

**2012 South Carolina INBRE Scientific Symposium**  
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**Abstracts**

## Oral Presentations

### **Jeffrey Triplehorn**

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#### **Stimulus-specific adaptation: why use a network if a single neuron can do the job?**

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Identifying neural mechanisms involved in filtering input is a major area of sensory neurobiology research since deficits in sensory filtering contribute to communication disorders, schizophrenia, and autism. Stimulus-specific adaptation (SSA) is a phenomenon described in vertebrates correlated with the auditory system's ability to filter inputs and detect novel events. Investigations into the mechanisms responsible for SSA have focused on neuronal interactions. SSA also occurs in the katydid auditory system, but at the level of a single auditory neuron (TN-1), making it an ideal system for investigating cellular mechanisms involved in SSA. TN-1 adapts rapidly to fast pulse rates, but responds to transient sound pulses presented during fast pulse rate stimulation if the carrier frequencies are different. Auditory afferents project to the TN-1 dendritic field in a tonotopic manner; therefore, different stimulus frequencies activate different TN-1 dendritic regions. Our dynamic dendritic hypothesis proposes that SSA in TN-1 can be explained by limiting adaptation neural mechanisms to dendritic regions activated by the acoustic frequencies comprising the fast pulse rates, leaving unstimulated regions free to respond to novel input. Pharmacological studies revealed two post-synaptic adaptation mechanisms: a transient calcium-mediated mechanism and a slow, tonic sodium-mediated mechanism. Based on these results, we hypothesized that the: 1) sodium-mediated mechanism occurs within the dendritic regions stimulated by fast pulse rates, reducing current enough to keep TN-1 from reaching threshold; 2) calcium-mediated mechanism occurs within TN-1's axonal region activated by spiking activity. Calcium and sodium imaging experiments demonstrated that: 1) fast pulse rates evoke transient calcium concentration increases in the axonal and dendritic regions; 2) fast pulse rates tonically increased sodium concentration within TN-1 dendritic regions only; 3) increases in sodium concentration were restricted to those dendritic regions stimulated by the acoustic frequencies contained in the fast pulse rates.

### **Onarae Rice**

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#### **The Dopamine D3 Receptor Antagonist SB-277011A Decreases Binge-Like Consumption of Ethanol in Mice**

O. V. Rice <sup>1</sup>, D. Schonhar <sup>1</sup>, J. Gaál<sup>2</sup>, E.L. Gardner <sup>3</sup>, C.R. Ashby <sup>4</sup> <sup>1</sup>Furman University, Department of Psychology, Behavioral Neuroscience Lab, Greenville, SC <sup>2</sup>MegaPharma Pharmaceutical Kft., Budapest, Hungary <sup>3</sup>Neuropsychopharmacology Section, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD <sup>4</sup>Department of Pharmacology, St. John's University, Queens, NY

The mesolimbic dopamine (DA) pathway plays an integral role in the reinforcing properties of many drugs of abuse, including alcohol (ethanol/EtOH). Further, it has been reported that selective and acute blockade of the DA D3 receptor by SB-277011A will attenuate EtOH preference, intake, and lick responses in EtOH preferring rats. However, alcohol consumption that leads to abuse is often marked by binge drinking - which is characterized as bringing ones blood EtOH levels to  $\geq 80\text{mg/dl}$  within two hours of the initial drink. It is unclear if brain mechanisms implicated in EtOH reward are equally implicated in EtOH binge consumption and abuse. Therefore, the present study examined the effects of SB-277011A on binge-like EtOH consumption in mice. Male C57BL/6 mice were trained to drink water, near beer (veh/no EtOH), or near beer containing 2% EtOH for two hours per day. Once stable drinking was observed, mice were pretreated daily, for 14 days, with (0, 7.5, 15, or 30 mg/kg i.p.) of SB-277011A 30 minutes prior to their drinking session. Total water and EtOH consumption was measured and blood EtOH levels calculated for the near beer and the near beer plus 2% EtOH groups. The results demonstrated that mice brought their blood EtOH levels to  $\geq 80\text{mg/dl}$  and that SB-277011A (15 and 30 mg/kg i.p.) decreased EtOH consumption (30 mg/kg significantly) each day without affecting water consumption. In addition, the animals' EtOH consumption returned to baseline drinking levels within 3 days after the cessation of SB-277011A pretreatment. These findings provide evidence that the DA D3 receptor plays an integral role in ethanol drinking behavior in mice and that SB-277011A has therapeutic potential in attenuating binge-like drinking in humans.

**Brandon Mattix**

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**Effects of Polymeric Nanoparticle Surface Properties on Interaction with Brain Tumor Environment**

Mattix, B., Willi, T.J., Reese, L., Pollard, S., Uvarov, O., Alexis, F.

The American Cancer Society predicts that in 2011 over 570,000 people will die due to cancer in the United States alone. Current cancer treatments include chemotherapy and radiotherapy, both possessing particular pros and cons in specific applications. One approach to improve the efficacy of cancer treatments is drug delivery to cancerous cells using nanocarriers. When injected into the body, the surface of the nanocarriers interacts with the biological environment, which controls the bioactivity of these drug delivery systems. However, there is a lack of understanding of the functional properties of chemical groups on the surface of nanocarriers that control interactions with the ECM, cellular uptake, and proteins inside the cells. This study proposes to determine the surface properties of nanocarriers that facilitate drug delivery to brain cancer cells and their tumor environment. As a proof-of-concept, we tested biocompatible and biodegradable poly(lactic acid)  $\text{â€} poly(ethylene glycol)$  (PLA-PEG-R) with twenty five different surface functional groups. Studies have been performed using two human brain cancer cell lines (U87 & U138) and four control cell lines (NCI-H460 human lung cancer cells, MCF-7 human breast cancer cells, HBMEC human brain microvascular endothelial cells, and HUVEC human umbilical vein endothelial cells). Interaction was performed to study three biological systems: ECMs, cells, and proteins. Results indicate that the functional properties on the surface nanocarriers are critical to control the bioactivity of drug delivery nanocarriers. The interactions of nanocarriers with the biological environment go beyond the conventional electrostatic interactions.

**Yun Xiang**

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**Nanoparticulate carriers to deliver SOD for anti-inflammation**

Yun Xiang<sup>1</sup>, Victor Maximov<sup>1</sup>, Vladimir Reukov<sup>1</sup>, Mark Kindy<sup>2</sup>, Alexey Vertegel<sup>1</sup> <sup>1</sup> Department of Bioengineering Clemson University, <sup>2</sup> Department of Neuroscience Medical University of South Carolina Enzyme superoxide dismutase (SOD) is well-known for its anti-inflammatory activity. However, the extensive application of SOD is hindered by its poor stability in vivo and often by inability to reach specific site of action. Use of nanoparticles and liposomes as the carriers can improve stability of protein drugs and, in the case of targeted nanoparticulate carriers, improve their delivery to the desired site. Both targeted [1] and untargeted [2] SOD conjugates have recently been investigated as potential therapeutic agents; however, no systematic study of enzyme performance upon attachment to nanoparticles and liposomes has been undertaken. The goal of this work was to study effect of different immobilization approaches on enzyme loading and antioxidant activity of SOD-nanoparticle and SOD-liposome conjugates. Comparison of the performance of nanoparticles with bulk-loaded and surface-immobilized SOD was also performed. In the case of surface immobilization, highest levels of SOD activity (~1,500 U/mL for nanoparticles and ~2,500 U/mL for liposomes) were achieved when using PEG-maleimide attachment chemistry. Activity of surface-conjugated SOD was found to be approximately twice as high as that of the bulk-loaded nanoparticles, even though the latter can be loaded with up to six times more SOD than the former. In order to achieve long storage duration for SOD-nanoparticulate carriers, lyophilization was applied. Cryoprotectants performance was analyzed immediately after lyophilization and the long term activity retention were measured to compare with non-lyophilized samples. In addition to surface immobilized SOD, variety of antibodies was also attached for targeting to central nervous system or respiratory epithelium. One specific antibody that has been used is anti-NR1 antibody. Its ability to bind to glutamate receptors on neural cells makes it a good candidate for targeting to the nervous system. Its targeting potential was verified by immunohistochemistry. 1. Reukov, V., V. Maximov, and A. Vertegel. *Biotechnology and Bioengineering*, 2011. 108(2): p. 243-252. 2. Reddy, M.K. and V. FASEB Journal, 2009. 23(5): p. 1384-1395.

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DiGeorge syndrome (DGS) is a complex and variable disorder that includes defects of the cardiovascular system, pharyngeal glands and head. Most of the tissues affected in DGS are derived from the pharyngeal arches, which are populated by migrating cranial neural crest cells (NCCs). The motility, guidance and survival of a subset of cranial NCCs that contribute to cardiac outflow tract (OFT) morphogenesis, cardiac NCCs, are under the control of the sonic hedgehog (Shh)-Forkhead-Tbx1-Fgf8-FgfR-Map kinase pathway. Fibulin-1 (Fbln1) is an extracellular matrix protein required for motility, guidance and survival of cardiac NCCs. Deficiency of Fbln1 in mice leads to cardiac and OFT abnormalities. We have performed preliminary DNA microarray gene expression profiling and qPCR to evaluate the transcriptomic effects of Fbln1 deficiency on the regulation of genes associated with the

Shh-Forkhead-Tbx1-Fgf8 pathway in E10.5 hindbrain (HB), pharyngeal arch (PA)-containing tissue. We observed down-regulation of a number of key Fgf8-related genes in Fbln1-deficient HB/PA-containing tissues including Fgfr 1 and 2 and forkhead box (Fox) transcription factors, Foxc1, Foxc2 and Foxa2. We have also evaluated the effects of Fbln1 deficiency on the expression of genes of the Shh-Fox-Tbx1-Fgf8 pathway in mouse embryo fibroblasts (MEFs) from E12.5 wildtype and Fbln1 null embryos. The results indicate that while Fbln1 deficiency reduces the expression of Foxc1, Foxc2, Foxa2, Tbx1 and Fgf8, it also acts to dramatically upregulate Shh expression. Together, the findings indicate that Fbln1 is a regulator of the Shh-Forkhead-Tbx-Fgf8-FgFR-Map kinase pathway acting to promote transcription of Foxc1/c2/a2, Tbx1 and Fgf8 as well as maintain Shh expression, and thereby promote cardiac NCC guidance, migration and survival.

