; ANG II group), both Ang II plus sitagliptin (ANG II+SITAG group) or vehicle (CONTROL). Enalapril treatment for 2 weeks normalized SBP. Sitagliptin had no effect on SBP in enalapril pretreated SHR rats. Chronic Ang II infusion significantly increased SBP and heart weight. Sitagliptin enhanced ( $p<0.05$ ) Ang II-induced hypertension and cardiac hypertrophy and tended to augment Ang II-induced renal hypertrophy ( $p<0.08$ ). We conclude that inhibition of DPP IV with sitagliptin augments Ang II-induced hypertension, cardiac hypertrophy and perhaps renal hypertrophy in genetically-susceptible animals. Further investigation of the hemodynamic, cardiac and renal effects of DPP IV inhibitors in diabetic hypertensive patients is advised.

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**METHODOLOGICAL CHALLENGES IN CLINICAL RESEARCH: MY EXPERIENCE-  
INTERRELATEDNESS OF DEPRESSION AND ANXIETY IN WOMEN WITH PELVIC  
ORGAN PROLAPSE**

**Objective:** Little is known about depressive symptoms in women with pelvic organ prolapse. Our objective is to assess depression symptoms in women with and without prolapse and evaluate the impact of these symptoms on Quality of Life.

**Methods:** This is an IRB-approved prospective study of women with Stage II prolapse or greater. Subjects were enrolled from a urogynecology practice and completed self-administered measures. One at baseline, two weeks, three months, six months, and 12 months after surgery.

**Results:** This study is currently ongoing and data is still being collected. Results are pending the conclusion of this study.

**Conclusions:** As an undergraduate intern, I was a key member of the research team. Through my experience, I gained an understanding of the basic science of pelvic organ prolapse as well as the clinical significance of prolapse and its treatment options. Through my involvement with the project, I have gained many new insights into conducting clinical research, specifically in the area of urogynecology, and more specifically in the area of pelvic organ prolapse. I have a new awareness of how physical health can have an impact on mental health. This study will not only prove that fact, but it will show that mental health can be cured through physical health improvement.

**Funding:** Magee-Womens Research Institute

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**GRAPHICS PROCESSING UNIT (GPU) ACCELERATED SEGMENTATION OF AXONS FROM CONFOCAL DATASETS**

We have developed new software prototypes for segmenting axon data attained using confocal microscopy for the purpose of the Connectome project. A project dedicated to the development of a connectonal map of the human brain in order to aid neuroscientists in modeling and understanding its function. We have built two prototypes to segment the axons, using the level set and shortest path method. After being given an initialization area by the user, the level-set method prototype is capable of providing its user with an interactive interface to semi-automatically segment the axons on a 2D slice. The result can then be automatically propagated to the next slice, using the current level set as an estimate for the initialization of the next. Our fast marching prototype, given seed points from the user, is capable of segmenting multiple axons simultaneously and label split axons with a different marker. In order to achieve interactive speeds, the implementation is completed using the GPU due to its substantial speed advantage over the CPU in parallel computation. Instead of passing individual pixels to the CPU, we can compute entire blocks of the image using the GPU. This parallel method has achieved a 120 times speed up in the level set method and a 24 times speed up in the fast marching method compared to CPU implementation using ITK. We are encouraged by our initial results and are currently working on adding anisotropy to the fast marching method to address the present limitations.

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**MEMBRANE TRAFFICKING, CELLULAR PROTEIN LEVELS AND LOCALIZATION AS A FUNCTION OF TRPML ION CHANNELS**

Lysosomal storage disorders (LSDs) disrupt degradation of endocytosed materials, leading to accumulation of material inside cells and to cell death. Mucopolipidosis type IV is a rare genetic disorder in which mutations in the gene coding for the transient receptor potential ion channel mucolipin 1 (TRPML1) induce lipid buildup in lysosomes, leading to defective motor and cognitive development. The exact functions of TRPML1 and its relative, TRPML3, are unknown. Identifying proteins whose cellular levels change as a function of TRPML activity may elucidate which processes the channels regulate and ultimately, the molecular underpinnings of LSDs.

