SCIENCE2010

TRANSFORMATIONS

THURSDAY AND FRIDAY, 7 AND 8 OCTOBER

UNDERGRADUATE RESEARCH POSTER RECEPTION

7 OCTOBER 2010

Welcome to the Science2010 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 Science2010 for graciously including this undergraduate research event in its 2010 program.

Office of the Provost
University of Pittsburgh
For more information about Undergraduate Research at Pitt, www.undergradresearch.pitt.edu

Suyesh Acharya

Bioengineering

Swanson School of Engineering

Faculty Mentor: Dr. Kong Chen and Dr. Robert Brychta, NIH

USING ACCELEROMETERS TO DETERMINE HUMAN MOVEMENT

Accurately and non-invasively measuring energy expenditure in the free living environment is a challenging task, but recent enhancements in size, memory, and performance has allowed accelerometers to become increasingly used for this purpose. Although the accelerometer method lacks the ability to precisely differentiate between similar activities like static postures and walking motions, improvements in detection of these events will increase the accuracy of energy expenditure models, and may have broader applications in behavioral monitoring. This study was performed to develop a model to identify the human movements most prominent in free living using raw accelerometer signals. Six males and nine females, aged 19-50 years, with height 153.5-186.6 cm and weight 54.3-85.6 kg participated in the following activities in varied order: Walking Upstairs, Walking Downstairs, Level Walking, Sitting Straight, Sitting Lean, Free Sitting, Laying Flat, Free Laying, and Jogging. Raw data collected from a tri-axial hip-worn accelerometer (ActiGraph GT3X) was used to develop an algorithm. Each activity was repeated several times to provide both "training" and "validation" data for the algorithm. The algorithm easily distinguished between low, moderate, and high intensity activities, identifying Sitting, Jogging, and Laying with high accuracy (above 86%) and Walking Downstairs, Walking Upstairs, and Level Walking with moderate accuracy (above70%). The study shows great potentials for detecting different human locomotion and postures from raw accelerometer signals. Testing even more subjects of a wider range of age and size at broader and faster walking speeds will allow for a more concise and accurate energy expenditure model to be developed.

Bahar Ahani

Bioengineering

Swanson School of Engineering

Faculty Mentor: Dr. Mitra Lavasani

ROLE OF MUSCLE-DERIVED STEM CELLS IN A MOUSE MODEL OF AGING

Aged individuals have a dramatically increased risk of numerous debilitating diseases including bone fractures, sarcopenia, cardiovascular diseases, diabetes and cancer. Growing evidence supports that DNA damage is one type of cellular damage that promotes aging. Using a mouse model of a human progeroid syndrome caused by defective DNA repair (ERCC1-deficient mice), we tested our hypothesis that muscle-derived stem cells (MDSCs) may be affected with aging. A modified preplate technique was used to isolate MDSCs from Ercc1-/Δ mice (mice expressing 10% of the normal complement of ERCC1-XPF with a maximum lifespan of 7 months) and Ercc1-/- mice (mice in which ERCC1-XPF is genetically deleted, with a maximum lifespan of 1 month). Using Live Cell imaging, the proliferation rate of Ercc1-/- MDSCs proved to be significantly slower when compared to wild-type (WT) MDSCs. WT MDSCs showed 80% myogenic differentiation while only 46% of Ercc1-/- MDSCs were able to fuse and differentiate into myotubes in vitro. To further investigate the myogenic potential of MDSCs in vivo, cells were injected into the gastrocnemius muscle of MDX/SCID mice, a mouse model of Duchenne Muscular Dystrophy (DMD). WT MDSCs fused with the host muscle fibers to form large dystrophin positive fibers showing higher regenerative index. Conversely, the Ercc1-/Δ MDSCs were significantly smaller in diameter and failed to fuse with the host muscle fibers. These results may suggest that the stem cells inability to proliferate and differentiate may contribute to the dramatically accelerated aging.

Funding: AG-NS-0303-05, NIEHS ES016114, NIH 1R21AG0344907-01A1

Abdul-Kareem Ahmed

Neuroscience

Arts and Sciences

Faculty Mentor: Dr. Bill J. Yates

DISCERNING THE NEURAL CIRCUITRY OF MOTION SICKNESS IN CATS EMPLOYING C-FOS GENE EXPRESSION

Motion sickness is elicited during conditions where multiple sensory inputs are present that provide contradictory information regarding body position in space. However, vestibular stimulation alone can induce motion sickness granted that the sensory inputs differ from those expected. The neural circuitry that produces motion sickness is not well established. In the present study, we determined the neural regions where a large fraction of neurons expressed the intermediate early gene c-Fos during motion sickness elicited by galvanic stimulation of the inner ear. Eight cats were chronically instrumented for delivery of current to the inner ear on both sides. During the experimental session, 0.5 Hz sinusoidal stimulation was delivered; the sinusoids on each side were 90 degrees out of phase, to provide a contradictory input to the two inner ears that is known to result in motion sickness. During the 90-minute stimulation session, the animals expressed a variety of indicators of motion sickness, including salivation, retching and immobility. One hour after stimulation was concluded, the animals were anesthetized and perfused transcardially with paraformaldehyde, and their brains were harvested for histological analysis. Sections through the brainstem and diencephalon were processed immunohistochemically to visualize c-Fos. High concentrations of Fos-positive neurons were present in the nucleus tractus solitarius, respiratory nuclei, periaqueductal gray, vestibular nuclei, parabrachial nucleus, raphe nuclei, and infratrigeminal nucleus. Presumably, all of these areas participate in generating motion sickness. Some of these areas contain a high density of serotoninergic neurons; we confirmed that Fos and serotonin were often co-localized in the same cells.

Funding: NIH, NIDCD

Ogechi Akalegbere

Biology

Arts and Sciences

Faculty Mentor: Deborah Jacobs-Sera

CHARACTERIZATION OF MYCOBACTERIOPHAGE BIGROCH

Bacteriophages were discovered in the early 20th century. With an estimated population of 1031 particles worldwide, these organisms have been used as tools for DNA recombination and genomics as well as the prevention of bacterial contamination in foods. Understanding the genomic makeup of bacteriophages and how they interact with their host is important to this investigation. Mycobacteriophages infect a Mycobacterium host. M. tuberculosis, the causative agent of tuberculosis (TB), is the prominent pathogen of this genus. Drug resistant strains of M. tuberculosis have become quite problematic to treat, calling for new approaches. Mycobacteriophages are being investigated as alternative treatments and as useful tools to manipulate the mycobacterial genomes. Understanding their host range is important in gathering knowledge on how and why they infect certain bacteria. Mycobacteriophage BigRoch was isolated from a soil sample collected in 2008. BigRoch was isolated on the host, M. smegmatis, which is a nonpathogenic relative to M. tuberculosis. At present, BigRoch has been purified, amplified, and its DNA has been extracted and analyzed. This poster describes

Funding: HHMI

Jordan Anderson

Bioengineering

Swanson School of Engineering

Faculty Mentor: Dr. Bridget M Deasy

STEM CELL MIGRATION ANALYSIS USING ROBOTIC IMAGING SYSTEMS

To study migration processes in vitro, we use a novel time-lapsed microscopy system that allows us to obtain unique data related to cell migration and cell chemoattraction. We used progenitor cells to determine how cells may migrate into a wound, and human mesenchymal stem cells (MSCs) to determine if the system could be used to study chemoattraction. We found that the system could detect significant differences in cell migration, depending on factors including distance from the wound or from the growth factor signal. Migration: The scratch wound assay, in which a confluent layer of cells is wounded by manually scratching a region of the plate to remove all cells, was used. Chemoattraction: Human MSCs were transduced to express a stimulatory growth factor and were cultured and pelleted. Non-transduced cells and the transduced pellet were plated. Live cell time-lapsed imaging was used to capture images at 10-minute intervals(1). Cells on the edge of the wound were observed to infiltrate about 1/3 of the scratch wound area by 36 hours. All cells moved in the direction towards the scratch wound, with no cells migrating inwards toward the confluent region. There was no difference in the mean directional persistence of cell migration for the 2 regions. In the chemoattraction experiment, MSCs in Region 1 appear to display random migration tracks. Some cells in Region 2 appear to exhibit non-random motion in the direction of the chemoattracting pellet. Cell velocity (um /min) was significantly higher for cells nearer to the GF signal as compared to cells farther from the signal. Live cell imaging greatly enhances cell science and tissue engineering by providing a unique dataset to quantify numerous temporal changes in cell populations. Our analysis shows that MSCs chemoattraction to various GFs can be determined readily and quantified to serve as a basis for design of scaffolds which include GF tags.

Yasir Arafat

Chemical Engineering

Swanson School Of Engineering

Faculty Mentor: Edward Lahoda

A COMPREHENSIVE APPROACH TO LIGHT WATER REACTOR FUEL RECYCLING AND LOWERING RADIOTOXICITY OF HIGH LEVEL WASTE: ASSESSMENT OF TECHNICAL REQUIREMENTS FOR PARTIOTION TECHNOLOGIES

A proper solution to the growing inventory of spent nuclear fuel has major implications on the future of nuclear power. The “open cycle” strategy currently adopted in the USA is a threat to the sustainability of the nuclear option and to its potential growth vs. other energy sources. The current trend can be reversed by adopting a proper strategy for closing the fuel cycle, with a judicious choice of isotopes to be separated from used nuclear fuel (UNF) and transmuted by further irradiation cycles. Many specific solutions have been proposed on this regard. This report is proposing a new approach, which involves redefining the specifics of the main systems involved (fuel type, reactor design and reprocessing techniques) to minimize the radiotoxic content of the final high-level waste (HLW) to be disposed. In a closed fuel cycle, although the transmutation rate of actinides depends on the specific properties of the system adopted for transmutation (e.g. fast reactors, accelerator-driven systems etc), the final waste will be predominantly characterized by the reprocessing plants. The efficiency that can be achieved in the separation and recovery of some critical isotopes is a key to reduce the HLW radiotoxic content. This paper focuses on identifying these critical isotopes, which are responsible for the long- and intermediate-term radiotoxicity in the conventional uranium-based fuel cycle and in a proposed alternative, the thorium-based fuel cycle. The isotopic composition at discharge from fuel typical of various spectral conditions (thermal and fast range) in either the uranium-based or the thorium-based fuel cycle has been calculated and its radiotoxic evolution with time analyzed using ORIGEN-ARP. The analysis reveals that thorium fuel produces negligible amount of transuranics (TRUs), which makes it the preferred option to minimize the issues associated with their partition and in-reactor transmutation.The various options available in current state-of-the-art reprocessing techniques and suitability to meet these specifics will be discussed in the final poster.

Funding: Westinghouse Electric Company

Alison Austin

History and Philosophy of Science

Arts and Sciences

Faculty Mentor: Dr. Nancy Kaufmann

EXPRESSION OF AQP17664 IN LIPID STORAGE SITES SUGGESTS A ROLE IN FAT METABOLISM

Intercellular glycerol transport is crucial for an animal's ability to maintain homeostasis and is necessary for survival. However, little is known about possible mechanisms in the cell membrane that transport glycerol. Evidence suggests that specific types of water channels called aquaporins also allow glycerol molecules in and out of the cell (aquaglyceroporins). Previous studies on mice involving an aquaglyceroporin called AQP7 have shown that these channels are involved in mammalian fat metabolism. AQP7 knockout mice showed impaired ability to release glycerol from adipocytes (Norikazu Maeda, PNAS). We are interested in learning more about the role of aquaglyceroporins in fat metabolism during early life stages by using Drosophila melanogaster as a model organism. I have investigated the expression of an insect homologue of AQP7 called AQP176664 in various drosophila tissues, focusing on adipocytes found in the fat bodies. Reverse Transcriptase- PCR technique was used to compare expression of AQP17664 with other aquaporins in the fat bodies of third-instar wandering larvae (a life cycle stage when the insect is actively feeding and storing fat). Results showed that AQP17664 is expressed in fat bodies, indicating that it may channel glycerol during fat metabolism. By learning more about how AQP1766 functions in the transport of glycerol, we can further understand metabolic pathways of pest insects such as the malaria carrier Anopheles gambia, which relies on stored glycerol for a source of energy.

Funding: Howard Hughes Medical Institute

Varun Badami

Neuroscience

Arts and Sciences

Faculty Mentor: Dr. Bill Yates

DISTRIBUTION OF HYPOTHALAMIC NEURONS WITH OREXIN OR MELANIN CONCENTRATING HORMONE IMMUNOREACTIVITY AND MULTISYNAPTIC CONNECTIONS WITH DIAPHRAGM MOTONEURONS

Prior work showed that neurons in the lateral, dorsal, and perifornical regions of the tuberal and mammillary levels of the hypothalamus participate in the control of breathing. The same areas also contain large numbers of neurons that produce either orexins (hypocretins)or melanin concentrating hormone (MCH). These peptides have been implicated in regulating energy balance and physiological changes that occur in transitions between sleep and wakefulness, amongst other functions. The goal of this study was to determine if hypothalamic neurons involved in respiratory control, which were identified in cats by the retrograde transneuronal transport of rabies virus from the diaphragm, were immunopositive for either orexin-A or MCH. In animals with limited rabies infection of the hypothalamus (<10 infected cells/section), where the neurons with the most direct influences on diaphragm motoneurons were presumably labeled, a large fraction (28–75%) of the infected hypothalamic neurons contained orexin-A. In the same cases, 6–33% of rabies-infected hypothalamic cells contained MCH. However, in animals with more extensive infection, where rabies had presumably passed transneuronally through more synapses, the fraction of infected cells that contained orexin-A was lower. The findings from these experiments thus support the notion that hypothalamic influences on breathing are substantially mediated through orexins or MCH.

Funding: NIH grants 5R01-DC003732-11 and 3R01-DC003732-11S1, and P40-RR-018604 from NIH's National Center for Research Resources

Rebecca Belan

Mechanical Engineering

Swanson School of Engineering

Faculty Mentor: Adrian Michael

ENZYMATIC SENSORS FOR DETECTION OF NEUROTRANSMITTERS IN OXYGEN DEPRIVED TISSUE

Electrochemical detection of neurotransmitters is an important method for understanding the brain. Single carbon fiber electrodes are widely used as their small size allows for detection without causing damage to the brain. In conjunction with enzyme trapping redox hydrogels, electrode arrays (daggers) are especially useful for the detection of neurotransmitters that are not themselves electroactive. To date no array has been made of similar scale to a carbon fiber electrode. For the past year, the Michael Group has been developing a triple-band platinum electrode array with aims of eventual in vivo use. To date, daggers with platinum bands with approximately 10x20μm cross sections have been produced. Previous work has established a procedure for manufacturing free standing polymer (Su8) structures through the use of lithography and aluminum as a dissolvable layer between the Si wafer and Su8 structure ("lift-off"). The structures produced through this procedure have a cross section of the desired size, and further corrected the curling issue and stiffened the electrodes. Signals have been successfully read in a potassium ferrocyanide electrochemical reaction and testing is continuing on to various neurotransmitters. This paper focuses on a redesign of the electrodes, to further stiffen them and make them easy to handle, as well as on a design of a holder for testing the electrodes.