### **Sibnath Ghatak**

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### **CCN1 promotes synthesis of HA, and HA-induced downstream cell survival signaling pathways necessary for the formation of the atrioventricular septal complex (AVSC)**

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Atrioventricular (AV) junctional morphogenesis and remodeling are central to heart development as indicated by the fact that so many clinically relevant, human congenital heart defects are linked to it. These include atrioventricular septal defect [AVSD, ~7.5% in humans], ventricular septal defect [VSD], and atrial septal defect [ASD]) as well as defects arising from septal misalignments. Endothelium to mesenchymal transformation (EMT) is a crucial first step to normal development of the AV cushions by producing the mesenchymal progenitors of inlet and outlet valves and septa. Hyaluronan (HA), a major component of the ECM plays a critical role in the expansion and swelling of the AV cushions by creating “spaces” for proliferation, migration and outgrowth of the AV cushions. Defects in the AV junction (AVSD and ASD or high VSD) were fully penetrant in CCN1 null mice. Moreover, the human CCN1 gene and AVSD susceptibility gene have been mapped to the same chromosome. The CCN1 KO mouse has similar defects found in global KO of Filamin A. These findings implicate CCN1 and HA as candidates for AVSD.

Our studies indicate that: 1) the expressions of HA and versican are higher at the sites of expansion or swelling of E-24 chick AV cushions. By stage E-32, the central mass of mesenchyme formed by the cushion fusion is intensely positive for HA. 2) CCN1 is expressed by all junctional tissues between ED 10.0 and 12.5. 3) CCN1/integrin signaling activates Has2 and stimulates HA production. 4) CCN1 phosphorylates signaling intermediates ErbB2, Erk and Akt associated with proliferation and survival in sheep AV valve cells. Conversely, silencing CCN1 expression reversed these effects. 5). CCN1 phosphorylates FLNA through Erk and Akt pathways involving ErbB2. Silencing CCN1 also inhibited phosphorylation of Filamin A. Thus, CCN1 and HA-induced signaling regulate post-EMT events that led to the formation the AV septal complex. Grant support: INBRE P20 RR016461 (SG and RRM).

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**Defining the *P. gingivalis* apo-Fur Regulon**

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Periodontal diseases are described as the bacterially-initiated conversion of a healthy gingival region into one characterized by inflammation (gingivitis) and the destruction of the supporting structures of the teeth (periodontitis). The Gram-negative anaerobe *Porphyromonas gingivalis* is now recognized as the major etiological agent of adult periodontal disease. Epidemiological evidence supports an association between cardiovascular disease (CVD) and periodontal disease. *P. gingivalis* requires iron in the form of hemin for growth. In many Gram-negative bacteria, iron complexes with the ferric uptake regulator (Fur) protein to repress gene expression. While this mechanism of regulation has been well studied, recent investigations have demonstrated that the Fur protein can also repress gene expression when not complexed to iron. This form of the protein is termed apo-Fur. Microarray analysis has identified apo-Fur regulated genes in *Helicobacter pylori*, *Campylobacter jejuni* and *Desulfovibrio vulgaris*. The identification of a Fur homolog in *P. gingivalis* that is capable of partially complementing an *E. coli* fur mutant indicates that the *P. gingivalis* Fur protein is functional. The *P. gingivalis* Fur protein displays 25% homology to the *H. pylori* Fur protein, and 24% homology to the *C. jejuni* Fur protein. Negative and positive regulation of genes by both hemin and iron have been identified in *P. gingivalis*, however, the precise molecular mechanisms involved in this regulation have yet to be identified. While the mechanism of conventional Fur regulation is currently being studied, apo-Fur regulation by this organism has not been examined. Studies are currently underway to generate a *P. gingivalis* fur mutant. Microarray analysis of the mutant will be performed to identify apo-Fur regulated genes in this organism, as well as genes regulated by iron independent of Fur. Understanding the role of apo-Fur and iron independent of Fur in regulating *P. gingivalis* genes may provide further insight into periodontal disease progression and how this progression contributes to other health risks such as cardiovascular disease.

**Lori Turner**

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**The effect of chloroquine on response to ceramide in prostate cancer cells**

Lorianne S. Turner, Lenton Holley, Frank Pezzimenti, Timothy Prince, and Krissy Smith.

Effect chloroquine on response to ceramide in prostate cancer cells over-expressing Acid Ceramidase  
Over-expression of the enzyme Acid Ceramidase (AC) has been observed in prostate cancer cell lines and primary tumors, and contributes to resistance to chemotherapy and radiation. AC over-expression increases the conversion ceramide, which is often produced as a pro-apoptotic response to stress, to sphingosine, which can then be phosphorylated to the pro-survival molecule sphingosine-1-phosphate.

In addition to the ability to metabolize ceramide produced in response to stress, prostate cancer cell lines over-expressing AC also have increased levels of autophagy and increased lysosomal density. Autophagy is a cellular mechanism for recycling proteins and organelles which can be used as a survival mechanism in response to stress. Therefore, in addition to the increased capacity to metabolize ceramide produced in response to insult, the increased basal level of autophagy results in cells that have a heightened resistance to cellular stress. Preliminary results suggest that cells over-expressing acid ceramidase have increased expression of the lysosomal-stabilizing protein KIF5B, which has been shown to be over-expressed in a highly invasive breast cancer line. These results also highlight lysosome stability as a potential therapeutic target for cancer therapy. We have evaluated the effect of chloroquine, an FDA-approved drug that localizes to lysosomes, on the response of these cells to ceramide treatment. Pre-treatment with chloroquine increased the sensitivity of the cells to ceramide treatment, possibly due to AC disruption. These studies suggest that chloroquine may increase the efficacy of treatments that induce ceramide.

### **Henry Bateman**

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### **Sparstolonin B Inhibits Angiogenesis by Downregulating Cell Cycle Regulatory Proteins**

Henry R. Bateman, Vanessa Rodriguez, Qiaoli Liang, Daping Fan, Susan M. Lessner, Univ of South Carolina, Columbia, SC

Sparstolonin B is a novel compound isolated from *Sparganium stoloniferum*, an herb historically used in Traditional Chinese Medicine as an anti-tumor and anti-spasmodic agent. Angiogenesis, the process of new capillary formation from existing blood vessels, is dysregulated in many pathological disorders, including diabetic retinopathy, tumor growth, and atherosclerosis. Previous work in our lab demonstrated potential anti-angiogenic effects of Sparstolonin B (SsnB). Functional assays showed that SsnB inhibits endothelial cell tube formation and cell migration in a dose-dependent manner. In addition, microarray experiments with human umbilical vein endothelial cells (HUVECs) and human coronary artery endothelial cells (HCAECs) demonstrated differential expression of several hundred genes in response to SsnB exposure (916 genes for HUVECs and 356 genes for HCAECs, fold change  $\square 2$ ,  $p < 0.05$ , unpaired t-test with a false discovery rate approximating 0%). Microarray data from both cell types showed significant overlap, including genes in pathways associated with cell proliferation and cell cycle. Flow cytometric cell cycle analysis of HUVECs treated with SsnB showed an increase of cells in the G2/M phase (from 8.2 to 21.2%) and a decrease of cells in the G1 and S phases (from 73.8 to 67.1% and from 18.0 to 11.9% respectively). Cyclin B1 (CCNB1) and Cyclin Dependent Kinase 1 (CDC2) are regulatory proteins that control cell cycle progression through the G2/M checkpoint. Both CCNB1 and CDC2 were downregulated in the microarray data. Real Time quantitative PCR confirmed that gene expression of CCNB1 and CDC2 was downregulated after SsnB exposure, to 13% and 17% of controls, respectively. The data suggests that SsnB may exert its anti-angiogenic effects by downregulating CCNB1 and CDC2, which are both essential for progression through the G2/M checkpoint. Overall, this study demonstrates the potential of SsnB as a novel pharmaceutical agent to inhibit angiogenesis.

**Heather Evans-Anderson**

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[evansandh@winthrop.edu](mailto:evansandh@winthrop.edu)**Microarray and Ultrastructure Analyses of Regenerative Myocardium**

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A common feature in both pediatric and adult heart disease is altered regulation of cardiac myocyte proliferation, which leads to structural and functional defects in the myocardium. The regulatory program directing cardiac myocyte proliferation must be well defined in order to develop well-targeted therapeutic strategies designed to correct heart disease in pediatric or adult patients. *Ciona intestinalis* is an invertebrate animal model system that is well characterized and has many advantages for the study of cardiovascular biology including: a conserved cardiac gene network, a relatively simple heart design, reduced genetic redundancy, and the ability to study large populations with relative ease in maintenance of the colony. Despite simplicity, heart development in *Ciona* is similar to early vertebrate embryos and provides a straightforward avenue for the study of signaling pathways, which are extremely complex in higher organisms. However, a striking difference between mammalian and *Ciona* hearts is that the *Ciona* myocardium is capable of regenerating cardiac myocytes throughout its lifespan, which makes the regulatory mechanisms of cardiac myocyte proliferation in *Ciona* very intriguing. In order to identify potentially important regeneration factors in *Ciona*, custom Affymetrix GeneChip arrays were conducted on adult *Ciona* hearts with normal or damaged myocardium. Hearts were injured via ligation or cryoinjury to stimulate regeneration. After a 24 or 48 hour recovery period, total RNA was isolated from damaged and control hearts. Initial results indicate significant changes in gene expression in hearts damaged by ligation in comparison to cryoinjured or control hearts. Ultrastructure analyses of injured myocardium using TEM were conducted in parallel to the microarray study. Preliminary results show changes in the myofibril arrangement and cellular organization in injured hearts versus controls. Further studies using immunohistochemistry to identify proliferation and apoptosis in cardiac myocytes of damaged and control hearts are currently underway. Taken together, these studies will coordinate differences in gene expression to cellular changes in the regenerative myocardium of *Ciona*, which will help to elucidate the regulatory mechanisms of cardiac myocyte proliferation.