Proteomic assays indicate that siRNA knockdown of TRPML in human cell lines induces changes in levels of several proteins relevant to lysosomal function, including Annexin II (ANXII), Cathepsin B (CatB) and Lysosomal Acid Lipase (LAL). To discern whether or not changes in the protein levels are associated with mislocalization, wild type and TRPML1- and TRPML3- knockdown (KD) cells were stained with antibodies specific against these proteins and against Acylglycerol kinase (AGK) and S100 and analyzed using confocal microscopy. Intracellular lipid traffic as a function of TRPML3 status was evaluated as well.

Confocal images corroborate protein immunoblot data for CatB and LAL, and downregulation of ANX II, AGK, and S100 is observed in KDs. Furthermore, we found changes in lipid traffic in TRPML-KD cells. These results suggest that protein production or handling, as well as lipid trafficking, varies as a function of TRPML activity.

Funding: HHMI Summer Undergraduate Research Fellowship

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**MODULATING THE NICHE IN MUSCULAR DYSTROPHY INCREASES HOST  
REGENERATIVE CAPACITY**

**BACKGROUND:** Cellular therapies have been suggested as a method to restore muscle function and healing capacity by reinstating the stem cell reservoir in the dystrophic skeletal muscle microenvironment. Recent studies investigating the trophic influence of donor stem cells on surrounding tissues have created an interest in examining this role as it applies factors dictating stem cell engraftment and myogenic differentiation.

**METHODS:** Proliferation Assay: Muscle derived stem cells (MDSCs) were harvested from mdx mice (MDSCmdx). MDSCmdx were co-cultured with MDSCs isolated from wild type mice (MDSCWT) in a transwell array, (MDSCmdx/MDSCWT, MDSCWT/MDSCmdx, MDSCmdx/MDSCmdx and MDSCWT/MDSCWT). The population in the bottom well was analyzed for all variables considered. This array allowed for paracrine effects to be observed without direct cell-to-cell contact. Proliferation across conditions was measured using Live Automated Cell Imaging for 60 hours. Differentiation Assay: Cells were plated using the above set-up, and maintained to >70% confluence (72 hours). After 72 hours, cell media was removed and replaced with differentiation-inducing media, and the cells were co-cultured for an additional 48 hours. Immunocytochemistry for myosin heavy chain and nuclei staining was used to calculate the myogenic fusion index (total nuclei within myotubes/total nuclei). ELISA for Vascular Endothelial Growth Factor (VEGF) was performed on culture supernatant and normalized to cell number.

**RESULTS/ CONCLUSIONS:** There was no difference in MDSCmdx proliferation in the presence or absence of MDSCWT. MDSCWT shows a trend toward significantly lower proliferation in co-culture with MDSCmdx, but is not statistically different at 60 hours. However, a significant increase in MDSCmdx fusion was observed in co-culture with MDSCWT cells, when compared to MDSCmdx/MDSCmdx controls. VEGF levels were significantly higher in MDSCWT/MDSCmdx compared to MDSCmdx/MDSCmdx. These results reveal that the consequential up-regulation of VEGF in the presence of wild type cells may in part improve MDSC fusion within the dystrophic niche.

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**SEX-RELATED DIFFERENCES IN OSTEOGENIC CAPACITY OF HUMAN UMBILICAL CORD STEM CELLS**