Funding: NIH #MH075989

Taylor Bissell

Bioengineering

Swanson School of Engineering

Faculty Mentor: Dr. Bridget Deasy

METHODS TO ISOLATE AND IDENTIFY QUIECSENT HUMAN MUSCLE DERIVED STEM CELLS

Stem cells populations are heterogeneous and contain dividing and non-dividing subpopulations. Included in the non-dividing subpopulation are quiescent cells, which are cells that are not currently dividing, but do have the potential to reactivate and re-enter the cell cycle. Quiescent cells are believed to be the most potent cells, and are thought to be the cells that survive transplantation during regenerative medicine, and contribute the most during the regeneration process. Human muscle derived stem cells (hMDSCs) were labeled with 5-chloromethylfluorescein diacetate (CMFDA) and sorted using flow cytometry into CMFDA positive (non-dividing) and negative (dividing) populations. The cells were observed using live cell imaging for 6 days, and the sequential jpegs were analyzed for proliferation data using image analysis software. Cells were also followed to determine lineage history and to determine if a cells was actively dividing. The sorted cells were cultured for a long-term expansion to determine longevity. The proliferation data used to determine the fraction of dividing cells in each subpopulation. 73% of the dividing cells were actively dividing, while 51% of the non-dividing cells were actively dividing. After 40 days in culture, both subpopulations were expanding at a similar rate with 12.4 doublings each. Identification of the subpopulations allows for an in depth understanding of the subpopulations. The quiescent cells may have the greatest proliferative capacity due to the fact that they are not actively dividing, and after transplantation, give rise to reparative progeny for successful regenerative medicine therapies.

Funding: National Institute of Arthritis and Musculoskeletal and Skin Disease (R03 AR053678)

Jennifer Boles

Neuroscience

Arts and Sciences

Faculty Mentor: Amy K. Wagner, MD

LEPTIN ASSOCIATIONS WITH POST TRAUMATIC SEIZURE AFTER SEVERE TRAUMATIC BRAIN INJURY

Posttraumatic seizures (PTS) are a common consequence of traumatic brain injury (TBI) and are also associated with mortality and poor outcome after injury. Leptin, a 16-kDa adipose derived hormone is known for its effects on metabolism, energy balance, reproductive regulation, the immune response, and neuroprotection. It has also recently been found to play a role in seizure activity. Rat models have been used to show that leptin prevents seizures by inhibiting hippocampal neurons by activating a large amount of Ca2+-activated K+ (BK) channels. However, in proconvulsant rodent models, leptin enhances NMDA receptor-mediated increases in intracellular Ca2+ levels and synaptic transmission in rat hippocampal cell cultures and brain slices. The purpose of this study was to explore the associations between leptin levels and the occurrence of PTS after TBI. This retrospective study included 145 adult Caucasians with a severe TBI. Time to first seizures and BMI were determined through a medical chart review. Leptin levels were determined with Cerebrospinal fluid (CSF) which was collected twice a day for 5 days post injury. Approximately 25% of the population experienced at least one documented seizure after injury. Higher mean leptin levels were associated with PTS occurring within one month post-TBI ($p=.005$). In addition, the average BMI for those who seized within a month was significantly higher than the average BMI for those who did not seize within the time period ($p=.046$). It appears that elevated average weekly leptin levels are associated with an increased risk of developing PTS.

Funding: DODW81XWH-071-0701

Sameul Brayer

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Amy K. Wagner

EXAMINING LEARNING STRATEGIES WITH THE MORRIS WATER MAZE SPATIAL MEMORY PARADIGM: A COMPARATIVE ASSESSMENT IN EXPERIMENTAL TRAUMATIC BRAIN INJURY

Historically the Morris water maze (MWM) has been utilized as a tool for assessing spatial learning and memory in rodents, by allowing animals to swim to an escape using proximal and distal cues for navigation. Questions remain about whether animals rely entirely on external cues for navigation, a spatial strategy, or also incorporate non-spatial search strategies, the effects of which may influence overall performance. We hypothesized that non-spatial pre-training prior to MWM testing would result in lower latencies for both acquisition and reversal trials during spatial testing. We further hypothesized that pre-training does not need to occur immediately prior to spatial testing and that training prior to the controlled cortical impact (CCI) model of traumatic brain injury (TBI) can be beneficial to MWM spatial memory performance two weeks post-injury. 39 Sprague-Dawley rats underwent CCI (2.8mm;4m/s) or sham surgery. 21 rats received non-spatial pre-training that was performed for 4 days prior to surgery with a dynamic hidden platform and static entry point. Motor testing occurred on post-surgery d1-d6 to ensure that motor ability returned to pre-surgical levels. MWM spatial acquisition trials were performed beginning 14d post-surgery. Significant improvements in swim latencies for acquisition trials were observed for the pre-trained CCI group compared to CCI rats without pre-training. This suggests that pre-training facilitated the use of learned behavioral strategy that was able to be utilized in rats with CCI. The work also highlights the importance of extra-hippocampal training and function as a relevant compensatory strategy for spatial learning in experimental TBI.

Funding: Department of Physical Medicine and Rehabilitation, University of Pittsburgh

Christopher Brett

Neuroscience

Arts and Sciences

Faculty Mentor: Amy K. Wagner, MD

OUTCOME AND INJURY SEVERITY ANALYSIS OF QUANTITATIVELY CATEGORIZED HYPOGONADOTROPIC HYPOGONADISM IN A POPULATION IN SEVERE MALE TRAUMATIC BRAIN INJURY (TBI) PATIENTS

Research has demonstrated derangements in chronic gonadal/adrenal serum hormone profiles in the first week following a TBI. Testosterone (TEST) and leutinizing hormone (LH) decline acutely to chronic depletion suggesting hypogonadotrpic hypogonadism. The purpose of this study was to examine hypogonadotropic hypogonadism serum hormone profiles in a male population after TBI with respect to outcome and injury severity. Male TBI subjects (N=38) had average hormone levels from blood samples taken acutely, every two weeks for the first 6 months and after 12 months post-injury This study defined hypogonadotropic hypogonadism quantitatively based on depleted TEST and LH serum levels with respect to normal hormone ranges according to the UPMC Laboratory. Injury severity and outcome were compared between hypogonadotropic hypogonadic subjects (HHS) and non-hypogonadotropic hypogonadic subjects (NHS). Injury severity measures included the Glasgow Coma Scale (GCS) and Injury Severity Scale (ISS). Outcome measures included Glasgow Outcome Scores (GOS), Disability Rating Scale (DRS), Functional Independence Measure (FIM), PHQ-9 depression scale, Quality of Life (QOL), and a cognitive composite measure. Injury severity was not significantly related to hypogonadism. Six month GOS, DRS, FIM, and cognitive composite scores for HHS were significantly worse ($P<0.05$) than NHS. Depression and QOL were not significantly different between HHS and NHS. NHS TEST, LH and E2 levels were consistently higher than HHS in the chronic phase. Male TBI patients with hypogonadotropic hypogonadism have worse outcomes and abnormal hormone levels chronically after injury.

Funding: Centers for Disease Control and Prevention (#R49/CCR323155), Department of Defense (#W81XWH-07-0701), & National Institute of Health (#P50NS030318)

Stephen Canton

Bioengineering

Swanson School of Engineering

Faculty Mentor: Debora Jacobs-Sera

ISOLATION, PURIFICATION, AND CHARACTERIZATION OF MYCOBACTERIOPHAGE AKOMA

Bacteriophages are viruses that infect bacteria. With over 1031 particles in existence, bacteriophages represent the most prevalent pool of unknown genetic information. These organisms are commonly found in areas with large populations of bacterial hosts. Mycobacteriophage Akoma, was isolated from a soil sample the North Oakland Area of the University of Pittsburgh campus. Using Mycobacterium smegmatis mc2155 as a host, my project is the characterization of Mycobacteriophage Akoma. This novel bacteriophage has been purified and prepared for sequencing. Once analyzed, its genome will be compared to over one hundred mycobacteriophages. The plaque morphology, physical structure, and restriction enzyme digest suggest that Akoma belongs to Cluster B. Using cluster specific primers, Akoma is believed to be in subcluster B3. This poster describes the physical and molecular characteristics known to date about Mycobacteriophage Akoma.

Funding: Howard Hughes Medical Institute

Aaron S Cantor

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Susan Kalisz

GARLIC MUSTARD-INVADED FOREST SOILS SUPPRESS FUNGAL HYPHAL GROWTH

Garlic mustard (Alliaria petiolata, Brassicaceae), hereafter GM, is a widespread invader of North American forests. Like some members of the mustard family, GM increases soil levels of allyl isothiocyanate (AITC), a powerful anti-fungal agent suspected to increase GM's invasive success. Low AITC concentrations are known to inhibit fungal spore germination in vitro, but the effect of GM's AITC on fungal growth within invaded soils is unknown. Therefore, we buried cellulose membranes in field plots with no GM (control) or with natural densities of GM at three sites for four weeks and then quantified hyphal colonization on the membranes using digital image analysis. We found that total hyphal length is significantly lower in GM vs. control plots, and mean hyphal length/image is significantly lower in GM vs. control plots at two sites. These results show that GM-infested soils suppress vital fungal growth and implicate that AITC produces this effect.

Funding: NASA Pennsylvania Space Grant Consortium

Evan Carder

Chemistry

Arts and Science

Faculty Mentor: Dr. Kazunori Koide

STUDIES TOWARD AN IDEAL FLUORESCENCE METHOD TO MEASURE PALLADIUM IN FUNCTIONALIZED ORGANIC MOLECULES

Detection and removal of toxic metals are crucial steps in the preparation of organic compounds. These steps, involving active pharmaceutical ingredients (APIs) and bioactive compounds, are particularly important for safe pharmaceutical practice and reproducible biological studies. However, detection of metals in synthetic organic compounds cannot always be achieved by currently used techniques, such as inductively coupled plasma mass spectroscopy (ICP-MS). False negative data for toxic metals are of particular concern due to the dangerous possibility of exposing the general public to tainted pharmaceuticals. Therefore, in order to ensure public safety, an alternative approach for quality control of synthetic compounds is warranted. Palladium, a common metal used in numerous organic reactions, is frequently found in synthetic compounds due to poor removal using typical purification protocols. Because ICP-MS is not likely to be a unified solution for the detection of palladium in all synthetic compounds, it is important to develop alternative methods that are sensitive, specific, rapid, and inexpensive. Furthermore, such methods must be able to detect residual palladium in the presence of APIs in a 5-10 ppm range, which is suitable for government regulations. Herein, we report an ideal fluorescence detection method, based off of the Tsuji-Trost deallylation reaction, which meets the necessary requirements for the detection of palladium in functionalized organic molecules. In our study, we account for the effects of sodium borohydride, temperature, phosphine ligand, and phosphate ions in kinetics. Such study has applications for synthetic chemists, pharmacists, and patients who are subjected to palladium-based drugs.

Funding: NSF

Anne Caruso

Neuroscience

Arts and Sciences

Faculty Mentor: Monica A. Perez

THE EFFECT OF FORCE DIRECTION ON MOTOR SKILL LEARNING

In healthy humans, bilateral isometric index finger forces in opposing directions exert differential changes in motor cortical function (Yedimenko and Perez 2010). It remains unknown whether the direction of force in one hand could influence motor skill learning by the contralateral hand. In this study, we examined the effect of index finger isometric forces in two different directions (abduction and adduction) with the right hand on the magnitude of motor skill learning by the left hand. During training, subjects were instructed to move a cursor following a series of target lines on a computer screen by performing 0-10% of their maximal abduction force with the left index finger while the right hand completed 30% of isometric index finger abduction or adduction. Each training period consisted of 10 sessions of practice. Within each session, three different targets were randomly presented eight times with a total of 24 figure presentations. There were 2 minutes of rest after each session was completed. We found that motor performance in the left hand improved to a larger extent when the right hand was performing abduction compared to adduction force. Similarly, the amount of error measured in the left index finger was significantly decreased when the right index finger completed abduction compared to adduction force. Our results suggest that motor skill learning in the non-dominant hand can be differentially affected by the direction of the force exerted by the contralateral hand.

Funding: National Institutes of Health

Brittany Charsar

Biological Sciences

Arts and Sciences

Faculty Mentor: Joe Martens

ISOLATION AND CHARACTERIZATION OF SPT16 MUTANTS THAT DEREPRESS SER3

Intergenic DNA comprises a major portion of eukaryotic genomes and is highly transcribed. Recently, transcription of intergenic DNA, leading to a non-coding RNA (ncRNA) product, has been shown to be a major player in the regulation of coding genes. Our lab has identified one ncRNA, SRG1, which has a regulatory role in gene expression, where the act of transcribing SRG1 represses its adjacent gene, SER3. In addition to SRG1, the essential yeast protein Spt16 plays a part in this repression of SER3 by maintaining nucleosome structure. Spt16 joins with other members of the FACT complex to reposition nucleosomes around RNA polymerase II during active transcription. Maintaining nucleosome architecture is important in denying other transcription factors access to SER3 promoter elements. To investigate the mechanism by which Spt16 represses SER3, a genetic screen was performed to isolate mutations in SPT16 that derepress SER3. spt16 mutants, generated through PCR-based mutagenesis, are being subcloned and retested for derepression of SER3 using a SER3-HIS3 reporter construct. Mutants that pass re-screening are then subjected to Northern analysis to assay endogenous levels of SER3. To begin categorizing spt16 mutants that derepress SER3, we are measuring Spt16 protein levels by Western analysis and testing for other phenotypes by performing growth assays on a variety of different media. Thus far, seven mutants have been identified that are expressed at wild-type levels and are specific to the regulation of SER3. Future experiments to characterize these Spt16 mutants will help us gain insight into how Spt16 functions mechanistically.

Funding: NIH

Olivia Creasey

Bioengineering

Swanson School of Engineering

Faculty Mentor: Dr. Rocky Tuan

EFFECT OF PARTIAL REPROGRAMMING ON IMMUNOPHENOTYPE OF ADULT STEM CELLS

Many tissue engineering approaches use adult mesenchymal stem cells (MSCs) to reconstitute or repair damaged tissues. For cartilage tissue engineering, a large number of stem cells are required. Lengthy in vitro expansion results in phenotypic changes in this cell population, namely decreased proliferation, down-regulation of MSC-associated surface markers, and decreased differentiation potential. We investigated how in vitro expansion changes the immunophenotype of MSCs, and whether partial reprogramming, which is the process of moving an adult cell along the pathway from limited multipotency toward pluripotency without returning it to a completely pluripotent state, returned late-passage cells to their early passage immunophenotype. MSCs from hip arthroplasy specimens with a passage number P8 or greater (late-passage MSCs) or P3 or less (early-passage MSCs) were fixed, permeabilized, and immunostained with antibodies to CD34, CD44, CD45, CD73, CD90, CD105, CD146, Oct4, Sox2, Nanog, SSEA3, SSEA4, TRA-1-60, TRA-1-81 (BD), Stro1 (BioLegend), and c-Myc (Abcam) to generate an immunophenotype. Immunostained cells were analyzed on a FacsDiva (BD), and the marker positivity was determined by analysis in FlowJo. Significant differences were determined using a two-tailed student's t-test. Nonstatistically significant trends were observed suggesting differences between early-passage and late-passage cells for the markers CD105 and CD146, and in particular SSEA4, which was higher in late-passage cells (96.5% positive) than in early-passage cells (3.04% positive). We observed no significant differences as a result of partial reprogramming for the markers that we examined. With a broader panel of markers, this technique may prove useful for selecting partially reprogrammed cells.