## Poster Presentations

### **Aja Moss**

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### **Methods Development of Metabolite Extractions from Cervical Mucus for Application in an NMR-Based Metabolomics Study of Human Papillomavirus (HPV)**

Kayla Felix, Aja Moss, and Arezue Boroujerdi

Human Papillomavirus is a member of the papillomavirus family of viruses and is established in the stratum epithelium of the skin or mucus membrane. There are more than 200 types of HPV, with up to 20% of these types being sexually transmitted. Persistent, high risk human papillomavirus (HR-HPV) infection is the major cause of cervical cancer. Most HPV infections clear spontaneously in a few months, and only about 10-15% persists. Currently, diagnosis of persistent HPV infection requires repeated, time consuming, and costly approaches which are not applicable to large scale screening. If markers of HPV persistence could be identified at the first visit, then subsequent care could be administered to the women who really need it, in a cost-effective and efficient manner. NMR-based metabolomics is a novel approach for biomarker discovery. This approach is proposed here as a means of initial screening to identifying biomarkers or characteristic metabolic profiles related to HR-HPV persistence. Furthermore, with the use of NMR and robust statistical analysis methods, the NMR-based metabolomics approach offers a non-targeted, quantitative identification of the metabolites present and can reveal unexpected properties of the metabolism of an organism that result from cellular adjustments to stressors. The first goal for our research is to determine the best method for polar metabolite extraction from human cervical mucus collected from women with and without infection by HR-HPV. Several methods including filtration of the mucus were tested; however, the NMR spectra of the cervical mucus were overwhelmed by compounds such as glycerol that were found on the sponges used to collect the sample. Although the presence of these compounds most likely masked many of the cervical mucus metabolites, there were some regions of the spectra that were not comprised by overlap. Lactic acid and sugars were identified in these areas.

### **Aaron Shepard**

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### **Protocol Development for a Metabolomics Approach to Understanding Cold Tolerance in *Drosophila Melanogaster***

Aaron Shepard, Daniel A. Hahn, Arthur S. Edison, (Arezue Boroujerdi)

It is known that genotype leads to phenotype, but exactly how genetic variation leads to phenotype variation is still unknown. *Drosophila melanogaster*, when exposed to cold temperatures experience, cold shock in which they temporarily become unconscious and are unable to move. *Drosophila* recover from cold shock after being exposed to warmer temperatures over time. The ability to recover from cold shock is determined genetically and varies among the 270 known lines of *Drosophila*. To better

understand the genetic changes caused by cold shock, protocol for a metabolomics study was developed and tested. Drosophila metabolites were extracted in an acetonitrile buffer and were then re-suspended in deuterium oxide for NMR spectroscopy. Both the 1-D <sup>1</sup>H and the 2-D COSY NMR experiments provided clear and cohesive data that could be used to observe metabolic changes due to cold shock. Currently, another extraction protocol is being developed based from the results of this experiment which focuses on separation the polar and non-polar metabolites.

### **Victoria DeSormeaux**

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### **Chemical Stress Response in Caenorhabditis elegans**

Victoria De Sormeaux, Jillian Harrison, Gregory Stupp, Ramadan Ajrendini, Arthur Edison, (Arezue Boroujerdi)

The bacterivorous, non-parasitic free living terrestrial nematode *Caenorhabditis elegans* is one of the most extensively studied organisms today. The nematodes small body size, compact genome and exposure to common pathogens has made it an ideal simple model for anatomical, cellular, genetics and neurobiological studies; however little is known of the worm's chemical biology. *C. elegans* like most small organisms utilizes chemical signals, known as pheromones, to communicate with like worms and its environment. Excreted pheromone composition varies according to the environment encountered by the worm, for instance pheromone complexity alters in response to stress such as low food/high population ratios, poisonous substances or sharp temperature changes in environment. This experiment tests homologous *C. elegans* populations' response to heat induced stress. We hypothesized that upon exposure to high temperatures *C. elegans* induces an up-regulation of hsp1 resulting in a variation in excreted pheromone composition. NMR analysis, using a Bruker 600 MHz spectrometer, of heat-shock worm exudates and non-heat shock worm exudates were compared and small variations in the peak patterns of the two groups observed. Further studies are currently being conducted. The complete analysis of the results of this experiment will be influential in the completion of other research endeavors as well as contribute significantly to the understanding of the correlation of genetics and behavior in *C. elegans*.

### **Ying Wang**

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### **Adhesive Strength of Atherosclerotic Plaques Depends on Collagen Content**

Ying Wang, Abigail Fulp, John Johnson, Michael Sutton, Susan Lessner

Atherosclerotic plaque rupture is a major cause of myocardial infarction and stroke. The adhesive strength of the bond between the plaque and the vascular wall, measured as local energy release rate, *G*, was used for quantitative plaque stability estimation. We hypothesize that adhesive strength varies with plaque composition in mice of different genotypes, and that it correlates with histological features

associated with plaque stability, such as collagen deposition and macrophage content in lesions. Mice which are genetically deficient in matrix metalloproteinase 12 (MMP12), a macrophage elastase, have previously been shown to demonstrate altered lesion composition. To estimate the energy release rate, G, peeling tests were performed on aortic plaques from apolipoprotein E knockout (apoE KO) and apoE MMP12 double knockout (apoE MMP12 DKO) male mice maintained on a Western diet for 8 months. For plaques in apoE KO mice, our experimental values for G averaged 19.1 Joule/m<sup>2</sup>. For plaques in apoE MMP12 DKO mice, G values averaged 12.1 J/m<sup>2</sup>. A two-tailed Mann-Whitney test showed a significant effect of genotype on measured G values ( $p < 0.001$ ). Histological studies confirmed that the plaques delaminated at the interface between the plaque and the underlying internal elastic lamina (IEL) in both strains of mice. Quantitative image analysis of Picrosirius Red-stained tissue sections demonstrated a positive linear correlation between local collagen content of lesions and G values in both strains of mice ( $p < 0.01$ ). The average collagen content for plaques in apoE KO (14.7%  $\pm$  13.8%) and apoE MMP12 DKO mice (9.8%  $\pm$  8.4%) was not significantly different ( $p > 0.05$ ). Immunohistochemical staining showed that macrophage content of aortic plaques is neither significantly correlated with G values nor significantly different between these two strains of mice. Overall, our results suggest that plaques adhere more strongly to the underlying IEL in apoE KO mice than in apoE MMP12 DKO mice.

#### **John Kaup**

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The position of Coordinator of Science Education at Furman University (a position created in January 2010 through NSF-RII and Furman matching funds in 2010 and co-funded by NIH-INBRE in 2011) has continued to grow its community impacts, including significantly enhanced connections with the public K-12 science sector. The addition of INBRE support through our 2011 award has provided seed money supporting outreach such as the Scope-On-A-Rope (SOAR) loaning program first pioneered at LSU. Focusing on three aspects of our outreach (Professional Development, Broadening Participation and K-12 /Community Connections), we present here both our successes and challenges, and highlight our ongoing and expanding commitment to enhance the scientific literacy of our community.

#### **Shana Roach**

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#### **Characterizing Collagen Fiber Angles in Mouse Aorta using Second-Harmonic Generation Microscopy**

Shana Roach, Susan M. Lessner University of South Carolina

Atherosclerosis or hardening of the arteries is a result of an accumulation of fatty materials such as cholesterol and other lipid substances in the artery wall. The fat and cholesterol build up leads to development of arterial lesions known as plaques, which narrow the arteries making it harder for blood to flow through them. In advanced atherosclerosis, plaque rupture often causes heart attacks or strokes. Collagen is a major structural protein in the artery wall, responsible for the overall strength of

the tissue. Studying collagen fiber distribution and alignment aids in understanding the structure and biomechanics of arteries. We collected samples from the thoracic and abdominal portions of mouse aortas in three strains of mice: ApoE knockout (KO), ApoE MMP9 double knockout (DKO), and ApoE MMP12 DKO mice. Collagen fiber angles were characterized by using Multiphoton Second-Harmonic Generation (MP-SHG) microscopy, which provides a method to visualize collagen fibers in tissue without staining. Using this technique, we can image collagen fibers throughout the thickness of the tissue and create a 3-dimensional reconstruction of fiber orientation. Our preliminary data suggests that the orientation of fiber angles varies with genotype. The ApoE MMP9 DKO mice appeared to have two fiber families at an angle to each other, whereas the ApoE KO mice demonstrate a single fiber family in a nearly circumferential orientation. We also found that there is no significant difference between the ApoE KO mice and ApoE MMP12 DKO mice in the abdominal portions of the aortas. In addition, when comparing the thoracic and abdominal portions in the ApoE MMP12 DKO mice, we also notice there is a lot of variability and a larger difference between regions than in the ApoE KO strain. To support these observations, an increased number of mice must be used to obtain better statistical analyses.

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### **Isolation and molecular characterization of Rem2 isoforms in the rainbow trout (*Oncorhynchus mykiss*): Tissue and central nervous system expression**

David M. Hollis, Yuri Sawa, Ashley Wagoner, Jason S. Rawlings, and Frederick W. Goetz. Furman University.

REM2 is a member of the REM, RAD, and GEM/KIR (RGK) subfamily of RAS superfamily proteins and plays an important role in brain development and function. In this study, two Rem2 isoforms were isolated from the rainbow trout (*Oncorhynchus mykiss*). The two genes, designated *O. mykiss rem2a* and *rem2b*, both encode 304 amino acid proteins with 61% and 62% identity to zebrafish (*Danio rerio*) Rem2, respectively, and each with 43% identity to mammalian (human) REM2. To our knowledge, this is the first incidence of Rem2 isoforms in a species that are the result of gene duplication. Both isoforms possessed similar tissue expression profiles with the highest levels in the brain. The *rem2a* gene has significantly higher expression levels than *rem2b* in all tissues assayed except the brain and head kidney. In the central nervous system, both isoforms showed similar expression levels with the highest levels occurring in the olfactory bulb, cerebrum, and midbrain, though *rem2a* expression is significantly higher in the spinal cord. Based on known functional roles of Rem2 in synapse development and stem cell proliferation, the characterization of Rem2 in rainbow trout could shed light on its role in adult vertebrate neurogenesis and brain regeneration. Supported by NIH Grant # P20 RR-016461.