Human umbilical cord stem cells (UCSCs) may be beneficial for use in cell therapy. Isolation of these cells from the umbilical cord results in a high harvest yield, and high expandability with stem cell phenotype stability. In this study, we examined human donor variability and dose-dependant effects of BMP4 on osteogenic capacity of UCSCs. Human UCSCs (n=8 donor populations) were stimulated with osteogenic media containing 0.1, 1, 10 and 100 ng/mL bone morphogenic protein 4 for 5-7 days and percent alkaline phosphatase (ALP) was examined. We subsequently prepared UCSC for the classic pellet assay (2.5E5 cells/pellet) for growth in control media, DMEM, or osteogenic media with 10ng/mL dose of (BMP4) for 10 and 28 days. Pellets were examined by micro-CT for matrix mineralization and were fixed in formalin for histological analysis. We observed that the most effective dose of BMP4 to increase ALP positivity varied with the donor population. In the pellet assay, we observed a larger amount of bone volume present in certain populations (UC80, UC82, UC84 and UC86). By day 28, we observed that these same populations did not continue to represent the most osteogenic cells, rather populations (UC79, UC81, UC83 and UC85) showed larger amount of bone volume present. To date, we have not detected any correlation between cell markers or ALP expression and mineralized matrix formation. Investigation shows that while all UCSC populations were capable of osteogenic differentiation, there was variability among populations. While previous studies showed remarkable phenotype consistency in the isolation of mesenchymal stem cells from the umbilical cord, UCSCs showed dose-dependant effects related to BMP4 concentration and length of stimulation. Findings have implications for individualized therapeutic approaches using mesenchymal stem cells. Whether autologous cells or allogeneic cells may be used in cell therapies, it will be important to examine the specific responsiveness of a given stem cell populations. Future studies are ongoing to examine the molecular differences among the populations with disparate osteogenic responses.

Funding: Children's Hospital of Pittsburgh

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TRACING DOMESTIC WATER INPUTS TO PITTSBURGH STREAMS

Sewer system overflow during wet weather directs contaminated water to local streams and rivers, specifically Pittsburgh's Nine Mile Run. Therefore, water quality monitoring is essential for successful restoration of the stream. Mineral sources of fluoride are not common in natural waters, yet fluoride is added to domestic drinking water to help strengthen teeth. Therefore, fluoride can be used as an inexpensive tracer to distinguish domestic water inputs to the stream from other water sources. In addition, temperature can also be used as a tracer and when coupled with the fluoride tracers, the effects of domestic water inputs can be better characterized. Further, as human activities vary through the day (e.g., morning showering), time periods where domestic water inputs to the stream are most frequent can be determined. Water samples from Nine Mile Run have been analyzed for chemical concentrations, including the concentration of fluoride to help characterize patterns in fluoride concentrations. Temperature loggers have also been deployed at multiple stations to detect temperature differences between the stream and the combined sewer overflow. Ultimately, this information can be applied to reduce flow in sewers during wet weather periods.

Funding: Pennsylvania Water Resources Research Institute and the University of Pittsburgh  
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**CHANGES IN CORTICOSPINAL EXCITABILITY DURING BILATERAL VOLUNTARY CONTRACTIONS IN DIFFERENT DIRECTIONS IN HUMANS**

In non-human primates, a large population of cells in the primary motor cortex (M1) displays activity that correlates to the direction of movement. In humans, little is known about changes in motor cortical function during bilateral voluntary contractions in different directions. In this set of experiments, transcranial magnetic stimulation (TMS) was used to examine motor evoked potentials (MEPs), short-interval intracortical inhibition (SICI), and interhemispheric inhibition (IHI) in the first dorsal interosseous (FDI) muscle during unilateral and bilateral index finger isometric voluntary contractions. Twelve healthy volunteers performed 10% of left maximal voluntary index finger abduction while the right index finger either remained at rest or performed 30% of the maximal voluntary abduction or adduction. Electromyographic (EMG) recordings in the left FDI were comparable across conditions. The size of the left FDI MEPs decreased during right index finger abduction and adduction. The magnitude of IHI and SICI targeting the left FDI (using the same stimulus intensity) was increased by contralateral adduction and abduction. These findings indicate that voluntary contraction of a finger muscle down-regulates corticospinal motor output in the contralateral active hand. During bilateral movements, motor cortical pathways mediating transcallosal inhibition and GABAergic intracortical circuits mediating SICI are modulated by voluntary contractions of different directions.