Fuding: Commonwealth of Pennsylvania Department of Health

Christopher Davis

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Karen Arndt

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

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. The Paf1 complex is a group of transcription elongation factors, consisting of Rtf1, Cdc73, Ctr9, Leo1, and Paf1, that associates with actively transcribing RNA polymerase II. Rtf1 is required for monoubiquitylation of lysine 123 in histone H2B, which subsequently directs the methylation of lysine residues in histone H3. Rtf1-dependent histone methylation aids in the maintenance of proper chromatin structure during transcription elongation. We have investigated the role of Rtf1 in this process by observing the effects of several different amino acid substitutions made within Rtf1. Particularly, these substitutions lie within a region known as the histone modification domain (HMD). The protein sequence of the HMD is highly conserved from the unicellular eukaryote S. cerevisiae to more complex organisms, including humans, suggesting its importance in eukaryotic transcriptional regulation. Accordingly, amino acid substitutions in the HMD lead to phenotypes associated with defects in transcription, including 6-azauracil sensitivity, loss of telomeric silencing, derepression of cryptic initiation, and an Spt- phenotype. We have assessed the ability of particular substitutions to disrupt methylation of histone H3 as well as ubiquitylation of histone H2B. We also show that the HMD is sufficient for ubiquitylation of histone H2B. Current work involves investigation of possible sources of extragenic suppression of rtf1 mutant phenotypes identified through a mutagenic suppressor screen.

Funding: National Institutes of Health and the Arnold and Mabel Beckman Foundation

Kyle DeHart

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Tia-Lynn Ashman

THE GREAT STRAWBERRY CAPER: SCIENTIFIC CURRICULUM DEVELOPMENT

Understanding science requires hands-on learning. We aimed to initiate the development of a biology curriculum that local area middle school teachers can utilize in their classrooms to teach students genetics and ecology. The basic premise is to use strawberries, purchased from local food markets, as a model system for hands-on, lab oriented activities. We began by addressing two questions:1. Is there phenotypic variation in store-bought strawberries? And is it attributable to the companies that supply them? If so it might indicate that they are different cultivars. 2: Can we use diagnostic microsatellite markers to a) successfully differentiate between berries from different companies if they indeed differ, or b) identify the cultivars? To answer these questions data was collected for several morphometric characters of berries and DNA was extracted from their sepals. PCR was conducted using two microsatellite primers known to be useful for fingerprinting strawberry cultivars and results were analyzed using GeneMapper. We discovered that there was a wide range of variation in strawberry size, seed number and dimensions, but that only the size differed significantly between the company sources. The results also showed that quality DNA could be extracted from the sepal tissue found on store-bought strawberries, even if it was old. Both microsatellite markers amplified products in the expected range in some of the samples. Combined these results provide promising framework for continued development and implementation of the middle school curriculum.

Fudning: Howard Hughes Medical Institute

Carly Deibler

Industrial Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Mary Besterfield-Sacre

EFFECTS OF TEAM DYNAMICS BEHAVIORS ON DESIGN ARTIFACT SCORES

The process of creating an innovative product for the first time in a design class can be a challenge for any student. While knowledge and diligent work are necessities, successful creation of the innovative product is also a function of team interactions. I examine which components of team interactions affect design artifact scores. I assert that positive team dynamics during different phases of the design process leads to higher-quality design and innovative projects. My objective was to identify the effects of three team dynamics behaviors on various design artifact scores. I also wanted to identify which subcomponents of team dynamics are most important to a design and at which stages of the project. I categorized team comments into the areas of team dynamics related to communication, collaboration, and decision making. I compared their team dynamics information to the actual scores received on their final product design to determine what, if any, relationship exists between the team interactions and the artifact scores. Data from 26 senior design teams from the University of Pittsburgh and the Rose-Hulman Institute of Technology was used in this analysis. My analysis also included a look at which phases of the project were crucial to team interaction in relation to the final output scores. The output scores on the final projects included the following criteria: Technical Performance & Standards, Documentation, Innovation, Working Prototype, and Overall Impact. I also examined the sum of these scores and the size of the teams in relation to the team dynamics categories.

Funding: Undergraduate Research Internship

Raphael Del Giorno

Neuroscience

Arts and Sciences

Faculty Mentor: Dr. Ariadne Letra

RECENTLY DESCRIBED MATRIX METALLOPROTEINASES ARE EXPRESSED DURING MOUSE PALATOGENESIS

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes responsible for degradation of the extracellular matrix and tissue remodeling during including embryonic development and palatogenesis. There are 23 MMPs described in humans so far which have been classified into different groups based on a combination of amino acid sequence homology, presence of specific peptide structural domains, and in vitro substrate specificity. MMPs are usually divided into collagenases, gelatinases, stromelysins, matrilysins, membrane-type metalloproteinases, and others, which include the more recently identified MMPs. Many MMPs have been detected during murine palatogenesis although no information is available about the expression of the more recently identified MMPs. We verified gene and protein expression of MMPs not yet described in mouse craniofacial tissues through real-time PCR and immunohistochemistry (IHC). For real-time PCR, we used full-length first strand cDNA derived from head and palate of mouse embryos at embryonic days (ED) 12, 13, 14, 14.5, and 15 and Sybr Green chemistry. Beta-actin was used as an endogenous control. Primers were designed using Primer3 software. For IHC, we used mouse head tissue sections at ED 13-15. The results showed differential temporal and spatial expression of MMPs 7, 10, 16, 25, 27, and 28 at critical stages for palatal development in mice, and correlate with the distribution of their substrates. Hence, the MMPs investigated in this study might also play a role during murine palatogenesis.

Funding: NIH/NIDCR R00-DE018954

Cassandra Delp

Acute & Tertiary Care

School of Nursing

Faculty Mentor: Dr. Mary Beth Happ

EXPLORING FAMILY'S PERCEPTIONS OF PATIENT COMMUNICATION IN THE ICU

Development and testing of effective communication strategies for critically ill patients who are unable to speak demands a better understanding of family perceptions of communication. To describe perceptions of communication by visiting family and friends regarding patient communication in the ICU. DESIGN: Cross-sectional self-administered survey of 133 family members or friends (visitors) of awake nonspeaking adult patients (orally intubated or tracheostomy without speaking valve). Family perceptions of communication quality, satisfaction with communication, and common methods used to communicate with their family member using a nine-item five point Likert type survey, administered ≥ 48 hours after patient admission to ICU. We received 57 completed surveys (response rate =42.9%). Three-quarters of respondents were women (n= 43), and were related to the patient as spouse (n= 20; 35%), sibling (n=16; 28%), and adult child (n=16, 28.1%). Respondents were equally satisfied (n=25) and dissatisfied (n=25) with communication. Forty nine percent (n=28) reported feeling uncomfortable communicating with the patient. Most reported feeling frustrated (n=36, 62%) and helpless (n=37, 64.0%) by not being able to communicate effectively with the patient. Although satisfaction ratings are equivocal, families report discomfort with communication and feelings of frustration and helplessness with the patient's impaired communication. The proportion of poor satisfaction ratings illustrates the need to develop and test new approaches for communication in this setting to reduce these negative experiences.

Funding: Robert Wood Johnson Foundation Interdisciplinary Nursing Quality Research Initiative grant # #66633 (Happ MB & Barnato AE)

Vincent DeStefino

Neuroscience

Arts and Sciences

Faculty Mentor: Dr. Bill Yates

RESPONSES OF NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA DURING CHANGES IN POSTURE: COMPARISONS BETWEEN CONSCIOUS AND DECEREBRATE ANIMALS

A region of the brainstem, the rostral ventrolateral medulla (RVLM), controls sympathetic nervous system activity and blood pressure. Prior studies in decerebrate or anesthetized animals showed that the RVLM integrates a variety of inputs, including those from baroreceptor and vestibular afferents; the latter signals are presumably involved in regulating blood distribution in the body during postural alterations. However, no prior studies have considered activity of RVLM neurons in conscious animals. The goal of the present study was to compare responses of RVLM neurons to moderate-amplitude body tilts in conscious and decerebrate cats. Single unit recordings were performed on RVLM neurons in 3 awake and 8 decerebrate cats. Individual neurons were assayed for 1) baroreceptor inputs and 2) modulation of activity during natural vestibular stimulation. A large fraction of RVLM neurons (51/110) responded to <15° tilts in decerebrate cats. In contrast, <2% of RVLM neurons in conscious animals responded to the same stimuli. Responses to vestibular stimulation were similar for cells that received baroreceptor inputs (exhibited cardiac-related activity or responded to carotid artery stretch) as those without such inputs. These data suggest that responses of RVLM neurons to body tilts are masked in conscious animals, perhaps because they are not beneficial during moderate-amplitude tilts. Future studies will examine the mechanism responsible for this response gating, and whether it can be modified during situations when postural changes that compromise blood pressure are more likely.

Fudning: National Institutes of Health (NIH)

Shannon Donofry

Psychology

Arts and Sciences

Faculty Mentor: Dr. Kathryn Roecklein

ASSOCIATIONS OF DRD4 7R AND DRD2 TAQ1A POLYMORPHISMS WITH BINGE EATING IN PATIENTS WITH SEASONAL AFFECTIVE DISORDER

The present study aims to investigate the relationship between variations in dopamine related genes, binge eating behavior, and weight gain in patients with seasonal affective disorder (SAD). Researchers have proposed the Reward Deficiency hypothesis to explain dopamine function as it relates to addictive behaviors such as overeating. According to this model, individuals whose dopaminergic systems are downregulated engage in rewarding behaviors to compensate for this deficit. To extend the work of Levitan (2004a), the Taq1A A1 restriction fragment length polymorphism (RFLP) linked to the dopamine-2 receptor gene has been included in the assay, in addition to the 7-repeat allele (7R) of the dopamine-4 receptor gene (DRD4), as both of these polymorphisms have been associated with blunted dopamine response and higher maximal lifetime body mass index (BMI) in multiple samples (i.e. Chang et al.,1996; Kaplan et al., 2008; Noble, 2000; Stice et al., 2008; Wang et al., 2001).Sixty- four male and female participants completed the SIGH-SAD, a modified version of the Hamilton Depression Rating Scale that includes a subscale structured to ascertain the presence of atypical depression symptoms seen in SAD. Additionally, participants completed the Questionnaire on Weight and Eating Patterns (QWEP), a self-administered survey designed to assess binge-eating behavior as defined in the DSM-IV (APA, 2000). Participants' height and weight were also measured in order to calculate BMI. DNA was collected using Oragene DNA self-collection units, and amplified with polymerase chain reactions (PCR). DRD4 PCR products were visualized through gel electrophoresis, while Taq1A genotypes were determined by utilizing high-resolution melt analysis (HRMA). Analysis and data collection is ongoing.

Tawny Duliba

Psychology and Neuroscience

Arts and Sciences

Faculty Mentor: Dr. Joseph H. Ricker

EFFECT OF PLEASANTNESS IN EPISODIC MEMORY WITH TBI AND HEALTHY POPULATIONS

Pleasant events have been found to be recalled better than unpleasant events by undergraduate volunteers (Walker, Vogl, and Thompson, 1997). The research has involved intact, healthy individuals, however it is not known whether individuals with Traumatic Brain Injury (TBI) exhibit the same pattern. This population supports this case because individuals with TBI are known to have memory complaints. Fifteen individuals with TBI (10 males) and thirteen intact controls (11 males) with no significant differences of age or education participated in a functional Magnetic Resonance Imaging (fMRI) study of episodic memory. They were presented multiple visual stimuli, such as words, shapes, letters, and pictures, and asked to rate the items as pleasant or unpleasant. The two populations were presented the stimuli again and were asked if they were reoccurring or not. Response times and accuracy values were collected during the recognition blocks and were then sorted based on whether the stimuli were rated as pleasant or unpleasant. In the TBI and healthy populations there was no significant difference of response time or accuracy between the groups. However, there was significance with pleasant responses being more accurately recalled. This study reinforces the idea that pleasant stimuli are more likely to be recalled than unpleasant stimuli and shows that this pattern can be found in persons with TBI.

Funding: NIH #R01 NS048178-01

Karthik Dwarki

Chemistry

Arts and Sciences

Faculty Mentor: Drs.Tony M. Plant and Paula Grabowski

HYPOTHALAMIC NEURONS EXPRESSING KISSPEPTIN, NEUROKININ B AND DYNORPHYN (KNDY NEURONS) CONTROL THE TIMING OF PUBERTY IN THE RHESUS MONKEY, A REPRESENTATIVE OF HIGHER PRIMATES.

Gonadotropin-releasing hormone (GnRH), a hypothalamic peptide that is secreted in a pulsatile fashion, provides the drive for the pituitary-gonadal axis that initiates puberty. Interestingly, in higher primates, robust GnRH pulsatility is also found in infancy. Thus, two hypothalamic postnatal switches control the timing of puberty in these species: one to turn off GnRH during infancy and the second to reactivate pulsatility late in juvenile development. In man, mutations of GPR54 are associated with delayed or absent puberty. The ligand for GPR54 is kisspeptin (KP), which is encoded by KISS1. In monkeys, expression of KISS1and release of KP in the hypothalamus increase at puberty. KP neurons also express neurokinin B (NKB), and mutations in this ligand and its receptor in man result in a phenotype similar to that seen in GPR54 mutations. The purpose of this experiment was to begin to determine whether decreased expression of hypothalamic KP and NKB underlies the arrest of GnRH pulsatility in infancy. Serial coronal 25-m hypothalamic sections (paraformaldehyde fixed) of three infant (GnRH on) and three juvenile (GnRH off) male monkeys were prepared. Sections (250-m between each) were stained for KP and NKB using a cocktail of primary antibodies (sheep anti-kisspeptin at 1:120K and rabbit anti-NKB at 1:6K) and the neuropeptides were detected with fluorescently tagged secondary antibodies. KP and NKB immunopositive perikarya were counted using a fluorescence microscope with a mobile stage allowing for systematic scanning of the arcuate nucleus. In infants, the number of KP perikarya/section ranged from 50-250, while only 15-150 perikarya/section were seen in juveniles. NKB was coexpressed in about 20% of the KP positive neurons and demonstrated no developmental change. These findings indicate that arrest of GnRH pulsatility during infancy, which leads to quiescence of the pituitary-gonadal axis during childhood, maybe due to a decrease in KP expression in KNDy neurons.

Funding: NIH R01 HD013254 and U54HD08160

Eussera El-Magbri

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Nancy Kaufmann

THE ELUSIVE ROLE OF THE BIG BRAIN AQUAPORIN IN BRAIN FUNCTION OF ADULT DROSOPHILA MELANOGASTER

Aquaporin-4(AQP-4) is a major cranial water channel near the interfaces of the blood-brain barrier(BBB) and the brain-Cerebrospinal fluid barrier(CSF) and is linked to the formation of brain edema. A promising new therapy for treating brain edema is by inhibiting AQP-4 expression. It is reported that Drosophila melanogaster has an aquaporin named big brain, which has regions of high-sequence homology to AQP-4. This aquaporin is shown to have a crucial function in fruit fly embryos during neurogenesis by controlling the number of neural precursor cells. However, big brain does not seem to be a water-permeable aquaporin. Better yet, little is known about big brain expression in adult flies, a key stage we chose to study in order to better understand aquaporins in mammalian brain physiology. We carried out controlled RT-PCR experiments with big brain primers to look for big brain expression in whole male adult flies, fly heads, and fly brains, and big brain seemed to be found in all three areas, especially the latter. Yet, the question remains as to why big brain is present in adult Drosophila. With further experiments, these new findings can potentially help answer the following questions: (1) what is the elusive function of the big brain “non-water-specific” aquaporin in adult flies, (2) what is the physiological significance of aquaporins, and (3) how can we control the regulation of fluid transport through manipulations of AQP-4 expression.