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[Brittany.Law@uscmcd.sc.edu](mailto:Brittany.Law@uscmcd.sc.edu)**Alterations in Cardiac Structure and Function in a Murine Model of Chronic Alcohol Consumption"**

Brittany Law, Wayne Carver

Epidemiological evidence has implicated alcohol abuse to account for a condition deemed alcoholic cardiomyopathy, a dilated cardiomyopathy presenting with significant fibrosis. Male, wild type, FVB strain mice were fed a nutritionally complete liquid diet supplemented with 4% ethanol v/v over a time course of 1, 2, 4, 8, 12, and 14 weeks. Controls were offered an isocaloric liquid equivalent and pair fed with their ethanol counterparts. Changes in cardiac physiology were assessed at respective timepoints via echocardiography. Additionally, the use of histological techniques, mRNA analysis, apoptosis determination, and immunohistochemistry were employed to research ethanol's physiological and structural changes on the heart over a time course. Left ventricular hypertrophy was observed in ethanol consuming mice early within the time course (1-8 weeks) followed by a reversion to normal or ventricular dilation by weeks 12 & 14. Throughout the study, an increase in cardiomyocyte hypertrophy, cardiac fibrosis, apoptosis, transforming growth factor beta and the presence of alpha smooth muscle actin was determined. The presence of cardiac fibrosis in mice chronically consuming ethanol chronically may play an important role in driving ventricular dilation. This fibrotic phenotype is correlated with alcohol's induction of transforming growth factor  $\beta$  and the promotion of apoptosis and myofibroblast transdifferentiation. Additionally, gene microarray analysis is being employed to correlate changes in gene expression with alterations in cardiac physiology and morphology in the hearts of mice chronically consuming ethanol. These studies will provide important insight into the molecular and cellular mechanisms of alcohol-induced myocardial remodeling.

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[schmidt@email.sc.edu](mailto:schmidt@email.sc.edu)**Contribution of New Features of REDCAT in Study of Macromolecular Structure and Dynamics**

Christopher Schmidt, Stephanie Irausquin, and Homayoun Valafar

Residual Dipolar Couplings (RDCs) are the only source of NMR data that encapsulate information, related to macromolecular structure and dynamics, in a biologically relevant timescale. An increasing number of instances support the importance of internal dynamics of proteins in their function. For example, the internal dynamics of human Thymidylate Synthase (hTS) are required for DNA synthesis in a number of organisms. This has led to its extensive use in the development of pharmaceutical agents aimed at a number of cancers. Although RDC data can in theory be used for such investigation, their elusive nature complicate their utility and therefore introduces challenges in their analyses. Here, we demonstrate a new feature of REDCAT, enhanced analysis of multiple structures in multiple alignment

media, in application to the study of internal dynamics of the hTS protein (1HVY). Here various models of dynamics were generated and subsequently used for generation of synthetic backbone N-H RDC data, to consider 3 classes of dynamics: discrete N-State rigid body dynamics, continuous rigid body dynamics, and uncorrelated dynamics. Molecular motion in 1HVY, was simulated in two known hTS mobile domains: between residues 107 to 128 (one of two loops inserted when compared to bacterial TS), and between residues 181 to 197 (the active site loop). hTS exists in two conformations, the “active” conformation similar to that seen in other species, and an “inactive” conformation where the active site loop is altered by 180 degrees. The dynamic averaging features of REDCAT were used to analyze the existing structure and the generated RDC data for all 3 classes of dynamics in order to assess the ability to not only identify regions of dynamics, but categorize the correct model of dynamics as well. Analysis of the averaged RDC data and the REDCAT files was conducted without prior knowledge of how the MDS was performed. For all 3 classes of dynamics, the residues involved in the motion, as well as the nature of the motion they underwent, was demonstrated clearly and accurately. Utilizing the VMD interface, it is possible to visualize fitness of the RDC data to the static structure. The analysis serves to predict the location and nature of the motion, which can then be easily visualized within the VMD program. Therefore, REDCAT can be used to identify and characterize internal motions from RDC data.

### **Fang Yang**

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### **Fast mixing in a non-parallel microfluidic chip using AC electrokinetic instability**

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Electrokinetic instability (EKI) flow can be use as an efficient tool for mixing in a lab-on-a chip system. In this report, we have fabricated a Metal directly formed sidewalls quasi T channels. And a parametric study was conducted to explore the effectiveness of manipulating EKI waves to control/enhance fluid mixing inside a quasi T channel. Firstly, mixing results in two cases have been compared: electrodes are placed at the sidewall and electrodes are located at the ends of the channel. Secondly, the mixing results in the microchannel with different angle between two electrodes were assessed in terms of scalar concentration distributions, the fluid mixing efficiency was found to increase rapidly at the angle of 5° between two electrode sidewalls, furthermore, we put fluorescent particles in one of the two streams and achieved a more clear visualization of mixing process in microchannel with 5° angle between two electrode sidewalls. Fourthly, the effectiveness of the applied voltage phase variation between two electrodes on the mixing process inside the quasi T channel were also explored for the further enhancement of the fluid mixing inside the quasi T microchannel. The measurement result revealed that the fluid mixing was found to be stronger under a 180° signal phase shift between two electrodes than that under a 90°. Finally, clearly mixing result under high frequency was also achieved in the electrode sidewalls quasi T channel.

**Karen Buchmueller**

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**Characterizing the Inhibition of HMGA by Netropsin and Distamycin**

Karen Buchmueller

Many diseases, most notably cancers, are characterized by abnormal patterns of gene expression, and therapies that target specific transcriptional regulatory complexes are promising approaches to treatment and prevention. However, the biophysical properties that underlie how small molecules physically disrupt proteins from binding to DNA are poorly understood. Understanding the dynamics by which small molecules compete against proteins for binding to DNA will aid in the development of more effective and selective protein inhibitors. Specifically, the overexpression of the High Mobility Group A (HMGA) family of proteins have been implicated in a variety of metastatic cancers. These proteins bind to DNA via an A/T hook motif, and binding of A/T hooks and the entire protein have previously been shown to be disrupted by distamycin and netropsin. We have further investigated the structural dynamics and the thermodynamic contributions of the competition between these small molecules and the A/T hook.

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**MiR155 deficiency in bone marrow promotes tumor metastasis through increasing the recruitment of MDSCs**

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Recent studies have shown that several types of tumor display increased miR155 expression, which is associated with a more invasive tumor phenotype, suggesting an oncogenic function of miR155. However, the role of host cell miR155 in tumor development and metastasis is unknown. Since miR155 plays a pivotal role in immunity, we aim to examine the function of bone marrow cell miR155 in tumor metastasis. To this end, we assessed the tumor metastasis in a Lewis lung carcinoma (LLC) implantation model. Lethally irradiated miR155<sup>-/-</sup> mice were reconstituted with wild type or miR155<sup>-/-</sup> bone marrow cells and then inoculated subcutaneously with LLC cells. We found that bone marrow miR155 deficient mice displayed more lung metastasis with significantly increased recruitment of myeloid-derived suppressor cells (MDSCs) to both primary tumor and metastatic lung. Increased HIF-1 $\pm$ , VWF and MMP2 expression in primary tumor indicated enhanced angiogenesis and a more invasive tumor phenotype in bone marrow miR155 deficient mice. Moreover, induction of S100A8/S100A9, which are mainly produced by bone marrow derived myeloid cells and believed to be metastasis-promoting, in metastatic lungs also indicated the relevance of the increased MDSC recruitment. Taken together, our data suggest that miR155 deficiency in bone marrow facilitates the recruitment of immature myeloid cells to primary tumor and lung, resulting in accelerated tumor metastasis. In light of the oncogenic role of cancer cell

miR155 in several tumors, our data present a predicament regarding to the development of anti-miR155 strategies for cancer treatment.

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### **microRNA-155 deficiency in bone marrow promotes atherosclerosis in hyperlipidemic mice**

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MicroRNA155 (miR155) plays a crucial role in the differentiation, function and survival of immune cells, including macrophages. Macrophages and other immune cells are determinants in atherogenesis. We examined the role of miR155 expressed by bone marrow-derived cells, especially macrophages, in atherosclerosis, and investigated the underlying mechanisms. First we found that microRNA-155 expression was increased in both mouse and human atherosclerotic lesions compared to normal aortic tissues. Next, we transplanted bone marrow cells from wild-type C57BL/6 or miR155<sup>-/-</sup> mice to lethally irradiated LDLR<sup>-/-</sup> mice and fed the mice with a high-fat diet for 12 weeks, and we found miR155 deficiency in bone marrow increased atherosclerosis in mice. Immunohistological analyses revealed that atherosclerotic lesions in miR155<sup>-/-</sup> bone marrow recipients contained more macrophages, more apoptotic cells and larger necrotic cores. miR155<sup>-/-</sup> bone marrow recipients also had reduced number of regulatory T cells in spleens. We further found that in macrophages FOXO3a and HIF-1 $\alpha$  are direct targets of miR155, and miR155 deficiency led to increased protein levels of FOXO3a and HIF-1 $\alpha$ , resulting in increased macrophage apoptosis and enhanced responses to hypoxia in the atherosclerotic plaques. In conclusion, miR155 deficiency in bone marrow promotes atherosclerosis hyperlipidemic mice, due to increased apoptosis of and enhanced hypoxic responses in macrophages, and decreased number of anti-atherogenic regulatory T cells.