Funding: NIH, NINDS

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**ROLE OF CAS PROTEIN AND THE MECHANISMS USED TO CONTROL EMT**

In vertebrates, the Cas protein family has four members: p130Cas, Efs/Sin1, HEF1/Cas-L, and HEPL. Cas proteins are adaptor proteins that play a significant role in intracellular signaling events. The Cas proteins assemble multiple effector proteins into macromolecular complexes, thereby activating downstream signaling cascades. Cas proteins are important in integrin-dependent signaling effectors, controlling apoptosis/anoikis and cell cycle progression. High levels of p130Cas is associated with higher risk of breast cancer recurrence and reduced survival. Recent studies also found that HEF1 is amplified in melanoma and, when over-expressed, promotes cancer progression and metastasis. To metastasize, a cancer cell has to undergo an epithelial mesenchymal transition (EMT), a transformation of a stationary epithelial cell into a fibroblast-like, migratory cell. EMT begins with a cancer cell losing its connections with the neighboring cells in the epithelial layer by deregulating E-cadherin, a cell-cell adherence molecule.

While HEF1-overexpressing cultured epithelial cells become motile and invasive, it remains unclear how HEF1 initiates and regulates EMT. We propose that HEF1 controls E-cadherin at the plasma membrane and initiates its internalization, thereby increasing a cancer cell's metastatic potential.

Funding: Fox Chase Cancer Center

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**INHIBITION OF CALCIUM/CALMODULIN-DEPENDENT KINASE II IS PROTECTIVE IN LIVER ISCHEMIA/REPERFUSION INJURY**

**Introduction:** Hepatic ischemia followed by reperfusion (I/R) occurs in the settings of trauma, transplantation, and elective liver resections. The mechanisms that account for inflammation and local organ damage are only partially understood but include deranged calcium signaling and activation of Ca<sup>2+</sup>/Calmodulin Dependent Kinases (CaMK). The purpose of this study was to investigate the contribution of CaMK II isoform to inflammation and damage after liver I/R.

**Methods:** In vitro, hypoxic (1% O<sub>2</sub>) primary rat hepatocytes were treated with a CaMKII-inhibitory peptide (AC3-I), and analyzed by CaMKII activity assay and western blot. In vivo, mice were injected with AC3-I or PBS and subjected to a model of warm hepatic ischemia/reperfusion and assessed for liver damage by serum ALT and inflammatory markers.

**Results:** In vitro, hypoxic activation of CaMKII is inhibited by AC3-I. We found decreased activation of the CaMKII target, MAPKinase p38, with CaMKII inhibition. In vivo, CaMKII is activated as early as one hour after reperfusion. Mice treated with AC3-I were protected from liver injury with decreased serum ALT (AC3-I:5175 ± 1088; PBS:8760 ± 610) and decreased necrosis and inflammatory cytokines compared to control mice. In addition, MAPKinase activation of ERK was decreased in the livers of mice treated with CaMKII inhibition.

**Conclusions:** CaMKII is activated in liver I/R and contributes to organ damage by activating MAPKinases such as p38 and ERK. Strategies to inhibit CaMKII may be considered in clinical settings of ischemic liver injury to decrease organ damage.

**Funding:** 2009 Summer Undergraduate Research Program

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## EWOD-PROPELLED ROBOT POWERED WIRELESSLY BY INDUCTIVE COUPLING

With the recent discoveries in the field of electrowetting-on-dielectric (EWOD) technology, miniaturized autonomous swimming robots are on the horizon with applications for environmental monitoring and bio-sensing/surgery and drug delivery inside the human body. To date, three separate EWOD propulsion mechanisms have been proposed and tested for swimming and floating robots. However, the previous experiments with these devices typically used wired power supplies or demonstrated a limited distance of propulsion. In order to facilitate total freedom of movement for swimming or floating robots, a new propulsion mechanism using EWOD actuated oscillating bubbles is integrated with a wireless power system. Key contributions in this paper are as follows: (1) we designed and realized a wireless power transmission system that specifically meets the requirements of EWOD actuation (high voltage ( $> 60$  V) at low frequency ( $< 100$  Hz)); (2) we achieved wireless EWOD operations with droplets and bubbles, including the wireless propulsion of a centimeter-scale boat.