Funding: Howard Hughes Medical Institute

Tracy Fan

Health and Community Systems

School of Nursing

Faculty Mentor: Ann M. Mitchell, PhD, RN, FAAN

ASSESSING A WELLNESS EDUCATION GROUP INTERVENTION (WEGI) FOR ADULTS WITH CHRONIC SEVERE MENTAL ILLNESS (CSMI)

Chronic severe mental illness (CSMI) is a common disorder in the United States, with adverse effects that include: poorer physical and mental health, significantly shortened life-expectancy, increased poverty, and poorer health literacy. Health promotion educational interventions have been shown to be highly effective, and nursing students are well-qualified to implement a unique Wellness Education Group Intervention (WEGI). The purpose of this project was to implement and evaluate an 8-week WEGI in order to educate adults who have CSMI with knowledge related to specific health promotion activities and skills for disease prevention, utilizing upper-class undergraduate nursing students enrolled in the psychiatric nursing course. Quantitative data on consumer satisfaction show that the vast majority (94%) of participants liked something about the program and were glad they came. Qualitative data from Focus Group review sessions following the WEGI show that participants expressed general satisfaction with having attended the program. They expressed the desire for the program to continue, while some participants also expressed the achievement of specific personal health benefits, including healthy lifestyle modifications. There were three primary areas of self-reported interest and benefit to the mental health consumers who participated in the WEGI: socialization and support; education and information; and motivation to change. The WEGI is a successful and easily-implemented wellness education intervention program for adults with CSMI living in the community. The WEGI could serve as a model for other multidisciplinary undergraduate training programs and for wellness education in similar communities.

Jeffrey Fein

Physics and Astronomy

Arts and Sciences

Faculty Mentor: Dr. Steve Dytman

Light Injection Calibration of the Photo-Multiplier Tubes for the MINERvA Detector

MINERvA (Main INjector ExpeRiment for v-A) at Fermilab is an experiment designed to measure properties of neutrinos, a type of subatomic particle, with atomic nuclei. This is done by shooting a beam of neutrinos at a detector, where they interact with nuclei. The products of these interactions deposit energy in the detector, which correspondingly produces light that is converted into an amplified measurable electrical signal via Photomultiplier tubes (PMTs). The PMT gain, which is the amount of charge produced per input photon of light, must be measured to calibrate the detector. To measure the gain, MINERvA needs calibration to match electrical signals from 30,000 PMTs to energy deposited by interaction products. An external Light-Injection system sends controlled pulses of light down fiber-optic cables into a box where the pulses are diffused over a collection of PMTs. Over the past fifteen months I worked with the University of Pittsburgh MINERvA group, measuring systematic errors of the gain. Calibration data was taken from the detector PMTs in compiled intervals of 3 to 15 minutes each for several months. The comparison of data from consecutive intervals established the gain stability over short intervals. I then measured the gain stability over longer time intervals and found that it did not vary significantly from that over short intervals. I determined that the gain was stable over a period of several weeks.

Funding: Department of Energy

Brandon Fields

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Lewis 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 (unc-112) encodes a member of an integrin-containing, transmembrane attachment complex that forms muscle-muscle attachments, anchors 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 by drugs or RNAi knockdown 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 an unc-112ts mutant within 24 hours of disruption. This paralysis can be prevented by mutation or RNAi knockdown of the immunoglobin-like protein(s) encoded by the gene dim-1. Our data suggest that either the attachment complexes are highly dynamic structures, or new attachment complexes must be continuously formed to support body growth even after adulthood is attained.

Funded by NIH grant 5R01-AR054342. EAO supported by the Pennsylvania Space Grant Consortium, the Univ. of Pittsburgh, the Biochemical Society (UK), a Barry M. Goldwater Scholarship, and the Howard Hughes Medical Institute. BDF supported by the Howard H

Heather Friedberg

Computer Science

Arts and Sciences

Faculty Mentor: Diane Litman

TURN-TAKING CUES IN A HUMAN TUTORING CORPUS

Human conversation is a seemingly simple, everyday phenomenon that requires a complex mental process of turn-taking, in which participants manage to yield and hold the floor with little pause in-between speaking turns. Many linguists suggest that this is due to subtle turn-taking cues that we subconsciously relay and process while talking with others. Identifying these cues can help improve spoken dialogue systems, which often fail in replicating human turn-taking patterns. In this research, turn-taking in a human tutoring corpus of spoken conversations between a teacher and a student is examined and compared to that in other domains. Student speaking turns were divided into segments based on pause intervals, and split into two groups: YIELD if the next segment was the tutor speaking, and HOLD if the student continued his turn. Prosodic features were calculated for each segment, and means were compared between the YIELD and HOLD groups. Significant differences between the two for a feature would suggest that this feature is a turn-yielding cue; that is, it signals to the other person that the speaker is giving up his turn to talk. Preliminary results suggest that while certain prosodic features can be significant turn-yielding cues, this varies greatly by person.

Funding: National Science Foundation

Maxwell Garber

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Linda Jen-Jacobson

FLANKING SEQUENCE EFFECTS AND ACTIVE SITE REPULSION ARE COUPLED AT THE ECORI PROTEIN-DNA INTERFACE

Sequence-specific protein-DNA interactions lie at the heart of most biological processes. Because of their stringent discrimination against incorrect DNA sites, restriction endonucleases provide excellent models for understanding the rules that govern specificity. We have previously shown that the binding affinity of EcoRI restriction endonuclease can be dramatically affected by (1) sequences flanking the recognition site and (2) active site repulsion (electrostatic strain) arising from the apposition of negatively charged residues (E111, D91) to the scissile phosphate. Here we present data that reveal a link between these two seemingly unrelated determinants of binding. Substitution of an active site residue produces a mutant EcoRI endonuclease (E111A) which no longer shows flanking sequence preferences; that is, the E111A protein binds cognate sites flanked by different sequences with similar affinity. The conformational properties of flanking sequence control the precise geometry of protein-phosphate contacts, which are structurally "coupled" to the positions of base-recognition and active-site residues. We propose that suboptimal flanking sequence and active-site repulsion thus cause reciprocally coupled micro-adjustments throughout the complex. Relieving strain in either the active site (E111A) or the flanking region (best sequence) consequently relieves strain everywhere, thus optimizing protein-DNA interactions. We do not yet know if other charge alterations (e.g. D91A) in the active site will produce the same effects.

Funding: Samuel D. Colella Award, Department of Biological Sciences Mentoring Fellowship

Linda Glah

Neuroscience

Arts and Sciences

Faculty Mentor: Amy Wagner, MD

VOLTAMMETRIC ASSESMENT OF AMANTADINE EFFECTS ON DOPAMINE NEUROTRANSMISSION

Amantadine, typically known as a NMDA antagonist, has been used to treat Parkinson's disease. Also, amantadine is used as a treatment for patients with traumatic brain injury (TBI), and is often administered in conjunction with other dopamine agents like L-dopa or methylphenidate. Although effective in the clinical setting, amantadine's actual mechanism of action on dopaminergic systems remains a mystery. We use a voltammetric model to measure real time evoked dopamine overflow in the caudate nucleus caused by stimulating neurons in the medial forebrain bundle of the rat brain. Dose response studies have been conducted in order to evaluate characteristic changes from the baseline level of evoked dopamine. Several doses have been studied including 10mg/kg, 20mg/kg, and 40mg/kg (n=5) in each group. These drug effects were observed for at least 60 minutes after administration. Results show that 10 mg/kg and 20 mg/kg have no significant impact on dopamine neurotransmission. However, 40 mg/kg caused an averaged 70% increase in dopamine release one hour after drug administration. Overall, amantadine can be considered a long acting dopamine enhancer with peak changes in evoked dopamine occurring at 60 minutes post-drug administration. This dopamine enhancement may explain clinical observations of improved cognition, mood, and motor control. Current studies are now evaluating an animal model of TBI where a 40mg/kg dose response is compared in injured and naïve rats.

Funding: Institute for Rehabilitation Research and NIH R21NS057348

Michael Gowen

Neuroscience

Arts and Science

Faculty Mentor: Dr. Michael Levine

ELECTROPHYSIOLOGICAL ALTERATIONS IN PREFRONTAL CORTICAL PYRAMIDAL NEURONS IN A MOUSE WITH DEFICIENT FULL-LENGTH DISC1 PROTEIN EXPRESSION

Schizophrenia is a complex disease, the etiology of which is determined by both hereditable and environmental factors. In a large Scottish family, a positive association between schizophrenia and a point mutation in the DISC1 (Disrupted In Schizophrenia-1) gene was found; this mutation causes a premature stop codon, leading to a truncated Disc1 protein. As Disc1 is a scaffolding protein involved in neurogenesis, cell growth, and migration, its disruption may cause changes in neural circuitry leading to the symptoms of schizophrenia. Using electrophysiological methods we recorded currents from brains in a knock-in mouse model with a disruption in the murine homologue of DISC1 to determine the effects of this mutation (Li et al., 2007). We recorded and compared spontaneous and evoked synaptic currents in cortical pyramidal neurons from DISC1 mutant mice and their wild type (WT) littermates using whole-cell voltage clamp electrophysiology. Inhibitory synaptic transmission was isolated pharmacologically using the selective glutamate receptor antagonists NBQX and AP5, which block AMPA and NMDA receptors, respectively, and by holding the membrane potential at +10mV. We found that the amplitude of evoked inhibitory synaptic currents in cells from DISC1 mutant mice was larger than in WT mice, whereas both the frequency of spontaneous synaptic currents was lower in the DISC1 mice, relative to the control. These findings suggest that spontaneous and activity-dependent GABAergic synaptic currents are altered; this may play a role in the expression of symptoms seen in patients with schizophrenia.

Funding: The Amgen Foundation

Akash Goyal

Economics

Arts and Sciences

Faculty Mentor: Dr. Amy K. Wagner

MANIFESTATION AND PATHOPHYSIOLOGY OF MYELIN BASIC PROTEIN AND S100 CALCIUM BINDING PROTEIN IN HUMAN SERUM AND CEREBROSPINAL FLUID AFTER SEVERE TRAUMATIC BRAIN INJURY

Traumatic brain injury (TBI) annually affects 2 million Americans, and 50,000 of these individuals die each year. Such TBI causes cellular damage, which releases proteins into cerebrospinal fluid (CSF) and serum that can be measured using Enzyme-Linked Immunosorbent Assays (ELISA). Establishing a correlation between levels of proteins in serum and CSF can establish the brain specificity of that protein and justify serum measurement as a part of clinical care. Previous studies have shown changes in levels of myelin basic protein (MBP) and S100 calcium binding protein (S100B) after severe TBI thus identifying them as potential biomarkers. With adequate transfer of the biomarkers across the blood brain barrier after injury, serum and CSF would correlate. Using ELISA kits, the concentrations of MBP and S100B were measured in serum and CSF of 31 and 60 TBI patients, respectively. Samples were collected from day of injury to 5 days following injury. Concentrations were compared to protein levels from healthy controls and were associated with various demographics to detect potential significance. Age ($p<0.05$), gender ($p<0.10$), 6-month GOS ($p<0.05$) showed potential significance in certain markers. CSF and serum levels showed preliminary correlation for S100B ($r=0.185$), however the population will need to be increased in future studies to make further inferences. Using this information and respective correlation between serum and CSF, it may be possible to better understand how serum and CSF MBP and S100B levels correlate with one another, whether levels present in serum versus CSF are more closely linked to outcome, and further establish the viability of these proteins as TBI biomarkers for use in clinical care.

Funding: CDC Grant: #R49/CCR323155 and DOD Grant: #W81XWH-07-1-0701

Feng Guo

Health Promotion and Development

School of Nursing

Faculty Mentor: Denise Charron-Prochownik, PhD, RN, CPNP, FAAN

A MIXED METHOD APPROACH TO DESCRIBE THE KNOWLEDGE OF MOTHERS OF ADOLESCENT DAUGHTERS WITH DIABETES REGARDING PRECONCEPTION COUNSELING AND PREGNANCY

Female adolescents with diabetes are at risk for reproductive-health complications. Preconception counseling (PC) can minimize these risks. Mother's perceptions and knowledge of PC and pregnancy with diabetes can positively influence their daughters' decisions/outcomes. In a previous RCT, we examined the effects of a tailored PC program (READY-Girls) on teens with diabetes, but did not include their mothers. The purpose of this mixed method pilot study was to describe the mothers' qualitative and quantitative knowledge of PC and pregnancy with diabetes. Paper-pencil questionnaires were administered to 7 mothers of adolescent-daughters with diabetes. Qualitative data were evaluated using content analysis with a coding scheme; 100% agreement was achieved for inter-rater reliability. Quantitative knowledge was measured using 28-items with 2-subscales. Mothers' mean age was 48yrs (45-53yrs), 86% Caucasian, 86% college graduates. Top qualitative themes to knowing PC: "nothing" (57%,n=4), general pregnancy (43%,n=3), and physician recommendations (29%,n=2). Top themes of knowing pregnancy with diabetes: "nothing/very little" (57%,n=4), good control (43%,n=3), general complications (43%,n=3), and infant complications (43%,n=3). Knowledge scale of PC had a mean score of 70% (50%-86%); and pregnancy with diabetes had a mean score of 67% (50%-75%). Mothers lacked knowledge in PC and diabetes with pregnancy. Both (mixed) methods supported these findings. Many mothers said they knew "nothing", or knowledge tended to be more general. Providing an educational intervention to the mothers like READY-Girls could potentially increase their knowledge and elicit maternal support that could help to empower their daughters to make informed decisions with reproductive-health behaviors and prevent long-term complications.

Funding: R01 HD044097-01 from NIH-NICHD

Yeong Han

Department of Pharmaceutical Sciences

School of Pharmacy

Faculty Mentor: Yijun Huang

DATA MINING OF PDB DATABASE FOR FRAGMENT-BASED DRUG DESIGN

Data Mining of PDB Database for Fragment-based Drug Design

Fragment-based drug design is a new approach that uses X-ray crystallography or other physical techniques to successfully screen fragment libraries for specific binding to a target protein. The knowledge of where specific chemical fragments bind to a protein is readily available through protein data bank (PDB) and Relibase. Querying the PDB using Relibase allow for the discovery of thousands of small molecule fragments that bind to specific active sites of any proteins giving way to creating higher affinity compounds. Two prominent fragments for drug-design are piperazine and para-chlorobenzyl motifs. Chemoinformatic studies of the databases give hundreds of ligands that contain either of those two fragments. The further investigation provides how the fragments are interacting with specific amino acids on target proteins. This study will give the insight of new directions for drug design based on these two fragments.

Kyle Holden

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Yong Li MD, PhD

SYNTHETIC MATRIX METALLOPROTEINASE INHIBITOR, GM6001, SLOWS MUSCLE HEALING PROCESSES AFTER INJURY

Matrix metalloproteinases are zinc-dependent endopeptidases that aid in tissue remodeling through the degradation of various components within the extracellular matrix. Previously, we have detected that MMP1 is effective in overcoming the barrier of fibrosis, which can hinder successful restoration of the damaged tissue, and ultimately lead to loss of function. To observe the mechanism behind MMP1 in the muscle healing process, the purpose of this experiment is to investigate regenerative processes and fibrosis formation in injured muscle through the use of the synthetic broad-spectrum MMP inhibitor, GM6001. C2C12 myoblasts were evaluated using immunocytochemistry to assess for the presence of stem cell markers (Sca1 and CD34) after MMP1 stimulation and after MMP1 stimulation followed by treatment of GM6001. Also, immunocytochemistry was used to evaluate the myogenic differentiation capabilities of C2C12 myoblasts that were either stimulated with MMP1 or treated with GM6001 after MMP1 stimulation. Lacerations to the gastrocnemius muscle were used to represent a skeletal muscle injury in the murine model; the areas of injury were treated with either MMP1 or GM6001 and assessed for fibrotic scar formation. The application of the synthetic MMP inhibitor, GM6001, resulted in increased fibrosis, decreased myogenesis, and the reduction of the percentage of cells expressing stem cell markers within a population in vitro. These findings have implications in furthering the knowledge of the roles of MMP1 in muscle healing and regeneration as well as providing possible insight into the mechanisms behind these processes.