### **Nicholas Grosseohme**

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### **Toward a Robust Calorimetric Methodology to Study Cu (I) Binding to Biological Molecules**

Nicholas Grosseohme, Destinee Johnson, Sharon Jenkins, and Zayed Almadidy

Copper is an essential nutrient for nearly all organisms and, as such, strict concentration regulation is required to avoid lethal consequences; in humans, non-homeostatic concentrations of copper results in Wilsons and Menkes Diseases. This metal has also been adapted by some pathogens (e.g. Mycobacterium tuberculosis) as a potent virulence factor. To date, nearly all thermodynamic information available describing copper interactions with biological molecules has been determined using indirect methods which preclude the possibility of determining additional thermodynamic driving forces. This is, in part, due to the inherent difficulties associated with aqueous solution chemistry of the biologically relevant oxidation state, Cu (I). The research described here outlines the development and

implementation of a rigorous stabilizing system for calorimetric titrations and complementary spectrophotometric titrations of Cu (I) over a wide affinity range. This methodology will enable a thorough characterization of the fundamental thermodynamic forces associated with Cu (I) binding and selectivity for several common structural motifs. ITC experiments described, carried out by the PI and undergraduate colleagues, are the first of their kind and will provide guidance to researchers wishing to investigate Cu (I) dependent systems. These experiments will provide a thermodynamic explanation for the metal specificities observed throughout all biological systems and define energies that govern allosteric communication through copper sensing transcription factors.

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### **2D-PDPA: Probability Density Profile Analysis as a Mechanism of Reducing the Cost of Protein Structure Determination**

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Traditional characterization of a protein structure by NMR spectroscopy is expensive and time consuming regardless of structural novelty of the target protein. In an effort to expand the applicability of NMR spectroscopy, the community is continually focused on development of new and economical approaches that enable study of more challenging, or structurally novel proteins. While many advances have been made in this regard, very little attention has been made on reducing the cost of structural characterization of routine proteins. Probability Density Profile Analysis (PDPA) is introduced by our lab to directly address the economies of structure determination of routine proteins and subsequently, identification of novel structures based on statistical analysis of a minimal set of NMR data. Our initial work with PDPA was conducted using 1D-PDPA and was based on unassigned RDC data from one medium alignment. 1D-PDPA established the feasibility of identifying homologous structures from unassigned RDC data, however it lacked the potential for large scale application. 2D-PDPA extends the analysis of 1D-PDPA by utilizing RDC data from two alignment media and successfully identifies an unknown protein using a library of ~1000 decoy structures. The additional set of RDC data address some of the inherent short coming of RDC data, such as inversion and varying insensitivity as a function of orientation of a structure in the AF (Alignment Frame). Parzen Density Estimation is used to generate a two dimensional PDP by considering both alignment media. PDPA operates by comparing a computed PDP (cPDP) to an experimentally determined pattern of data distribution (ePDP). This comparison has been conducted in an equally spaced grid mechanism between the two PDPs. 2D-PDPA calculates PDP for every rotation and a scoring method is used to find the best structures in terms of the similarity to the ePDP. The main contribution of our method demonstrates the correlation between scored PDP and bb-rmsd of the corresponding structure also is a viable method for ascertaining a protein's structural novelty within 3Å..., relative to the existing library of structures. A hybrid approach of 2D-PDPA based on selection of the best computed structure can be envisioned, to extend the sensitivity of 2D-PDPA by using more than two structural dataset while maintaining low cost of the process.

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**PI-3 Kinase mediates Zinc-induced Mitophagy in neuronal cells**

Wendell Jones, Krissy Smith, Latha Malaiyandi, and Lindsey Smith

Zinc in neural tissue is normally highly regulated, however, evidence suggests that the accumulation of free zinc ions ( $Zn^{2+}$ ) in response to injurious stimuli can induce neuronal death. The imbalance of cellular  $Zn^{2+}$  is implicated in pathologies such as stroke, epileptic seizures and Alzheimer's disease. Although many targets are associated with  $Zn^{2+}$ -induced neuronal death, recent studies have suggested that  $Zn^{2+}$  can give rise to and accumulate in autophagic lysosomes. Autophagy is a programmed intracellular mechanism used to remove dead or non-functional organelles. Our previous findings suggest that mitochondria may be an important intracellular target for  $Zn^{2+}$ , which could contribute to overall energy failure of the neuron. Despite progress in understanding  $Zn^{2+}$ -induced pathologies, the mechanism(s) of mitochondrial autophagy (mitophagy) remains unclear. Here, we hypothesize that  $Zn^{2+}$  induces mitophagy in cultured neuronal cells. After exposing cells for two hours to  $Zn^{2+}$ , we visualized mitochondria and autophagic lysosomes using fluorescent microscopy and measured the degree of co-localization between signals. Our results show that  $Zn^{2+}$ -induced mitophagy and cell death occur at similar concentrations. Other studies provide evidence that autophagy is regulated by many diverse signaling pathways, including phosphatidylinositol 3-kinase (PI3-kinase) and mitogen-activated protein kinase (MEK). To determine a downstream mechanism, we used protein kinase inhibitors to demonstrate that  $Zn^{2+}$ -induced mitophagy is mediated through activation of PI3-kinase, but not through MEK. These findings provide new insight into the mechanism of  $Zn^{2+}$ -induced neurotoxicity.

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**Analysis Pipeline for Simultaneous Study of Structure and Dynamics of Challenging Proteins from Residual Dipolar Couplings**

Stephanie Irausquin, Christopher Schmidt, Mikhail Simin, Homayoun Valafar

Studies which further contribute to our understanding of protein structure-function relationships have indicated a need for investigations conducted in a physiologically relevant timescale and environment. Residual Dipolar Couplings (RDCs), have been recognized as a possible method for probing the structure and dynamics of proteins on timescales ranging from pico to microseconds, when most biologically important functions occur. Despite their promise, very little success has been demonstrated due to challenges presented during the separation of structural and motional information. Here we present a pipeline comprised of software tools (msTALI, REDCRAFT and REDCAT) specifically designed to address different aspects of RDC data extraction and analysis, utilizing a protein which undergoes

conformational switching. Thymidylate Synthase (TS) is required for DNA synthesis in a number of organisms and its inhibition in rapidly dividing cells eventually leads to apoptosis. This has led to its extensive use as a target for chemotherapy aimed at a number of cancers. Although TS is highly conserved among different species, X-ray crystallographic studies reveal that human TS (hTS) differs from that of bacterial TS's in three regions: the N-terminus which is extended by 28 residues, an insertion of 12 residues at position 117, and an insertion of 8 residues at position 146. Another feature unique to hTS is that its active site loop (residues 181 to 197) can exist in two conformations (“active” and “inactive”). These characteristics identify hTS as an ideal target for both the therapy of cancers, and the study of internal dynamics. By first utilizing msTALI, we were able to summarize existing hTS structural information and select a representative structure, which was used to create Molecular Dynamics Simulations of hTS that modeled existing constraints in the literature. The resulting trajectories were then used to generate synthetic RDC data for the implementation of two categories of internal dynamics (rigid body and uncorrelated). Following identification of the type of dynamics: REDCRAFT was capable of reconstructing rigid body, 2 and 3 state dynamics with less than 1.5Å of accuracy; and REDCAT was able to identify the correct model of uncorrelated dynamics from an ensemble of alternate decoy models of internal dynamics. In conclusion, our method combines Bioinformatic tools with computational biology approaches and is applicable to other challenging proteins.

#### **Andrew Shedlock**

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#### **Mobile DNA, digital transcriptomics, and the evolution of amniote genome structure and function**

Andrew M. Shedlock

The era of genomics has reinforced Barbara McClintock’s original intuition that mobile DNA elements can have a large impact on phenotypic diversification, and indeed the essential role of transposable elements in the evolution of novel gene regulatory pathways is still being revealed by advances in high-throughput sequencing methods and digital transcriptome profiling. In the case of comparative vertebrate genomics, analysis of the large, active and diverse repetitive DNA landscape in reptiles has been essential for accurately reconstructing ancestral genomic states in amniotes while lending insight into the scope and evolutionary dynamics of the vertebrate genotype-phenotype relationship. Recent publication of the first lizard genome assembly (*Anolis carolinensis*) and the imminent completion of both the first turtle genome (*Chrysemys picta*) and the first crocodilian genome (*Alligator mississippiensis*) provide a timely opportunity to accurately map the first marine reptile transcriptome. The feasibility at the College of Charleston to massively parallel sequence loggerhead turtle (*Caretta caretta*) cDNAs has broad implications for advancing our predictive theory of eukaryotic genome evolution now lacking in comparative biology. In particular, RNAseq results from divergent tissue types in cohorts of experimentally reared loggerheads spanning the pivotal temperature for sex determination can open a critical window on lineage-specific signaling patterns inherent to adaptations to a fully pelagic marine life history. It is also expected to reveal patterns associated with human activities creating stressors on turtle health as measured by highly up- and down-regulated genes linked to physiological responses to environmental disturbances.

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**Parameter identification of polyethylene glycol diacrylate (pegda) hydrogels using a single invariant constitutive model**

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Cells interact with their extracellular environment by mechanical force exchange, mediated by the tension generated in the cytoskeleton. This interaction influences many biological processes, including cell migration. Therefore, by quantifying cellular traction forces during cell-material interactions, we can better understand the role of these forces in cell biology. Recent advances in optical strain measurement include the combination of digital volumetric correlation (DVC) with laser scanning confocal microscopy (LSCM). This approach has been used to quantify cell-generated forces throughout the volume of a transparent hydrogel seeded with fluorescent microspheres. To calculate the traction forces generated by the cells using the above techniques, it is important to understand the intrinsic mechanical properties of the hydrogel. In our study, we use polyethylene glycol diacrylate (PEGDA) hydrogels. To model the response of our system, we employed a single invariant constitutive model shown to be capable of accurately representing the mechanical response of polyvinyl alcohol hydrogels. In this study, we describe approaches used to control the mechanical properties by changing the initial concentration of the PEGDA. For a given hydrogel, we performed uniaxial compression experiments. The parameters within the single invariant constitutive model were estimated and the strain energy function (SEF) was fully characterized. To use this hydrogel system to measure cell-generated traction forces, we add fluorescent microspheres to the PEGDA hydrogels to track cell-induced deformations by DVC. The single invariant constitutive model provided a good linear fit to our compression test data. The two parameters within the single invariant model were determined and the resulting strain energy function can be used to describe the intrinsic mechanical properties of the PEGDA. One of the model parameters was sensitive to PEGDA concentration but the other parameter appeared to be concentration-independent. In this study we have shown that the mechanical properties of the PEGDA hydrogel can be modeled using the single invariant constitutive model, which is a more suitable model than a linear elastic model. Using this model, we have identified material parameters describing the behavior of the PEGDA hydrogel in uniaxial compression. We also show that the mechanical properties of the PEGDA hydrogels can be varied by changing the initial PEGDA concentration.