Funding: Grant of Research Advisory Committee, Children's Hospital of UPMC

Daniel Holohan

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Jeffrey Hildebrand

Designing Proteins to Spatially Regulate Actomyosin Contractility

Shroom 3 (Shrm3) is an actin-binding protein that influences epithelial morphogenesis during development by establishing contractile actomyosin networks. Shrm3 regulates these networks by recruiting Rho-kinase (Rock) to the tight junctions (TJs) that are located at the apical domain of the lateral membrane, where it causes contraction by activating myosin II. To determine if Shrm3 can be bypassed in this pathway, we sought to generate a version of Rock that is directly targeted to the TJs. To accomplish this, we generated a chimeric protein consisting of the localization domain of Shrm3 & the kinase domain of Rock. Individually, it was shown that Shrm3 localizes to F-actin stress fibers and adherens junctions in epithelial cells. Furthermore, it was shown that constitutively active Rock increases the formation of F-actin in cells, while dominant negative Rock decreases the presence of F-actin. In future experiments, the chimeric protein construct will be transfected into epithelial cells to see if the it directly targets the kinase domain to TJs and causes apical constriction. The morphological changes will then be compared to those induced by the Shrm3 and Rock constructs.

Funding: Biological Sciences Department through HHMI

Christopher Horvat

Math

Arts and Sciences

Faculty Mentor: Bard Ermentrout

SPIRAL WAVES IN FLEXIBLE MEMBRANES

Rotational spiral waves are a common find in many disjoint areas of the life sciences. Especially notable is experimental evidence for their existence in both heart and brain tissue. For modeling these organs, it is often convenient to use the mathematical study of flexible membranes. Here, we present that spiral wave formation is evident for even small-scale specific flexible membrane problems, using a number of different models.

Funding: NSF RTG

Jennifer Huling

Bioengineering

Swanson School of Engineering

Faculty Mentor: Dr. Frank Witte

THE DEVELOPMENT OF A HISTOCHEMICAL STAINING METHOD FOR DETECTING ACETYLCHOLINESTERASE IN SOFT TISSUE, BONE AND CULTURED CELLS

Magnesium metal offers a viable option for a biocompatible and biodegradable material. In bone, the corrosion of magnesium causes temporarily increased bone growth by increasing osteoblast activity. The enzyme acetylcholinesterase (AChE), typically thought to be involved with cholinergic synapse function, has been found to play a role in osteoblast function. The goal is to be able to monitor AChE as an indicator of osteoblast behaviour and a measure of the change induced in bone because of magnesium corrosion. To develop a staining method for the detection of AChE, soft tissue and bone samples, as well as cultured cells, were stained using the partially established Karnovsky and Roots method and immunohistochemical methods. Karnovsky and Roots staining was found to only work on frozen sections of tissue. The mouse-anti-AChE clone ZR3 antibody was found to be ineffective as a primary antibody, but the clone HR2 was found to work with both enzyme and fluorescent staining methods in frozen sections and those embedded in paraffin and plastic. Further studies can apply the developed method of positively identifying AChE in bone to help determine AChE's role in osetoblast behaviour and how magnesium corrosion stimulates increased bone growth.

Funding: Hannover Medical School

Alex Ireland

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Jeff Brodsky

The Identification of Small Molecule Polyomavirus Inhibitors Based on a Novel Scaffold

Polyomaviruses are common viral agents in the human population, and can become pathogenic in immunosuppressed populations. Because most treatments non-specifically target DNA replication, no treatments for polyomavirus infection without undesirable side-effects are currently available. However, all polyomaviruses express a Large Tumor Antigen (T Ag), which is a multi-domain, 708 amino acid protein. The replication of SV40 (Simian Virus 40), a member of the Polyomaviridae family, requires two domains within the viral-encoded T Ag: The ATPase domain, which serves as a helicase for viral DNA, and the N-terminal J domain, which stimulates the activity of host-encoded Hsp70 molecular chaperones. Previous screening of pyrimidinone-peptoid hybrid compounds in our laboratories identified MAL2-11B as an inhibitor of viral replication, Hsp70 stimulation, and T Ag ATP hydrolysis (IC50 ≈ 50 μM). Therefore, we proposed that the MAL2-11B scaffold might be co-opted to identify more effective inhibitors of T Ag ATPase activity and viral replication. We now report that a MAL2-11B tetrazol derivative inhibits T Ag ATP hydrolysis (IC50 ≈ 20 μM). The same derivative also displays significantly more potent inhibition of T Ag-mediated Hsp70 ATP hydrolysis than MAL2-11B. Moreover, the tetrazol is more effective at inhibiting the replication of viral DNA in SV40-infected cells, possibly because the derivative is anticipated to possess a greater ability to permeate the membrane than MAL2-11B. We are currently screening additional tetrazol-based MAL2-11B derivatives to elucidate a structure-activity relationship. We suggest that these may provide a specific, novel therapeutic treatment for polyomavirus infection and related disease.

Funding: Beckman Scholars Program

Helmet Karim

Health Information Management (HIM)

School of Health and Rehabilitation Services (SHRS)

Faculty Mentor: Leming Zhou

MANUALLY ANNOTATING GENES IN MULTIPLE DROSOPHILA GENOMES USING EVIDENCE-BASED APPROACH

As sequencing technology improves, we have access to an increased amount of fully sequenced genomes. The first and most important step in analyzing these genomes is gene annotation, that is, accurately identifying the locations of genes along the genome and determining the function of these genes. After annotations of genes within a species genome, many further analyses can be performed to obtain more meaningful understanding of the genome. In this work, we have manually annotated sequences from a few Drosophila species (D. erecta, D. majovensis, D. grimshawi) using strictly computational methods and an evidence-based approach. This particular organism is selected because Drosophila species are extensively used in basic science and biomedical research, twelve genomes of Drosophila species have been fully sequenced, and the genome of Drosophila melanogaster is well annotated. This manual gene annotation project utilizes ab initio gene finding programs, the accurate D. melanogaster gene models, the BLAST search programs, and the UCSC genome browser to determine the precise locations of each coding exon in genomic sequences of multiple Drosophila species. The obtained gene models for these species are then compared to their ortholog genes in D. melanogaster to determine the function of these genes and gene exon-intron structure changes. This comparison can also show sequence divergence of multiple Drosophila species at different evolutionary distances in both coding and non-coding regions. The obtained results in this project have been submitted to a central database and more data will be accumulated to perform further analysis.

Fudning: NSF IIS-0938393 and a funding from HHMI.

Joseph Kaus

Chemistry

Arts and Sciences

Faculty Mentor: Dr. Lillian Chong

EFFICIENT EXPLICIT-SOLVENT MOLECULAR DYNAMICS SIMULATIONS OF MODEL MOLECULAR RECOGNITION SYSTEMS

Protein binding is a rare event because a long time is spent waiting for the event to occur, rather than the duration of the event itself. This makes it difficult to study using standard molecular dynamics simulations. The goal of this research is to develop a computational approach for simulating these rare events. This approach is known as the weighted ensemble (WE) approach. This technique enhances the efficiency of computer simulations of rare events. It uses computer time more efficiently by spending less time simulating molecules that are not likely to bind, and more time simulating molecules that are likely to bind. It does this by tracking how close the molecules are to binding, making copies of the simulations that are closer to binding, and terminating simulations that are farther from binding. Each simulation is assigned a statistical weight, so making copies of a simulation or terminating simulations does not bias the results. This technique gives the pathways of binding (which is a movie of all of the different ways the molecules bind), as well as the relative rate at which the molecules bind. Here we compare the results and efficiency of WE approach to that of standard molecular dynamics simulations on four model systems. Our results reveal that the WE approach is at least four times more efficient at sampling these molecular association events.

Funding: NSF CAREER; Brackenridge Fellowship; Office of Experiential Learning

Matthew Keddie

Chemistry

Arts and Sciences

Faculty Mentor: Alexander Star

CARBON NANOCAPSULES: TAILORED LOADING THROUGH SYNTHETIC CONTROL

The synthesis of nitrogen-doped carbon nanotube cups through chemical vapor deposition (CVD) has created an intriguing form of multi-walled carbon nanostructure. By performing CVD with metal catalyst, ethanol, and a nitrogen source of ammonia, individual segments are synthesized in a “stacked cup” conformation. We have observed using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) the diameters of such architectures are contingent upon metal catalyst diameter. In addition, we have discovered individual segment length is highly dependent upon nitrogen concentration. Based on this work, we have tailored the volumetric parameters of these nanostructures. Furthermore, separation of size-controlled segments is promoted via mechanical grinding, where individual cups are isolated from their fibrous shell. Two individual cups can be cross-linked using glutaraldehyde to form a nanocapsule, upon which particles can be enclosed. The capsule lengths are highly dependent on the quantity of particles that can be contained. Based on their size, shape, and inherent hollow interior cavity, these materials possess limitless potential to be utilized as media for energy storage and biomedicine. This methodology serves as a foundational understanding of employing an engineered system as an eventual segue into the realm of drug delivery.

Funding: The National Energy Technology Laboratory

Devin Knisely

Acute Tertiary Care

School of Nursing

Faculty Mentor: Dr. Paula Sherwood

LONGITUDINAL PATTERNS OF DEPRESSION IN PATIENTS FOLLOWING ANEURYSMAL SUBARACHNOID HEMORRHAGE

Depression is common following aneurysmal subarachnoid hemorrhage (aSAH). Most studies use population-average analyses masking identification of distinct groups/patterns of change over time; data vital to designing and implementing interventions. This analysis used group-based trajectory analysis with data from persons with aSAH to determine patterns of depressive symptoms over the year following hemorrhage. Data from adult patients (n=193) with a moderate to large aSAH and no pre-existing neurologic disease were recruited at insult (R01–NR001339). Depressive symptoms (Beck Depression Inventory II) were assessed during face-to-face interviews at 3 and 12-months following hemorrhage. Group-based trajectory analysis (SAS) was conducted using clinical/statistical criteria to determine number and shape of trajectory groups. Analysis identified two distinct linear trajectories. Most participants (75%, n=145) had low levels of depressive symptoms at 3-months (mean=6.46, SE=1.1), scoring below the established cutoff for being at high risk for clinical depression. One-quarter (25%, n=48) were at moderate risk for depression at 3-months following hemorrhage (mean=20.56, SE=1.95). Neither mean significantly changed from 3 to 12-months following hemorrhage (p=0.86, 0.70 respectively). Data suggest two distinct patterns of depressive symptoms exist. One-quarter of the aSAH population may be at moderate risk for depression, remaining unchanged at 12-months. Future analyses should identify characteristics of each group to allow targeted interventions.

Funding: NIH R01 NR004339

Melissa Knox

Psychology

Arts and Sciences

Faculty Mentor: Judith A. Erlen

DIARY USE AND MEDICATION ADHERENCE IN CONTROL SUBJECTS

Diary keeping of medication taking can assist self-monitoring, often a component of adherence interventions. The purpose of this study is to examine diary use and medication adherence in a control arm of a randomized study. This sub-study of 97 subjects from a control condition of a randomized clinical trial on behavioral interventions to improve HIV medication adherence examines the use of diaries and their relationship to changes in medication adherence. Seventy-eight subjects recorded their medication taking using paper diaries over 18 months. Adherence was measured with a self-report medication adherence scale (SMAS) and electronic event monitoring (EEM) recorded at baseline and 18 months. Open ended exit interviews assessing self-perception of improved adherence were completed after final follow-up at 18 months (n=74). Change in adherence for each measure was categorized as either 1) improved or 2) maintained or diminished. Findings demonstrate no relationship between diary keeping and adherence. While participants reported improved adherence during the study with the SMAS (t=1.758, p=.083), EEM indicated a decrease in adherence (t=-2.404, p=.018). Neither measure related to diary use (SMAS: Χ^2=1.557, p=.212; EEM: Χ^2=.084, p=.772). On interview, 39 of the control subjects reported improved perceived adherence from study participation; this also was not related to diary use (Χ^2=.056, p=.813). Diary keeping was unrelated to medication adherence in the control group in this clinical trial suggesting that diary use was not an intervention. Because of subject perception, future medication adherence studies should examine the role of diary keeping on control subjects.

Funding: NIH, NINR 1R01 NR04749

Belinda Lao

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Lydia Daniels

LEPTIN IN ASSOCIATION WITH NEUROINFLAMMATION AND OUTCOME AFTER SEVERE TRAUMATIC BRAIN INJURY (TBI)

The hormone leptin is usually recognized for its role in metabolic regulation and energy balance. However, leptin has been shown to exhibit neuroprotective and anti-apoptotic functions in the human brain, as well as a potential role in the formation and modulation of the immune response. This study investigates the cerebrospinal fluid (CSF) levels of leptin in association with pro- and anti-inflammatory markers and markers of cell adhesion (sICAM-1 and sVCAM1) in severe adult traumatic brain injury (TBI) patients to determine trends in relative marker levels for informing prognosis and effective management of the inflammatory response following TBI. CSF was collected from the 14 patients within an acute period of 5 days (144 hours) after the TBI, through an EVD (extraventricular drain) every 12 hours. Eleven healthy control samples were obtained from volunteers who met the established criteria for health and demographic. The leptin concentration was tested using ELISA (Enzyme-linked immunosorbent assay), while cytokines (IL-1, IL-6, IL-8, IL-10, TNFa), sVCAM-1 and sICAM-1 were measured by xMap Luminex technology. Patient medical records were utilized to document incidence of sepsis and other complications in TBI patients. The aim of the study is to describe the relation between leptin and the formation of immune response in TBI patients while controlling for factors like septic complications and demographic background.

Funding: DODW81XWH-071-0701

Naomi Latorraca

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Michael Grabe

MEMBRANE BENDING STABILIZES TRANSMEMBRANE PROTEINS CONTAINING CHARGED RESIDUES

The lipid bilayer of cellular and organellar membranes deforms to stabilize the inclusion of transmembrane (TM) proteins. Previous studies have demonstrated that membrane bending helps to expose proteins' charged residues to the polar extracellular environment and phospholipid head groups. The present study uses a computer search algorithm to generate a membrane boundary curve that most stably accommodates the inclusion of a simple TM -helix. The algorithm then uses continuum electrostatics, which assigns constant dielectric values to the extracellular and bilayer environments, to calculate the peptide's minimum free energy of insertion into the bilayer based on the membrane's configuration. Peptides used in these simulations consisted primarily of hydrophobic residues, with a central position containing one of each of the twenty amino acids. The individual insertion energies obtained for these peptides permitted the construction of a biological hydrophobicity scale, which ranks the set of amino acids in terms of their individual apparent free energies of insertion. The data compare favorably with existing hydrophobicity scales, falling within range of both experimental and computational values. Additionally, the algorithm was tested on other simple peptides, including those with multiple charged residues. This novel method demonstrates the sensitivity of the membrane's ability to find energetically favorable accommodations for proteins positioned at the water-lipid interface, providing a computationally efficient way to model essential aspects of protein-membrane interactions.

Funding: Howard Hughes Medical Institute 2010 Summer Undergraduate Fellowship to N.L. and NSF CAREER Award to M.G.