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**Leading-Edge Protein and Genomic Services at the MUSC Proteogenomics Facility**

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The MUSC Proteogenomics Facility is an SCINBRE supported resource that provides regional investigators with expertise, instrumentation and turnkey services for a range of genomic and protein-related technologies. The Facility currently offers Genomics services that include array-based

transcriptomic profiling (mRNA, microRNA) and SNP genotyping analysis services; we are also excited to offer next-generation sequencing to our list of services in 2012 following our recent acquisition of a Life Tech/ABI Ion Torrent PGM sequencing instrument. Protein-related services include multiplex analysis of cytokines, phosphoproteins and microRNAs, and biomolecular interaction analysis using a BIAcore 3000 surface plasmon resonance instrumentation. Bioinformatics services cover a full spectrum of microarray-related analyses as well as training in online and stand-alone informatic software, statistical analysis, promoter and transcription factor analyses and bioinformatic tool development. Since the formation of our Facility in 2003, the Facility has steadily increased the number of services provided per year as well as the number of investigators assisted. In FY2010-11, our Facility assisted >50 investigators at institutions across SC, including Clemson University, Coker College, MUSC, USC, and Winthrop University, conducting approximately 40 services/usages per month. In addition to our technical services, the Facility also provides training and consultation, whether for post-run data analysis or for preparation of grant application materials. Detailed description of current services and fees can be found at the MUSC Proteogenomics Facility website, <http://proteogenomics.musc.edu/>.

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Analysis of a Discontinuous RNA Enzyme Enzymes are Nature's catalysts: they accelerate chemical reactions by several orders of magnitude, allowing even inherently slow reactions to proceed with the speed needed to sustain life. One of the challenges of bio-related research is to understand the power of enzymes. Until a few years ago, we believed that ribozymes (enzymes made entirely of RNA) were formed by stretches of contiguous nucleotides that adopt complex three-dimensional structures in the cellular environment; however, recent work on the hammerhead ribozyme showed that 'discontinuous' ribozymes, in which two RNA regions distant in sequence recombine in-vivo to form an active ribozyme, are also present in Nature. In this project, we have started the investigation of a putative discontinuous group I intron. Introns are particular ribozymes that excise themselves from the surrounding RNA. Group I introns use an exogenous guanosine molecule to initiate this process. This 'cut and paste' process (splicing) allows, for example, formation of sequences that meaningfully encode for proteins. One of our collaborators recently reported the existence of a minimal group I intron (the 'PaSSU intron'), that lacks structural elements conserved in larger group I introns. This intron splices when long flanking regions (exons) are present, but not when these exons are minimal. It is therefore possible that regions outside the intron assist the splicing process. If these preliminary data are confirmed, this intron will represent a novel class of discontinuous ribozymes. The goal of this project is to find the minimal length of the flanking regions that allows proper splicing.

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**Testing Structural and Computational Models of Enzymes Active Sites through Chemical Modifications: Metal Ion Interactions in the Hammerhead Ribozyme**

Thomas Struble, Marcello Forconi

Synergy between functional tests, structural determinations and computation is emerging as a requirement for a deep understanding of biological systems. In particular, recent advances in molecular dynamics have provided the basis for the development of a rigorous treatment of the solvent structure, the hydrogen-bond networks, and the metal ions interactions in the active sites of many enzymes. With the aid of structural data derived from a plethora of high-resolution structures, we have now a formidable opportunity to feed the aforementioned synergy with carefully planned functional tests that validate and refine models derived from computational and structural work. Recent computational and structural works on the full length version of the hammerhead ribozyme have put forward novel models for the catalytic mechanism of this RNA enzyme. In one of these models a metal ion involved in catalysis is ligated to the N7 atom of residue G10.1 in the ground state of the reaction, but loses this coordination in the transition state. Thus, the contact between the metal ion and the N7 would be anticatalytic. A ribozyme modified to disrupt such ground state contact would be predicted to display enhanced activity relative to the unmodified ribozyme. To test this model, we have constructed a variant of the hammerhead ribozyme containing a CH group in place of the N7 of G 10.1, and we have measured its reactivity using single-turnover kinetics. Our preliminary results suggest that this model is incorrect, and that the contact between the N7 atom of residue G10.1 and the active site metal ion is maintained in the transition state of the reaction of the full-length hammerhead ribozyme.

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**Functional characterization of SdsA1, an alkylsulfatase from *Pseudomonas aeruginosa***

Avery Zierk, James Holt and Marcello Forconi

Functional characterization of SdsA1, an alkylsulfatase from *Pseudomonas aeruginosa*  
SdsA1 is a 72-kDa protein used by the pathogenic bacteria *Pseudomonas aeruginosa* to hydrolyze sodium dodecyl sulfate (SDS), a common surfactant used in soap and detergents that fights bacteria and other microorganisms by disrupting the interactions that hold the cells membrane together. To date, the information about this enzyme is basically limited to its crystal structure, published in 2006, which proposed a reaction mechanism and a set of interactions important for catalysis. However, there is no functional data to support these proposals yet. We have undertaken a comprehensive study to understand the mechanism of catalysis by this enzyme. To date, we have expressed and purified SdsA1, and tested for sulfatase activity using a dye-based assay. Here, we will discuss the set of experiments that will allow us to distinguish between different mechanisms, to determine and assess the importance of individual active site interactions in catalysis. Our work will provide important information regarding an enzyme used by a pathogenic bacteria, and will also have implication for a system biology approach

aimed at engineering microorganisms for the bioremediation of SDS-contaminated sites. Further, future comparison between the catalytic strategies used by SdsA1 and other enzymes belonging to the same protein superfamily of SdsA1, the metallo-beta-lactamase superfamily, will elucidate factors that contribute to the evolution of new substrate specificities using a common three-dimensional scaffold.

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### **Cloning and Protein Expression of Fox-O from *Ciona intestinalis***

Zayed Almadidy, Heather Evans-Anderson and Nicholas E. Grosseohme

Cloning and Protein Expression of FOXO from *Ciona intestinalis* Cardiovascular-related diseases are the leading cause of fatalities in the United States, responsible for nearly one million deaths each year. These conditions often times lead to damaged myocardial tissue which results in irrevocable loss of heart function. Forkhead (FOX) transcription factors (TFs) have been implicated to have a potential role in myocardial repair and are known to function in the regulation of mitosis, proliferation, cellular differentiation and apoptosis. All FOX TFs feature a winged helix DNA binding domain (DBD) that functions to mediate target gene expression. It has also been established that the activity of FOX TFs function in tandem via protein-protein interactions. In addition, FOX TF activity can also be mediated by posttranslational modifications that include phosphorylation, acetylation and ubiquitination. FOXO is a subgroup of these transcription factors that are regulated by growth factors through PI3K/AKT signaling pathways. The phosphorylation of FOXO, mediated by various kinases, initiates the binding of 14-3-3 proteins, which results in nuclear exportation and thus inhibits target gene expression. The goals of this project include a full sequence cloning of the FOXO DBD and a complete biochemical characterization of interactions between the FOXO DBD and its respective DNA or protein partners as well as any post-translational modifications that may affect such interactions. For such an experiment, *Ciona intestinalis* (*C. intestinalis*) specimens were utilized as they hold the simplest form of the chordate gene. To this end, we have amplified the DBD of FOXO from *C. intestinalis* and ligated it into a pET15a expression plasmid. *E. coli* BL21 (DE3) cells were transformed with this construct and used for protein expression. Currently, a chromatography based purification strategy is being generated. Upon purification, FOXO DBD-DNA interactions will be explored using electrophoretic mobility shift assays and quantitatively measured through fluorescence anisotropy. The ultimate aim of our research is to define the mechanisms by which the FOXO DBD functions to regulate gene expression in *C. intestinalis* during heart development and myocardial repair.

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**Comparative study of large axons in the abdominal connectives of mantids (Mantodea) and cockroaches (Blattaria)**

Marissa L. Paolini<sup>1</sup>, David D. Yager<sup>2</sup>, Jeffrey D. Triplehorn<sup>1</sup> <sup>1</sup>College of Charleston, Department of Biology, Charleston, SC <sup>2</sup>University of Maryland, College Park, Department of Psychology, College Park, MD Insect nervous systems lack myelinated axons and must increase axon diameter for faster action potential propagation. In abdominal connectives, large diameter axons are typically associated with the wind-sensitive cercal system that mediates escape and flight behaviors. These large axons carry information from the rear of the animal to the thoracic motor centers. Since not all insects fly or exhibit escape responses, large diameter axons likely vary based on these behaviors across species. We compared: 1) the nine largest axons in the abdominal connectives in five cockroach species and twenty mantis species; 2) the four largest axons in the dorsal and ventral intermediate tracts (DIT and VIT) due to their behavioral relevance: the VIT initiates escape responses while the DIT mediates continued escape running and flight maintenance and 3) morphological characters related to the cercal system. Initial results suggest cockroaches that exhibit escape responses possess similar, potentially adaptive, characteristics that are not present in non-escaping cockroach species. In mantids, sexual dimorphism in flight is reflected in the DIT. In *Parasphendale affinis*, DIT neurons occupy more connective space in flying males than in non-flying females, but the DIT dimorphism does not exist in *Hierodula grandis* where both sexes fly. Our data set includes several morphological and neural characteristics that can be mapped onto existing cockroach and mantis phylogenies to trace the evolutionary development of large diameter axons in these groups.