Cory Leeson

Bioengineering

Swansons School of Engineering

Faculty Mentor: Dr. William Wagner

SPATIAL CONTROL OF GENE EXPRESSION IN A POLYMERIC SCAFFOLD

Tissue engineering and regenerative medicine are promising approaches to restore healthy tissue after injury – such as myocardial infarction. One important approach to encourage regeneration is to create healthy heart muscle in vitro for implantation in the place of damaged myocardium. Although controlling cell behavior within a scaffold is a challenge, such control could allow the generation of complex tissue constructs. Control can be achieved by inducing expression of desired genes. Inducer molecules, such as RheoSwitch Ligand 1 (RSL1), can provide control of target genes in cells transfected with the corresponding gene expression system. In this project, a biodegradable elastomer important for tissue engineering was utilized to construct a highly porous scaffold with defined regions where RSL1 was either present or absent. Cells grown on this scaffold expressed the reporter green fluorescent protein (GFP) predominately in regions of the scaffold that contained RSL1.The normalized green/blue ratio (GFP to blue nuclear stain) of image intensity data across the center of the scaffold confirmed a localized control of GFP expression. The ability to spatially control gene expression within a cell seeded scaffold may open opportunities to drive cell behavior toward the generation of complex tissue constructs.

Funding: National Institute of Health

Christopher Lippert

Mechanical Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Gregory Reed

Sample Survey of Smart Grid Approaches and Technology Gap Analysis

Research and development in the field of “smart grids” is advancing at an ever expanding rate, with an increasing number of industry participants and other key constituents internationally, including government entities and educational institutions. This survey was undertaken with the intent of determining representative approaches from various participants, and combining them into an overarching view of the industry as a whole. As a result, the more practical and efficient methods of improving the electrical grid were revealed, as well as gaps within the existing technology and standards. The most apparent gaps were in the following main areas: common communications; improved transmission and distribution controls; real-time information and incentives for both the end-user and the utility; self-healing grids; energy storage and renewable integration; and improved standards for the industry. In particular, our research group has begun development of a computer model to simulate the performance of energy storage for load management on a distribution substation. Using the loading data from a substation, it will model the effects of installing various levels of energy storage on that substation. This will allow for the determination of ideal energy storage sizes for a particular substation, which will be essential to the economic implementation of the smart grid.

Funding: Mascaro Center for Sustainable Innovation (MCSI)

Hei Ma

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Zhou Wang

GENETIC ANALYSIS OF FACTORS INFLUENCING ANDROGEN RECEPTOR LOCALIZATION USING YEAST AS A MODEL SYSTEM

Androgen receptor (AR) is a key molecule in the development and progression of prostate cancer, which is a major disease among men in the United States. In normal prostate, AR regulates androgen response genes expression in a ligand-dependent manner. In the absence of ligand, AR is localized in the cytoplasm and is transcriptionally inactive; however, it is translocated into the nucleus and becomes active after ligand binding. It has been shown that in castration resistant prostate cancer, AR remains in the nucleus regardless of ligand availability. The mechanism of how this occurs remains unknown. In order to gain more insight into the pathway mediating AR intracellular trafficking, we seek to identify molecular factors that influence this process using S. cerevisiae (budding yeast) as a model system due to its genetic tractability. Previous work in our lab has identified a novel nuclear export signal (NES-AR) in AR that is necessary and sufficient for AR cytoplasmic localization. Yeast expressing a fusion protein containing GFP, NLS-SV40, and NES-AR was subjected to transposon mutagenesis and the localization of such protein was examined under fluorescence microscopy. Mutants defective in mediating cytoplasmic localization of the fusion protein will be identified. The results from this study will give us a better understanding of AR localization pathway in hopes of developing treatments that modulate AR activity in humans to prevent or delay prostate cancer progression.

Funding: NIH R37 DK51193 and R01 CA 108675

Catherine Madden

Chemistry

Arts and Sciences

Faculty Mentor: Christopher Matranga, National Energy Technology Laboratory

SYNTHESIS AND CHARACTERIZATION OF A SERIES OF PORE FUNCTIONALIZED PILLARED-LAYERED METAL ORGANIC FRAMEWORK TYPE MATERIALS FOR CO2 CAPTURE

Pillared-layered compounds are an exciting material which show promise as CO2 capture agents. They belong to a general class of compounds called Porous Coordination Polymers (PCPs) which are formed by reacting transition metal ions with various organic linker molecules. The chemistry used to synthesis these compounds creates a material whose pore size, network type, and chemical makeup is controlled in a directed and rational manner by the choice of metal ions and organic linkers. In this regard, pillared-layered compounds are closely related to metal organic frameworks (MOFs), but typically have distinct performance and stability advantages over traditional MOFs. One of the largest obstacles in making new PCPs for CO2 applications is creating the organic linkers needed for the pore walls of the structure. The addition of different functional side groups on these linkers allows one to modulate the chemical affinity of the PCP pore wall towards CO2 or other impurity gases in the flue of a coal fired power plant. To this effort, several novel functionalized bi-pyridyl derivatives containing -NH2, -NO2, -CH3, -F, -C(O)H, -C(O)OH, and -OCH3 groups were synthesized by either an amidification reaction or the Suzuki coupling reaction. These linkers were then incorporated into a pillared-layered material to create new pore- functionalized PCPs. Each of these structural analogs was evaluated for CO2 capture and separation applications using isotherm and porosimetry techniques. From this work, the systematic effect of adding these functional groups to the bi-pyridyl linkers was assessed and will be used to optimize these CO2 capture materials.

Funding: Mickey Leland Energy Fellowship (Department of Energy)

Michael Mihalevic

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Jeffrey Brodsky

CHARACTERIZATION OF THE ROLE OF SMALL UBIQUITIN-LIKE MODIFIER (SUMO) IN CELLULAR QUALITY CONTROL

Molecular modifiers function to confer new properties upon their target proteins. For example, binding of a particular modifier can either activate or deactivate a protein, or even change its distribution in the cell. An interesting attribute of these molecules is the impact that they may have on protein stability and cellular quality control in response to cell stress. Upon binding of molecular modifiers a protein could be protected from, or targeted for, degradation. One prominent molecular modifier is ubiquitin; its binding to a protein can act as a signal for proteasome-mediated degradation. We propose that Small Ubiquitin-like Modifier (SUMO) is involved in the cellular stress response. In our experiments we subjected cells with mutations in the SUMOylation pathway to assays that measure various types of cellular stress. We observed that the strains were vulnerable to exposure to the reducing agent dithiothreitol (DTT), which unfolds proteins in the Endoplasmic Reticulum, as well as chemicals that induce general protein misfolding and osmotic stress. Additionally, we performed cycloheximide chase assays to evaluate if the degradation of specific substrates in these mutant backgrounds was altered. Results of the experiments revealed that deSUMOylation plays an important role in the degradation of a model misfolded substrate CPY*. Future studies aim to elucidate the function of SUMO in protein degradation. For example, cycloheximide chases and immunoprecipitation assays will reveal if CPY* is SUMOylated or if the defect degradation occurs indirectly.

Funding: Chancellor's Undergraduate Research Fellowship

Kaitlin Mitchell

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Graham Hatfull

IDENTIFYING MYCOBACTERIOPHAGE GENES CYTOTOXIC TO MYCOBACTERIA

Bacteriophages, viruses of bacteria, have evolved to effectively take over and utilize the cellular machinery of their host. While the major processes of phage infection are well known, we sought to identify single phage gene products that interact with host proteins to inhibit cell growth. Genes of unknown function from mycobacteriophage Giles were chosen for screening with a cytotoxicity assay. Giles was chosen for this experiment because there is a large amount of information known about its gene requirements and expression acquired through work in our lab. Individual genes were cloned into a nitrile-inducible vector system, allowing for controlled gene expression. Twenty-four constructs were made and transformed into Mycobacterium smegmatis mc2155, a non-pathogenic relative of M. tuberculosis, and are being tested for their effect on cell survival. The LysA gene from phage L5 was used as a positive control, as it encodes a protein shown to lyse cells. Genes identified as cytotoxic will then be tested on M. tuberculosis. The study of such genes and the interactions of their gene products with host proteins will allow us to discover potential drug targets and aid in future development of anti-tuberculosis drugs.

Funding: HHMI

Leah Nissle

Biological Science

Arts and Sciences

Faculty Mentor: Arundhati Ghosh, Ph.D

TEACHING TECHNIQUES IN UPPER-LEVEL UNDERGRADUATE ANIMAL PHYSIOLOGY COURSE

A pilot study was done in an upper-level undergraduate Animal Physiology course in order to determine the effectiveness of various teaching techniques. Students were mostly juniors and seniors, preparing for Medical, Dental, Veterinary Schools or Graduate schools. Students were given two types of worksheets: one with direct questions purely from the textbook and lectures, the other with a case-based questions (having to do with a real life situation or in association with the organ system studied that week in the lecture). After completing each worksheet, students were surveyed about their thoughts on if the assignment helped them retain and understand the information for the exam, and if the assignments prepared them for a real life situation or a patient, among other things. Many students felt that the text-based questions were effective in helping them prepare for an exam, but that the questions did not prepare them for a real life situation. More students felt that the case-based lessons were effective or very effective than were the text-based questions. Based on the students' opinions, the case-based lessons were more successful in teaching the topics on the exam. Case-based studies have prepared them better for the professional schools.

Alexis Nolfi

Bioengineering, Psychology

Swanson School of Engineering, Arts and Sciences

Faculty Mentor: Steven Abramowitch, PhD

BONE TISSUE ENGINEERING AS A SCIENCE AND MATH LEARNING MODEL FOR YOUTH OUTREACH EDUCATION

The Pittsburgh Tissue Engineering Initiative (PTEI) has created an outreach program to encourage middle and high school students to pursue continued education in the science and math fields. PTEI's program in Pittsburgh, "A Starfish Can Grow A New Arm, Why Can't I?", and North Carolina A&T State University's program "Nano-to-Bio Summer Camp" have allowed approximately seventy students to develop quantitative, analytical and technical skills in a simulated laboratory environment. Based on current research, modules were created to engage and involve students in tissue engineering (TE) activities. Original bone tissue engineering modules were established to teach the major components of the TE triangle including signals, scaffolds, cells and blood. One of the main objectives was to teach the students the properties and materials of a tissue engineered scaffold. Students created a scaffold using poly(lactic acid) and performed an assay exploring the toxicity of various scaffold degradation products. Campers were taught the importance of adding signals such as growth factors to scaffolds in order to promote bone formation. Another main objective was to explore the mechanical properties of artificial materials such as joints and blood. Lastly, students explored the structural properties of bones and how these properties are used to design bone grafts in regenerative medicine. Students were required to take pre and post tests evaluating their knowledge of tissue engineering. These tests indicated a mastery of basic tissue engineering principles. This confirmed that the new and previously used modules were relevant and exciting to students. Future work will include improvements to the activities and development of new activities based on student feedback and integrating the modules in middle and high school classes. New models may also be developed to incorporate additional subject matter.

Funding: University of Pittsburgh BioE REU Summer Program

Amenawon Ogiefo

Biological Sciences

Arts and Sciences

Faculty Mentor: Graham Hatfull and Deborah Jacobs-Sera

The Microbiological Chacterization of Mycobacteriophage DeadP

Bacteriophages are viruses that infect bacterial hosts. They are the most common and diverse organisms on Earth, and collectively comprise the single largest pool of genetic information. Mycobacteriophages are important because of their ability to infect the mycobacteria, the most notable of the genus being Mycobacterium tuberculosis, the causative agent of human tuberculosis. Mycobacteriophage DeadP was isolated from a soil sample in the South Oakland region of Pittsburgh, PA. Mycobacteriophage DeadP forms small, turbid plaques with a medium-sized 'halo' on a lawn of M. smegmatis, a fast-growing, non-pathogenic relative of M. tuberculosis. This study describes the isolation, purification and microbiological characterization of Mycobacteriophage DeadP through electron microscopy and restriction digest analysis, as we prepare to send DeadP for 454 pyrosequencing.

Funding: Howard Hughes Medical Institute

Yewande Olugbade

Biological Sciences

Arts and Science

Faculty Mentor: Dr. Anthony Kline

PERSISTENT DELETERIOUS EFFECTS ON SPATIAL LEARNING FOLLOWING CHRONIC ADMINISTRATION OF HALOPERIDOL OR RISPERIDONE AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Antipsychotic drugs (APDs) are provided to alleviate clinical traumatic brain injury (TBI)-induced agitation, albeit the effects on functional recovery remain unclear. Previous studies have shown that haloperidol (HAL) and risperidone (RIS) impair motor/cognitive function. Still unknown is how long the adverse effects induced by these APDs remain after their cessation. To address this issue, fifty-nine anesthetized male rats received a cortical impact or sham injury and then were randomly assigned to four treatments (HAL 0.5 mg/kg, RIS 0.45 mg/kg, vehicle; VEH 1 mL/kg, or bromocriptine; BRO 5.0 mg/kg; a positive control for D2-receptor action) that began 24-hours after surgery and were administered i.p. every day for 19-days. Motor/cognitive tests were conducted on days 1-5/14-19. Treatments were discontinued on day-20 and the rats were re-evaluated at 1 and 3 months. During the treatment phase, both APDs significantly impaired cognition vs. VEH and BRO. Furthermore, BRO was better than VEH (p=0.0042). At 1-month, both APDs still exhibited significant cognitive impairment vs. VEH and BRO. At 3-months, only the HAL group was still impaired vs. VEH, but both APDs continued to be deficient vs. BRO. No differences were revealed between the APD groups. Motor deficits/recovery did not differ among groups. These data replicate previous reports of HAL and RIS impeding cognition after TBI and extend those results by showing that the deleterious effects persist 3 months after discontinuation. The benefits observed with BRO suggest that the adverse effect of the APDs is mediated by D2-receptor antagonism. Evaluation of non-D2-antagonist APDs is needed.

Daniel Perchy

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Huinan Liu

MAGNESIUM ALLOYS AS NEXT-GENERATION BIODEGRADABLE MEDICAL IMPLANTS

Biodegradable magnesium implants may offer a viable alternative to traditional surgical treatments for torn tendons and ligaments. Magnesium can be implanted at the site of injury and then degrade benignly over time. As the implant degrades, the injured tendon or ligament can re-grow by cell proliferation and extracellular matrix production, eventually restoring tissue function. In order to optimize the growth of connective tissue cells on magnesium, the effects of surface microstructure and chemical environment on implant degradation and cell function must be studied. It is important to understand how cells would grow and differentiate on magnesium with various properties. My experiments investigated the degradation of pure and yttrium-alloyed magnesium samples over time. Samples were immersed in either water, phosphate buffered saline solution, cell medium, or cell medium supplemented with fetal bovine serum to study their degradation in different environments. The change of weight and surface appearance of magnesium and magnesium-yttrium alloy samples was measured and monitored until the samples were fully degraded. The pH variation of immersion solutions was tracked to understand how the degradation of magnesium affects the local physiological environment. This study provides a basis for the design of magnesium alloys as next generation biodegradable medical implants .

Funding: NIH, University of Pittsburgh

Nicholas Perri

Chemistry

Arts and Sciences

Faculty Mentor: Tara Meyer

ELECTROPLASTIC ELASTOMER DEVELOPMENT: QUANTIFICATION OF IRON ION CROSSLINKS

Electroplastic elastomers, materials whose stiffness can be altered by electrochemical stimulation, should prove valuable for a wide range of applications. Using hydrogel copolymers of 4-polystyrenesulfonic acid and sodium acrylate we have developed a composite material that can be reversibly converted between a soft and a hard state by the reduction or oxidation of metal ion (Fe2+/Fe3+) crosslinks. In order to optimize control and to verify reproducibility, it is important to quantify the number of crosslinking metal ions. We have therefore developed an analysis method to determine the total iron content of our electroplastic elastomers. Cross-linked hydrogels were treated with concentrated hydrochloric acid in order to break down the polymer network and release the Fe3+ bound to the monomers. By exploiting the characteristic peak for Fe3+ at 362 nm, the quantity of iron in the sample was determined by UV-Vis spectroscopy. Control studies were conducted to verify the accuracy of the method.

Funding: National Science Foundation

Lindsay Plavchak

Biological Sciences

School of Arts and Sciences

Faculty Mentor: Dr. Jeffrey Brodsky

CHARACTERIZING THE ROLE OF MOLECULAR CHAPERONES IN THE DEGRADATION OF THE EPITHELIAL SODIUM CHANNEL

The Epithelial Sodium Channel (ENaC) is an ion channel found in the epithelial membrane of both the kidney and the lungs, where it tightly regulates sodium reabsorption within the body. Because salt and water homeostasis is of such critical importance, even slight errors in ENaC function can lead to major health concerns such as hypertension, hypotension, pulmonary edema, and Cystic Fibrosis-like symptoms. ENaC is composed of three homologous subunits – α, β, and γ – that assemble in the ER to form the mature ENaC protein. However, formation is highly inefficient, oftentimes leading otherwise functional subunits to be degraded through a process known as Endoplasmic Reticulum Associated Degradation (ERAD). To determine how ENaC is degraded, we have made a yeast expression system. Previous results established that Jem1 and Scj1, two ER lumenal Hsp40 chaperones, play a role in ENaC degradation. Jem1 and Scj1 facilitate ATP hydrolysis by the ER lumenal Hsp70 chaperone, BiP. Furthermore, we found that Lhs1 and Sil1, two nucleotide exchange factors (NEFs) for BiP, may play a role in ENaC subunit degradation. These results led to the hypothesis that the Hsp40s and NEFs work in combination to facilitate ENaC degradation. To test this hypothesis, the extent of αENaC degradation was assayed in a newly created lhs1∆jem1∆scj1∆ strain. Results reveal that the combined effect of deleting Lhs1, Jem1, and Scj1 further stabilizes ENaC in comparison to the single deletions, indicating a synthetic effect. Consistent with these data, the lhs1∆jem1∆scj1∆ deletion is synergistically sensitive to agents that induce ER stress.

Funding: Howard Hughes Medical Institute Academic Year Undergraduate Research Fellowship; American Heart Association Summer Undergraduate Research Fellowship

Christopher Rovensky

Chemical and Petroleum Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Amy E Landis

BIOFUELS ON MARGINAL LANDS

Biofuel-based phytoremediation is being evaluated as a beneficial means to repurpose potentially contaminated underutilized urban marginal lands. Biofuel crops, such as the canola, corn, soybean, and sunflowers studied, are able to accumulate high concentrations of heavy metals that typically contaminate soil in brown- and greyfields. Students have been traveling to sites shared through the generosity of a local Pittsburgh nonprofit community group for the past three years to collect soil samples before planting and shortly after harvesting occurred. Soil samples collected from marginal lands throughout Pittsburgh were dried, ground, and digested using acid digestion. Soluble digested samples were analyzed with atomic absorption spectroscopy (AAS) to determine the heavy metal concentrations for 9 metals. Heavy metal concentration data has been collected for several sites since 2008, and new sites have been identified for the 2009 and 2010 growing seasons. Furthermore, the team collaborated with ALCOA and Pennsylvania Department of Environmental Protection researchers to determine the ability of plants to remediate contaminated and highly acidic mine refuse. A greenhouse was constructed at the University of Pittsburgh, and preliminary tests are under way using mine refuse provided by ALCOA and crops grown by the University of Pittsburgh team of researchers. The biofuel crops could improve soil conditions at the sites, as well as alleviate the economic and social blight associated with vacant urban lands.

Funding: NSF CBET Award #0933249 and NSF MCSI REU Program

Andrew Savinov

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Graham F. Hatfull

INVESTIGATION OF THE PUTATIVE PHOSPHOESTERASE ACTIVITY OF BXB1 GP47

Bxb1 is a temperate bacteriophage that infects and forms lysogens of Mycobacterium smegmatis. The Bxb1 lysogeny system depends on site-specific DNA recombination catalyzed by Bxb1 serine-integrase. Bxb1 gp47 protein, the Recombination Directionality Factor (RDF), alters integrase site-specificity to promote excision and inhibit integration. Bxb1 gp47 is an unusual RDF with no functionally characterized homologs, although 15 other mycobacteriophages encode closely related proteins. The presence of a homolog in phage L5 in particular suggests a secondary role for this protein, because L5 encodes a tyrosine-integrase and the known requirements for recombination do not include the Bxb1 gp47 homolog L5 gp54. Our prior BRED phage mutagenesis work showed that gp47 indeed has a secondary role in vivo (in lytic propagation of Bxb1) and that this secondary role is separable from the excision-promotion activity. L5 gp54 and Bxb1 gp47 align well with the consensus sequence for the calcineurin-like phosphoesterase domain, which consists of four main motifs. We are investigating the hypothesis that Bxb1 gp47 has a phosphoesterase activity. We have purified His-tagged variants of wild-type gp47 and two phosphoesterase motif mutants; purification of functional proteins was tested by RDF activity assays. Preliminary phosphatase assays showed no activity from wild-type gp47 in the standard reaction buffer. Further assays are now underway with various divalent metal ions added, as calcineurin-like phosphoesterases are predicted to be metal-dependent. If the putative phosphoesterase activity and dependence on intact motifs is confirmed, further work will focus on correlating these results with the lytic-cycle viability of motif mutant Bxb1 phages.

Funding: US Steel Foundation Undergraduate Research Award (Arts and Sciences Research Award, to AS)

Amy Scarbrough

Biological Sciences

Computer Science

Arts and Sciences

Faculty Mentor: Dr. Michael Grabe

WEIGHTED ENSEMBLE SIMULATION OF ALTERNATING ACCESS IN SODIUM-HYDANTOIN TRANSPORTER MHP1

Many transport proteins use the energy in sodium gradients to drive the uptake of small molecules. These proteins perform functions as diverse as the withdrawal of neurotransmitters from the synaptic cleft, the removal of sugar from the gut, and the salvage of nucleobases and related metabolites. It is thought they operate via an alternating access mechanism in which substrate is bound in an outward-facing conformation followed by a transition to an inward state that delivers the cargo to the cell. X-ray structures of both states exist, but the long time required to see such slow events makes studying this transition using traditional molecular simulations prohibitive. We employed the weighted ensemble method, which focuses computational efforts on rare events, to study transitions in the benzyl-hydantoin transporter Mhp1. With this method, which we accelerated using the computational efficiency of graphics processing units, we have observed thousands of transitions. Our analysis of these trajectories using root mean squared deviation calculations and techniques such as protein fingerprinting reveals insights into the mechanism of alternating access, which we believe will impact our understanding of the operation of these proteins and their roles in human diseases.

Funding: Department of Biological Sciences, through HHMI

Lauren Schmidt

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Graham Hatfull

CHARACTERIZING THE MYCOBACTERIOPHAGE DAVINCI PARTITIONING GENES: SIMILARITIES TO MYCOBACTERIOPHAGE REDROCK AND BACTERIAL HOMOLOGUES

Many bacteria and plasmids encode partitioning proteins that are essential for normal chromosomal segregation during replication. There are three essential components of these partitioning systems: parS, a cis-acting regulatory site, and parA, and parB, both protein-encoding genes. Some bacteriophages also encode these proteins to ensure stability of the extrachromosomal lysogenic state. The E. coli phage P1 has long been known to have a partitioning system, but only recently has this system been discovered in mycobacteriophages. Only five sequenced mycophages have a partitioning system: Redrock, DaVinci, Gladiator, Hammer and Blue7. It is not known whether these phage homologues function in the same way as their bacterial counterparts, and characterizing the mechanism of actions of these genes will give insight into the lysogenic state of mycophage DaVinci. Attempts at knocking out parA have been unsuccessful, and in order to complement the parA knockout pNIT with parA will be used. pNIT is an inducible plasmid allow control over levels of expression of the protein. A parA-GFP fusion on pNIT will be constructed to perform real-time fluorescent microscopy experiments to give insight into the function of parA.

Funding: HHMI

Katelyn Shaffer

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. John Rosenberg

FUNCTION WITHOUT STRUCTURE: STUDYING α-SYNUCLEIN AS A MACROMOLECULAR COSOLVENT (OSMOLYTE)

α-synuclein is a natively unfolded protein whose deposits accumulate in several neurodegenerative diseases, appropriately called “synucleinopathies;” e.g. Parkinson’s disease (PD). α-synuclein appears to be a pleitropic effector. One suggested role is in vesicular trafficking. Another is based on the observation that it stimulates protein phosphatase 2A (PP2A) activity, both in vivo and in vitro. Conventional thinking on natively unfolded proteins argues they adopt a folded state when bound to a suitable macromolecular partner, preserving the “structure determines function” paradigm. A crystal structure recently obtained in our laboratory and resulting work suggests a different paradigm, one in which α-synuclein can have a biochemical function while in a structurally disordered state by acting as a macromolecular cosolvent (or osmolyte), where it participates in the solvation of other proteins. We have been using dynamic light scattering (DLS) to characterize an interaction between molecules of a GST- α-synuclein fusion protein that appears to be mediated by α-synuclein. This biologically active fusion protein forms a (polydisperse) collection of particles dominated by tetramers. The 2D HSQC NMR spectrum of the fusion protein shows that α-synuclein is unstructured under the conditions that support the formation of tetramers. Conventional osmolytes perturb the size and behavior of the fusion protein in DLS. GST alone in DLS forms a well-defined stable dimer. GST-fusions of α-synuclein’s 3 modular domains show behavior unique to each domain in DLS.Our results are consistent with the idea that disordered α-synuclein contributes to the formation of a composite solvation shell surrounding the GST.

Funding: HHMI

Aditee Shinde

Arts and Sciences

Faculty Mentor: Dr. Robert Gibbs

EFFECTS OF CHOLINERGIC LESIONS AND ESTRADIOL ON ESTROGEN RECEPTOR MRNA EXPRESSION IN SPECIFIC REGIONS OF THE RAT BRAIN

Studies show that estrogen therapy can have beneficial effects on cognition in animals and humans, and may protect against neurodegenerative disorders such as dementia and Alzheimer's disease. However, studies also suggest that beneficial effects of estrogen on cognition decrease with age and time following menopause. Some of these diseases have been associated with signaling deficits in cholinergic functions in the brain, yet estradiol has shown to significantly enhance cholinergic functions and improve performance in many cognitive tasks. However, the mechanism through which estradiol can enhance the cholinergic functions is unknown; perhaps it may exert its effects by binding to any of three particular estrogen receptors, ERa (alpha), ERb (beta), and GPR30. For this project, I evaluated the effects of basal forebrain cholinergic legions and estradiol treatments on levels of estrogen receptor mRNA (messenger Ribosomal Nucleic Acid) in different regions of the brain. The hypothesis is that cholinergic lesions lead to decreased estrogen receptor expression of mRNA in the brain, accounting for the lack of estrogen.

Jessica Soe

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. William Saunders

IDENTIFICATION OF THE REQUIREMENT FOR MITOTIC MOTORS IN STRESS GRANULE DYNAMICS

Stress Granules (SGs) are cytoplasmic domains containing RNA binding proteins, 40S ribosome subunits, stalled initiation complexes, and RNA aggregation that form under stressful conditions. The non-translating messenger RNAs in SGs are being repaired, degraded, or stored due to initiation-related damage. In order to identify how stress protein complexes form into SGs, we observed if microtubules contribute to the highly dynamic nature of SGs. Microtubules have been shown to be required for SG formation, which was confirmed in cells treated with sodium arsenate, which induces cells to form SGs and there was a 30% decrease in SG assembly. Microtubule inhibitors were also tested for SG dissolution. Cells treated with nocodazole, which disrupts microtubule polymerization, demonstrated faster dissolution of SGs suggesting microtubules delay SGs disassembly. We investigated if mitotic motors may function in SGs formation. Both mitotic motors, Eg5 and Kid were found to localize to SGs. SiRNA studies were conducted and a 17% reduction in SG assembly was observed when Kid was knocked down, while a 32% reduction was observed when Eg5 was knocked down, but Eg5's ATPase activity was not required for assembly. We inquired whether Eg5 helps dissociate SGs by treating cells with monastrol, a specific inhibitor to Eg5, and a significant lag was observed during SG dissolution after Eg5's ATPase was inhibited. This demonstrates Eg5 is also required for SG dissolution and its ATPase activity is required for this process. In summary, the identity of two mitotic motors in SG formation, coalescence and or dissolution is a novel SG component and paves the way the identification of other families of molecular motors in SG dynamics.

Funding: NIH

Yeohan Song

Neuroscience

Arts and Sciences

Faculty Mentor: Donald B. DeFranco, PhD

EFFECTS OF HYPERGLYCEMIA AND DEXAMETHASONE IN A MODEL OF NEURODEVELOPMENT

Background Hyperglycemia, or elevated blood glucose levels, in pregnant women can lead to impairments in the development of fetal organ systems, including the nervous system. In diabetic individuals, the hypothalamic-pituitary adrenal axis is overactive, leading to hypersecretion of endogenous glucocorticoids. Glucocorticoid therapies have been shown to induce hyperglycemia in both diabetic and non-diabetic patients, and elevated glucocorticoid levels have been found to mediate deficits in cognitive function due to chronic stress. The exact mechanisms by which hyperglycemia and glucocorticoids interact to affect neurodevelopment have yet to be determined. Methods Cell cultures of the neuroblastoma line SH-SY5Y were used as an in vitro model to determine the impact of hyperglycemia and glucocorticoids on neuronal growth and function. Results Undifferentiated SH-SY5Y cells treated with ≥50 mM glucose exhibited lower viability after 24 h of treatment and showed decreases in both Ki67 labeling and BrdU incorporation. Treatments of these cells with ≥100 nM dexamethasone, a potent synthetic glucocorticoid, were associated with reductions in combined Ki67 labeling and BrdU incorporation. Future Directions The effects of hyperglycemia and dexamethasone on apoptotic mechanisms and signaling pathways in SH-SY5Y will need to be further studied.

Funding: NIH Grant R01 DK078394

Craig Stevenson

Chemical and Petroleum Engineering

Swanson School of Engineering

Faculty Mentor: Prof. Goetz Veser

A NANOSCALE APPROACH TO CARBON CAPTURE

Anthropogenic CO2 emissions are widely recognized as a leading cause for climate change. Capturing CO2 from concentrated point emissions, such as fossil fuel power plants, is hence crucial for curbing atmospheric CO2 concentrations. However, current CO2 capture technologies, typically based on the use of liquid amines, result in large energy penalties and strongly increased cost of energy (up to 85%). In order to overcome some of the shortcomings of liquid amines, much work has recently focused on the use of silica-based solid sorbent materials, either via impregnation with amines or via grafting of amine groups onto silica. In particular nanostructured silica materials are of great interest due to very high CO2 capture capacities of amine-impregnated "nanosilicas", in some cases even exceeding that of liquid amines. In our work, we are evaluating novel hollow silica "nanobubbles" recently synthesized in our laboratory for the confinement of CO2 sorbent materials. First results indicate that these materials hold great promise for CO2 capture via nanoconfined liquid sorbents, with current sorption capacities of up to 125 mg CO2/g sorbent and the potential for further increases based on tailoring of the silica nanostructure. Materials synthesis, characterization, and CO2 sorbtion results will be presented in the poster.