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**Comparative study of stimulus velocity encoding by the population of wind-sensitive interneurons in insect cercal sensory systems**

Clare A. McGorry<sup>1</sup>, Caroline N. Newman<sup>2</sup>, and Jeffrey D. Triplehorn<sup>1</sup> <sup>1</sup>College of Charleston, Department of Biology, Charleston SC <sup>2</sup>Department of Psychology, College of Charleston, Charleston SC The wind-sensitive cercal sensory system is an ancestral structure of insects. Previous work suggests that this system has been modified within each species based on its evolutionary and life history. This variation includes cercal morphology, the number of sensory receptors, the number and size of wind-sensitive primary interneurons (WSIs) that transmit wind information to the thoracic motor areas that generate behavior (i.e. terrestrial and/or aerial escape responses) and the behaviors that wind stimuli elicit in different species. This variation may result in natural differences in sensory processing and encoding of wind stimulus properties across species. The present study begins an extensive comparative investigation of variation in sensory processing and the mechanisms underlying these differences using the cercal sensory system as a model. Using extracellular recordings, we measured neural responses

generated by the WSI population to 300 ms wind puffs with velocities between 0-300 cm/s. We generated stimulus-response (S-R) curves for three closely-related cockroach species (*Periplaneta americana*, *Gromphadorhina portentosa*, and *Blaberus* sp.) as well as two more distantly-related species (mantis *Tenodera aridifolia* and cricket *Acheta domesticus*) to assess how encoding of wind stimulus information by the cercal system varies across species. Wind stimuli elicited more neural responses in species that exhibit strong wind-evoked escape responses (*P. americana* and *A. domestica*) than those that do not (*G. portentosa* and *T. aridifolia*). However, wind elicited the most neural responses in *Blaberus* sp., which has a weaker wind-evoked response than *P. americana* or *A. domestica*. Furthermore, *Blaberus* sp. exhibited the least inter-individual variation in the S-R curves. Future studies will examine: 1) whether the differences in the S-R curves across species begins at the level of the primary afferents and 2) whether the differences in WSI activation translate into corresponding differences in motor neuron activation, which may contribute to differences in the wind-evoked behavioral responses across species.

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### **The Role of the Protein C System in Prostate Cancer**

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We investigated the thrombomodulin (TM)-protein C system in prostate cancer (CaP). During coagulation, thrombin bound to TM on endothelial cells activates protein C into activated protein C (APC) with the aid of the endothelial protein C receptor (EPCR). We determined if TM and EPCR are also expressed in two prostate cancer cell lines, PC-3 and DU-145, and their ability to activate protein C into APC. We then determined how each of the proteins involved in this system regulate CaP cell proliferation and invasion in vitro. We first demonstrated that TM and EPCR are expressed by both PC-3 and DU-145 cell lines using Real-Time PCR and Western Blotting. We then localized TM and EPCR to both cell lines using confocal microscopy. PC-3 and DU-145 TM and EPCR were able to activate protein C into activated protein C in the presence of thrombin. Protein C, APC, and thrombin did not affect PC-3 or DU-145 proliferation. In an invasion assay, protein C, APC, and thrombin alone did not affect CaP cell invasion. Since APC has been shown to affect tumor cell invasion in ovarian cancer by competing with uPA for PAI-1, we determined if APC also regulates tumor cell invasion in CaP by competing for PAI-1. In the presence of uPA and PAI-1, APC competed with uPA for binding to PAI-1, enabling uPA to increase the invasiveness of DU-145 cells. We conclude that the CaP cell lines PC-3 and DU-145 make TM and EPCR, receptors necessary for the generation of APC. We also conclude that although protein C, APC, and thrombin alone do not affect CaP cell proliferation or invasion, in the presence of CaP cell TM and EPCR and exogenously added thrombin, protein C, PAI-1 and uPA, APC is generated. APC then binds to PAI-1, freeing uPA to facilitate CaP tumor cell invasion.

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### **A pro-inflammatory splicing of Cyld in vascular smooth muscle cells**

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Inflammation, cytokine production, and vascular smooth muscle cell (VSMC) proliferation are pivotal for arterial repair, usually culminating in excessive neointimal formation and decreased luminal diameter associated with adverse clinical outcome. A prevalent notion is that the activation of nuclear factor (NF)- $\kappa$ B pathway predominantly mediates the vascular inflammatory responses. However, inactivation of NF- $\kappa$ B pathway could either inhibit or promote vascular lesion formation via yet undefined mechanisms. Herein, we report that a splicing variant of cylindromatosis (CYLD), named bCYLD, preferentially promotes the inflammatory cytokines such as TNF $\alpha$ -induced migration and exaggerates vascular lesion formation independent of NF- $\kappa$ B. In rat aortic smooth muscle cells, knocking down all splicing variants of CYLD enhanced NF- $\kappa$ B activity but predominantly inhibited TNF migration. To further address the role of CYLD variants in inflammatory activation of VSMCs, transgenic mice of VSMC specific over-expression bCYLD (bCYLDvtg) were generated utilizing rat smooth muscle heavy chain genomic region from -4.2 to +11.6 kb (within the first intron) (rSM-MHC)-driven human bCYLD. The transgenic mice had no observable abnormality, with a normal body weight, heart rate and blood pressure. Of note, the inflammatory neointimal formation that was induced by perivascular cuff placement was dramatically increased in the bCYLDvtg mice, related to the wild type (WT) mice. At a molecular level, bCYLD inhibits IKK activity; however, did not affect NF- $\kappa$ B activity in the inflamed VSMCs. Collectively, our data reveals that bCYLD is a novel mediator of abnormal VSMC growth via suppressing IKK independent of NF- $\kappa$ B thereby leading to neointimal formation at a setting of vascular inflammation.

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### **Characterizing Mechanical Heterogeneity in Vascular Smooth Muscle Cells**

Aesha Desai, Dr. Sandra Deitch, Dr. Delphine Dean. Most tissue-level mechanical models assume homogeneous mechanical properties within a single cell type. However, measurements of cellular mechanical properties show large variability in whole-cell mechanical properties between cells from a single population. This heterogeneity has been observed in many cell populations and with several measurement techniques but the sources are not yet fully understood. Cell mechanical properties are directly related to the composition and organization of the cytoskeleton, which is physically coupled to neighboring cells through adherens junctions and to underlying matrix scaffolds through focal adhesion complexes. As such, we believe that this high level of heterogeneity can be attributed to varying local microenvironment conditions throughout the sample. To test this hypothesis, cardiomyocytes and vascular smooth muscle cells were cultured under several conditions that limited the variability in their microenvironment. First, cells were cultured on aligned collagen and fibronectin matrices (more uniform

extracellular matrix). Next, cell-cell and cell-matrix interactions were limited by using antibodies to N-cadherin and integrin  $\beta 1$ . Finally, these experiments were replicated on gels and under tension conditions to more closely mimic the native cellular microenvironment. Under each of these conditions, cellular viscoelastic mechanical properties were characterized through AFM testing and cellular structure was analyzed through immunofluorescence imaging. The results of these studies provide insights from a basic science perspective about the impact of the cellular microenvironment on cell behavior. Additionally, researchers may use these results to consider heterogeneity in the cellular microenvironment in vivo, especially in disease conditions where there is often elevated disorganization, and incorporate realistic levels of cellular heterogeneity in tissue-level mechanical models. Such models may help to better understand tissue behavior in both health and disease. Our future work involves observing response of cardiac cells on blocking cell-cell and cell-matrix interactions and performing western blot analysis to give a quantitative measure of phenotypic differences between cells from one sample versus another.

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### **Lowering NMR Data Requirements in Protein Structure Determination using REDCRAFT**

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De novo protein structure determination from orientational constraints is a challenging task that is necessary to extend the current limitations of protein structure determination by NMR spectroscopy. Although the information content of Residual Dipolar Couplings (RDCs) is capable of performing de novo protein structure determination, it is not clear how much data is required for such successful characterizations. As a result, RDC data is conventionally used only for protein structure refinement protocols. Yet another challenge is in establishing their utility in structure determination of deuterated proteins, which if properly addressed, may prove beneficial in extending the size of proteins amenable for study by NMR spectroscopy. Our lab has previously demonstrated the success of REDCRAFT as a viable approach for protein structure determination using only RDCs. Here we list our most recent enhancements to REDCRAFT's core capabilities, specifically as they relate to de novo structure determination and deuterated proteins. REDCRAFT's search for the optimal structure is dramatically different from other existing approaches. In effort to improve its efficacy for structure determination from sparse RDC data, the following enhancements have been made: atomic collision detection using Ca-estimated Van der Waal radii; solution space decimation; structure filtration based on estimated relative order tensors; program hooks that allow structure minimization using Levenberg-Marquardt without a known Order-Tensor; and template-assisted determination of protein structure. In order to establish the plausibility for backbone protein structure determination from {N-H, Ca-Ha} we first quantified the information content of the 2-vector RDC data set. Using NMR-determined 1GB1 as a reference structure, we randomly generated 5000 structures as a function of their bb-rmsd. Each structure was evaluated based on bb-rmsd, RDC and NOE fitness. The observed funneling effect supports our claims of structure determination from a 2-vector RDC data set. As a confirmation REDCRAFT was able to perform de novo structure determination using experimental {N-H, Ca-Ha} or {C-N, N-H, C-H} RDC data from the 1P7E and 1D3Z proteins, producing structures which varied from their corresponding X-ray structure by 1.02Å... and 1.57Å... respectively.

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**Cocaine affects central neuropeptide systems to influence anxiety during withdrawal**

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Anxiety during withdrawal from drugs of abuse is a major contributor to relapse. Although multiple systems likely influence this phenomenon, the nonapeptides oxytocin and vasopressin have emerged as potential contributors to anxiety during cocaine withdrawal. To better understand the role of these neuropeptides in this anxiety, we used an animal model of cocaine addiction, in which rats self-administer cocaine during daily 6 hour sessions for 14 days; a group of yoked saline animals served as controls. Following self-administration and 2 days of abstinence, we assessed anxiety levels using the elevated plus maze and defensive burying test. We then used multiple protein quantification techniques to assess both neuropeptide and neuropeptide receptor expression in brain regions associated with anxiety and cocaine addiction. Animals that self-administered cocaine exhibited increased anxiety-like behavior; we are currently quantifying neuropeptide and associated receptor levels in multiple brain areas including the extended amygdala and striatum.