Funding: NETL

Kimberly Stuchul

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Michael Palladino

MODELLING TRIOSEPHOSPHATE ISOMERASE DEFICIENCY IN DROSOPHILA

Triosephosphate isomerase (TPI) deficiency is a progressive neurodegenerative condition most often resulting in death before 5 years of age. This autosomal recessive disease is caused by missense mutations and remains poorly understood. Previously it has been shown that the Drosophila TPI missense mutation M80T (TPIsgk) causes protein instability leading to higher protein turnover. In order to further investigate mutant TPI protein stability and understand the neurodegenerative aspect of TPI deficiency, five TPI missense mutations known to cause human disease (I170V, E104D, C41Y, F240L, and V231M) and a catalytically inactive mutation (K12M) were generated in Drosophila melanogaster using genomic engineering. Briefly, the TPI locus was replaced with an attp and LoxP site using homologous recombination gene targeting. PGE-TPI-attB plasmids bearing the wild type Drosophila TPI gene (control) or various mutations were generated using site-directed mutagenesis. Additionally, we have engineered PGE-TPI-attB capable of expressing the human TPI protein. Genomic engineering was performed by using site-directed recombination via the attP-attB recombination system. The engineered loci will allow in vivo analysis of each disease-causing mutation, which will lead to a better understanding of human TPI deficiency pathogenesis.

Funding: NIH

Jiaxiang Tao

Civil and Environmental Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Albert C. To

Computational Modeling of Nanoporous Aluminum

Nanoporous metals have gained a significant momentum for their substantial applications in today's new construction material because of their very low relative densities. However, previous research has shown that nanoporous metals are weak, brittle, and undergoes a catastrophic failure when they are subjected to tension. In order to prevent such "catastrophic failure", Molecular Dynamics simulation is applied to further investigate the failure mechanism of nanoporous aluminum, with different porosities, under uniaxial tensile loading. The general computational results demonstrate that ligament size and joint stiffness of nanoporous aluminum structure govern its softening rate. To be more specific, high porosity structure with small ligaments and joints soften much slower and local stress is uniformly distributed across the structure with no specific high stress localization and, thus, undergoes less catastrophic failure. In contrast, for the low porosity structure with large ligaments and joints, stress is highly localized on a small region, causing progressive softening and more catastrophic failure. More evidences have proven that the joint size mainly governs the softening rates, because the high compliance of joints allows the overloading stress, due to the rupture of weakest ligament, more effectively spreads through the entire structure.

Funding: University of Pittsburgh Undergraduate Summer Research Internship Program

Hendrik van Hemmen

Civil and Environmental Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Xu Liang

Design of Wireless Sap-flow Sensor Housings

Describes the design of a system to attach remote sap-flow sensor systems to trees at the Audubon Center north of Pittsburgh. The system used prior to this design suffered from problems with water-tightness, rigidity, animal damage, and bulkiness.

Funding: Swanson School of Engineering, REU program

Michael Volkwein

Civil and Environmental Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Jorge D. Abad

COMPARISON AND ANALYSIS OF HYDRODYNAMIC MODELS FOR RESTORATION PROJECTS: THE CASE OF POOL-RIFFLE STRUCTURES

Naturalization of urbanized streams through the use of pool-riffle structures creates a more hospitable environment for fish and a consistent depth during high discharges while providing self-maintenance of the structure. Whereas during low flow sediment builds up in pools, a pool-riffle structure with sinusoidal narrowing banks allows higher shear stresses to develop in pools during high flows thereby maintaining a pool-riffle structure over time. To design these self maintaining structures, hydrodynamic models are used to optimize the parameters necessary to facilitate this shear stress reversing process. 1D models are often favored in place of resource intensive 3D models. However, the 1D and 3D pool-riffle models do not always agree. 2-D models can be used to attempt to validate the 1D models and describe their limitations. 1-D modeling was completed for four channel designs at eight levels of discharge using HEC-RAS. The pool-riffle design displayed the self-maintenance mechanism for four flow rates. Shear stresses in the pools did appear to exceed the stress at the riffles during high discharges. 2D depth-averaged modeling was done using River2D and initial results show that the mechanism may not be effective. This discrepancy should be compared to 3D models in more detail. Industry uses 1D modeling most frequently for its cost effectiveness, showing that 1D models may not be sufficient in pool-riffle design could be significant.

Funding: Swanson School of Engineering and the Heinz Endowment Award

Katelyn Walzer

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Jon P. Boyle

THE CODING REGION OF ROP43 MAY CONTRIBUTE TO INCREASED TRANSCRIPTION IN TOXOPLASMA GONDII

Toxoplasma gondii infects over 60 million Americans and causes serious diseases in immunocompromised hosts. These individuals suffer from the pathogenic effects of T. gondii when chronic infections (caused by bradyzoites) are reactivated in T. gondii tissue cysts, initiating acute infections (caused by tachyzoites). The ROP42 subfamily of genes is upregulated during tachyzoite-to-bradyzoite differentiation and may therefore play a role in this process. Our goal is to determine the minimal nucleotide sequence required for upregulation of these genes during differentiation by using luciferase assays. We observed that including the first 270 nucleotides of the coding region of ROP42 increases the luciferase activity 21-fold during the conversion of tachyzoites to bradyzoites compared to 4.2-fold when using the promoter alone. This suggests that the nucleotide sequence in the 270 bp region is involved in transcriptional regulation of ROP42 and/or that the peptide sequence it encodes may alter the stability of the luciferase fusion protein. We examined whether a similar construct from a closely related paralog, ROP43, behaved similarly in strain CTG of T. gondii. Our results indicate that there is a 14.2-fold increase in the upregulation of luciferase activity when the promoter and 270 bp fragment is present. This is in contrast to a 7.7-fold increase when only the promoter is present. Further experiments will be done to determine if the upregulation caused by the 270 bp fragment works equally well in a more virulent strain of T. gondii, ME49. We also want to find the minimal sequence within the 270 base pairs that causes an increase in transcript abundance, transcript stability, and/or protein stability.

Funding: Summer Undergraduate Research Fellowship (HHMI-funded), Chancellor's Undergraduate Research Fellowship (University Honors College)

David Wang

Chemistry

Arts and Sciences

Faculty Mentor: Dr. Lillian Chong

COMPUTER SIMULATIONS OF AN ARTIFICIAL ZYMOGEN DESIGNED TO COMBAT HIV

Molecular switches are biomolecules with functions that can be turned “on” or “off” in response to signals from the surrounding environment such as pH, ligand binding, or enzymatic modification. One strategy for engineering molecular switches involves the design of “alternate frame folding.” In this design, two proteins are fused together such that they share an overlapping sequence, resulting in alternate frames of the protein sequence that fold in a mutually exclusive fashion. This design has been used to create a zymogen for the treatment of AIDS where the alternate frames correspond to two different forms of barnase: one active and the other inactive. The inactive form can be shifted to the active form upon cleavage by HIV protease, thereby unleashing the toxic enzymatic activity of barnase only in HIV-infected cells. Since the “on” and “off” states of this switch are partially unfolded, it is difficult to determine the structures of these states using X-ray crystallography or NMR spectroscopy. An alternative approach is to use computer simulations with residue-level detail. Here we use such simulations to characterize the “on/off” states of the barnase zymogen as well as its mechanism of conformational switching. Understanding this mechanism can aid in the design of future molecular switches.

Funding: University of Pittsburgh Startup Funds

Kurt Weiberth

Biological Sciences

Arts and Sciences

Faculty Mentor: Christopher J. Guerriero

Determining the Role of Chaperones in Endoplasmic Reticulum-Associated Degradation

Proper protein folding is vital for a cell's survival and when secretory proteins misfold, one mechanism to eliminate them is known as endoplasmic reticulum-associated degradation (ERAD). The ERAD of some proteins has been linked to diseases; therefore, better understanding the mechanisms of ERAD can lead to treatments of these diseases. In this study, the chaperones and components for ERAD were examined using the protein Ste6p*, a native yeast protein with 12 transmembrane units that is a well-characterized ERAD substrate. Currently, the relationship between chaperone requirements for ERAD and the number of transmembrane domains in a substrate are unknown. The goal of this study was to determine the chaperones required for substrates containing different numbers of transmembrane domains but the same degradation signal. To do this, a chimeric protein, called Chimera A, was constructed containing two transmembrane domains and the misfolded domain of Ste6p*. The rate of degradation was monitored in yeast treated with a chemical inhibitor of ERAD. Following treatment, Chimera A was stabilized by approximately 5-fold. This data supports the use of Chimera A to study the specific chaperones and other requirements for ERAD.

Funding: Howard Hughes Medical Institute Summer Undergraduate Research Fellowship

Paul Werntges

Civil and Environmental Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Piervincenzo Rizzo

OPTIMAL ANGLE DETERMINATION FOR THE DETECTION OF SYMMETRIC GUIDED WAVE MODES

Underwater structures that are used in everyday life include ships, submarines, and underwater pipes. Very often the inspection of these structures requires temporary termination of service or the employment of rugged technology that can withstand underwater conditions. In recent years the use of Guided Ultrasonic Waves (GUWs) for the Nondestructive Evaluation (NDE) and the Structural Health Monitoring (SHM) of waveguides has drawn much attention. In this study GUWs are exploited to inspect plates immersed in water. The novelty of the present project is twofold: for the first time waves are generated by means of pulsed laser; and the optimal angle of the sensing system for the detection of the symmetric (So) mode is determined. A pulsed laser was used to generate stress waves in an aluminum plate immersed in water, and an immersion piezoelectric transducer detected the propagating waves. Signals were detected starting at 0° with respect to the vertical axis, and rotated toward the point-source in three degree increments, up to 45°. The time waveforms were extracted from an oscilloscope, and processed using Matlab software. Our results confirm theoretical predictions of wave speed and dispersion frequencies for the symmetric S0 mode. Furthermore, our experimental optimal angle conclusion is consistent with the prediction made using Snell's Law. It is because of these parallels that we can conclude that our experiment was a success.

Funding: NSF

Whitney White

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Howard Chang, MD,PhD, Stanford University

METASTASIS ASSOCIATED LUNG ADENOCARCINOMA TRANSCRIPT 1 ASSOCIATES WITH THE MLL COMPLEX

While much has been discovered about RNA, long intergenic noncoding RNAs (lincRNAs) are still an area of research that is vastly unexplored. In the past couple of years, thousands of lincRNAs have been identified, yet the function of many of them still remain unknown. Of the long intergenic noncoding RNAs that have been characterized, most serve a role in gene or epigenetic regulation. Malat-1 is a long intergenic noncoding RNA that has been shown to be linked with increased metastasis in various carcinomas. While it has potential to serve as a marker for survival, its function currently remains unknown. Previous research suggested that Malat-1 might be associated with the MLL complex, a protein complex that regulates epigenetic activation. In our studies, we have shown that Malat-1 associates with various proteins of the MLL complex using RNA immunoprecipitation. By identifying Malat-1's binding partners we hope to uncover some information about its function and the roles long intergenic noncoding RNAs play in cancer.

Funding: HHMI ExROP

Emily Wolff

Civil and Environmental Engineering

Swanson School of Engineering

Faculty Mentor: Dr. Radisav Vidic

MARCELLUS SHALE FLOWBACK WATER TREATMENT FEASIBILITY STUDY WITH ACID MINE DRAINAGE

In recent years natural gas extraction from the Marcellus Shale formation, which underlies much of Pennsylvania and some neighbouring states, has become feasible due to developments of the hydraulic fracturing process. Hydraulic fracturing, known as “fracing” in industry, is an extremely water intensive process requiring 3 to 5 million gallons of freshwater to frac one well on average. The reuse of water recovered after the hydrofracturing, i.e. flowback, is limited by the low recovery, the high salinity and expensive treatment costs. The reuse of flowback water requires the removal of toxic heavy metals (barium, strontium) and other ions that can cause scaling and corrosion in the gas well. The objective of this study is to develop a sustainable management approach for Marcellus flowback water that will allow its reuse for hydrofracturing subsequent wells. In particular, the study is focusing on the feasibility of mixing flowback water with acid mine drainage (AMD) which will serve as make-up water and remove barium and strontium through sulfate precipitation. The work presented in this poster shows the initial steps in understanding the chemistry of different flowback waters and AMD mixes by studying precipitation kinetics and crystal size, morphology and composition.

Funding: U.S. Department of Energy

Narayana Yelleswarapu

Psychology

Arts and Sciences

Faculty Mentor: Dr. Anthony E Kline

The effects of the 5-HT1A and 5-HT7 receptors on behavioral recovery after traumatic brain injury

Previous data from our laboratory has shown that the 5-HT1A receptor agonist 8-OH-DPAT improves behavioral recovery after experimental traumatic brain injury. It has generally been thought that 8-OH-DPAT is mediating its beneficial effects via the 5-HT1A receptor. However, because 8-OH-DPAT also has partial agonist activity at the 5-HT7 receptor, it is possible that activation of this receptor may be playing as role in the benefits observed. To elucidate this possibility, we evaluated the effects of antagonists for the 5-HT1A and 5-HT7 receptors. Specifically, sixty-eight rats received either a controlled cortical impact (CCI) or sham injury and then were randomly assigned to the following groups: 8-OH-DPAT (0.5 mg/kg), 8-OH-DPAT + SB 269970 HCl (5-HT7 antagonist; 2 mg/kg), 8-OH-DPAT + WAY 100635 (5-HT1A antagonist; 0.5 mg/kg), or saline vehicle (1 mL/kg). Each group was tested on motor (beam) and cognitive (Morris water maze) tasks on days 1-5 and 14-19, respectively. 8-OH-DPAT alone, or when combined with the antagonists showed cognitive improvement relative to vehicle controls, but there was no statistical difference among the groups. The data did not provide the clear cut distinction of what receptor is mediating the benefits observed with 8-OH-DPAT. It is possible that both receptors are playing a role. Evaluation of a 5-HT7 receptor agonist is warranted to further elucidate the potential mechanisms and our understanding of 8-OH-DPAT and its effects.

Funding: NIH

Ally Young

Biological Sciences

Arts and Sciences

Faculty Mentor: Dr. Karen Arndt

INVESTIGATING THE FUNCTION OF RKR1 THROUGH A HISTONE-BASED GENETIC SCREEN IN S. CEREVISIAE

Protein ubiquitylation regulates many important processes in eukaryotes. Uncontrolled ubiquityation has been associated with a number of diseases, including cancer. Rkr1 is a previously unstudied ubiquitin ligase that has been linked to transcription and chromatin function in yeast. To elucidate a function for Rkr1, we devised a screen to find genetic interactions between Rkr1 and histone mutations using a plasmid library encoding alanine substitutions at all histone residues. Each of these plasmids was transformed into a rkr1 deletion strain, which also contained a plasmid encoding the only wildtype source of histone in the cell. Transformants were forced to lose the wildtype copy by counter-selection. We hypothesize that a histone mutation that affects a process for which Rkr1 also plays a role will result in a genetic interaction when both Rkr1 and the histone residue are compromised. The screen has recently been completed for histones H2A and H2B. Candidates found were re-transformed, subjected to dilution assays, and sequenced to verify mutations. Of the 12 candidates identified, 2 are known lethal mutations. Another is the mutation of H2B K123, which is known to be synthetically lethal with the deletion of rkr1. Interestingly, many candidates have published phenotypes associated with defects in transcription, including sensitivities to hydroxyurea, 6-azauracil, and mycophenolic acid. The candidate residues also form an obvious patch on the surface of the histone molecule when mapped. Future analysis will investigate the role of Rkr1 in pathways associated with these mutations. Finally, the entire screen will be repeated for histones H3 and H4.

Funding: Arts and Sciences Undergraduate Research Award