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**The Hedgehog Pathway in the Basal Bilaterian *Isodiametra pulchra* (Acoelomorpha)**

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The goal of this work is to characterize the hedgehog (Hh) pathway in the acoelomorphan species *Isodiametra pulchra*. This organism was chosen because it is one of the most primitive bilaterians, we have access to its transcriptome (unpublished), and it is a completely ciliated animal. This latter feature is important because Hh signaling may well have evolved in connection with the ciliated epidermis-- the primary cilium is known to be an important Hh signaling center during vertebrate development. In order to study Hh signaling in *I. pulchra*, we have taken both morphological and molecular approaches. First, we have carried out titrated exposure experiments of *I. pulchra* to Cyclopamine (Hh signaling antagonist) or Purmorphamine (Hh pathway agonist). The animals that were exposed to Purmorphamine looked healthy, but did seem to have developed more eggs after 24 hours than those in the control group. The animals that were exposed to Cyclopamine showed significant phenotypic changes after 24 hours. The worms were lethargic, shrunken, and had a profoundly deformed epidermis. These results are consistent with the twin hypotheses that Hh activation upregulates stem-cell division, and that Hh signaling is necessary to maintain epidermal integrity. We are in the process of testing these hypotheses. Second, our lab has identified and sequenced genes that code for components of the Hh pathway. We have identified the definitive hedgehog protein, a KIF7 orthologue, one of three possible candidates for PTCH (the Hedgehog receptor), and the most likely candidate for SMO, the seven-pass-GPCR family member that acts downstream of PTCH. In order to determine whether our

choice of PTCH orthologue is correct, we propose to take advantage of small-molecule-inhibitor effects on Hh signaling. Accordingly, we believe that PTCH expression, which is upregulated during Hh activation, will decrease under the influence of Cyclopamine, and increase under the influence of Purmorphamine. We propose to use RT-PCR to measure PTCH mRNA expression. To date, we have developed and tested RT primers for Hedgehog and for two “housekeeping” genes (beta-Actin and EF1alpha), and we are in the process of setting up preliminary experiments to utilize these.

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**Circadian Synchronization of the Cell Cycle in *Aeolosoma* (Annelida)**

Emily Bowie, Courtney Patenaude, & Julian Smith III

In many organisms, the cell cycle is at least partly synchronized to a circadian rhythm. At least two possible hypotheses have emerged to explain this. First, consigning sensitive portions of the cell cycle to periods of low aerobic activity may be one way of protecting the cell’s genetic material. A second hypothesis is that consignment of the sensitive portions of the cell cycle to the scotophase may be a way of reducing light-mediated DNA damage. In order to investigate these hypotheses further, we determined whether the cell cycle in *Aeolosoma* (a small, transparent annelid common in freshwater habitats) exhibits a circadian rhythm. If so, it might be expected that the stem cells present in *Aeolosoma* will divide more frequently at night than during the day. *Aeolosoma* reproduces prolifically by asexual fission in culture, and has proven useful in our lab for studying stem cells and the mitotic cycle. *Aeolosoma* were cultured under a clock-shifted photoperiod of 12L/12D; with ZT=0 (lights-on) at 11:00 AM. After the *Aeolosoma* acclimated to this new circadian rhythm, they were killed by rapid ejection onto a liquid-nitrogen chilled metal block. For M-phase analyses one group was frozen at ZT=20 (relative 3:00AM-- “dark” group) and a second group, at ZT=8 (relative 3:00PM--“light” group). Mitotic cells were labeled with anti-phosH3 and the nuclei of the cells were stained with Hoechst 33342. For S-phase analyses, we labeled animals with EdU (200µM) for one hour before freezing, starting at ZT=0, at ZT=8, and at ZT=20. Counts of mitotic cells were scaled to animal length; counts of S-phase cells were taken from uniform volumes of the body wall in mid-body, fission plane, and posterior daughter zooid. Mitoses were significantly (approximately three-fold) higher in the dark group (compared to the light group), and the dark group exhibited statistically more fission planes S-phase was significantly elevated in the fission plane of animals labeled starting at ZT=0 compared to the other two labeling times. These results show that the stem cells are more likely to undergo mitosis at night than during the day, and that S-phase may be triggered by entry into the photophase, at least in the fission plane. *Aeolosoma* may be useful as a model system for studying circadian timing of the cell cycle and asexual fission.

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**Crystallization and Preliminary X-ray Diffraction Analysis of Clostridium papyrosolvens C71 Xylanase**

Elizabeth Bales, Jason Hurlbert

Clostridium papyrosolvens C71 xylanase catalyzes the hydrolysis of the xylan component plant hemicellulose. Xylans are made of  $\beta$ -1,4-linked xylopyranose units with arabinofuranose and glucuronopyranose substitutions along the backbone that serve to limit xylanase activity by physically blocking access to the xylopyranose backbone. This enzyme has been targeted for structural studies due to the fact that initial functional assays have indicated it may hydrolyze arabinofuranose substituted xylans better than homologues from Bacillus and Erwinia species, which makes it an interesting candidate for inclusion in the enzymatic repertoire of biocatalysts used in the industrial production of ethanol. An initial crystallization condition was identified from a commercially obtained sparse matrix screen. This condition contained 0.1M ammonium acetate, 0.1M BIS-TRIS pH 5.5 and 17% w/v Polyethylene Glycol 10,000. Crystals of the xylanase as well as co-crystals of the xylanase and the Ligand, X3, were grown and harvested. Once the crystals were harvested the well solution was exchanged for a cryo-protectant solution made of 50% well solution and 50% glycerine. A Rigaku R-Axis II diffractometer equipped with a rotating anode x-ray source and image plate detectors was used to collect data from the crystals. A 180 degree dataset was collected with 5 minute exposure times and a Phi rotation of 0.5 degrees per image. The resulting data was processed to 2.1 angstroms and the crystal belonged to the orthorhombic space group, C2221 with unit cell parameters:  $a=66.020$ ,  $b=76.520$ ,  $c=150.550$  and  $\alpha=\beta=\gamma=90.00$ .

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**Crystallographic Structure Characterization of Clostridium papyrosolvens Xylanase CpC7: Molecular Replacement using Homology Models**

James Smith, Dr. Jason Hurlbert

The enzyme CpC71 is a xylanase belonging to Glycosyl Hydrolase (GH) Family 30 cloned from the anaerobic bacterium Clostridium papyrosolvens. This enzyme has the ability to bind and hydrolyze beta-1,4-xylan chains bearing arabinose and glucuronic acid substitutions. This feature makes the enzyme of potential value in biocatalytic processes designed to breakdown woody biomass and agricultural waste for conversion to ethanol. The enzyme is only the third xylanase of family GH30 characterized structurally, and previously described structures of Erwinia chrysanthemi Xylanase A (XynA) and Bacillus subtilis Xylanase C (XynC) are specific to 4-O-methylglucuronate (MeGA) substitutions (PDB entries: 1NOF and 3GTN, respectively), with little tolerance for arabinose substitutions. CpC71's novel specificity garners particular interest in solving the enzyme's structure to determine differences in its binding mechanism. The primary focus of our work was to produce a viable initial molecular replacement (MR) phasing solution for the enzyme using lowest energy homology models. We have previously created homology models of CpC71 using an approach involving two different computational algorithms and

used the top five models during phasing to obtain an initial MR solution. The structure was solved to 2.01 angstroms and the active site was compared to those of XynA and XynC. Structural data indicate three primary mutations to the arabinose-binding subsite along the  $\beta$ 8- $\alpha$ 8 loop. This initial structure will serve as a basis for mutagenic and functional studies to investigate the role of the putative arabinose binding subsite in the enzyme.

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### **Cloning and Expression of Human Sphingosine Kinase**

Jason Hurlbert

Lipid signaling molecules such as ceramide, sphingosine, and sphingosine-1-phosphate (S1P) have recently been shown to be increasingly important in determining cellular fate. The sphingosine kinase family of proteins helps regulate the balance of these molecules in the cell and one of the two family members, sphingosine kinase 1 (SK1), is overexpressed in various types of cancers, specifically cancers of the breast, colon and lung, which are the three most lethal types of cancer affecting South Carolinians. Increased expression of SK1 has been associated with tumor angiogenesis and resistance to radiation and chemotherapy. These observations have made hSK1 an attractive target for the development of new therapeutic agents for the treatment of cancer. Such efforts depend upon a knowledge of the three dimensional structure of the protein afforded by x-ray crystallographic analysis. Our laboratory has used NIH-INBRE support to initiate structural studies of human sphingosine kinase 1. The resulting structure may then be used by collaborators within the University and at other state institutions to develop inhibitors of the proteins that may be used as therapeutic agents in the treatment of cancer. At present, we are in the process of optimizing the expression of recombinant hSK1 in bacterial cultures. Through bioinformatic analyses, we have identified the core domain of the protein likely to fold into a stable structure, have amplified the target sequence from human cDNA and cloned it into two different prokaryotic expression vectors. Expression trials of the constructs were performed in four different bacterial hosts under varying growth conditions and no soluble hSK1 was produced as determined by MALDI-TOF MS. An Escherichia coli codon-optimized expression construct encoding the hSK1 target was designed and similar expression tests revealed small amounts of hSK1 produced at 25C in E. coli C41(DE3) cultures. We are currently cloning the codon-optimized hSK1 sequence into other prokaryotic expression vectors in order to boost the amount of soluble protein obtained to levels sufficient to initiate crystallization trials.

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### **Mechanisms of Epidermal Cell Replacement: is Hedgehog signaling involved?**

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Summary: The broad goal of this research is to understand the mechanisms by which the production of new epidermal cells in multicellular organisms is regulated. These mechanisms are relatively well-understood in mammals, relatively poorly understood in lower vertebrates, and nearly unstudied in most invertebrates. However, it seems very likely that the most fundamental molecular mechanisms underlying epidermal replacement (and other developmental processes) in vertebrates will be those found most frequently in the primitive invertebrates on which my lab works. Accordingly, I have chosen to focus on the possible involvement of the hedgehog pathway in two readily-cultured invertebrate organisms—*Aeolosoma headleyi*, an annelid, and *Isodiametra pulchra*, an acoelomorph. In the former organism, my lab has established the general location of cycling cells using S- and M-phase labeling techniques, identified a pronounced circadian rhythm to the cell cycle in this organism, and has begun moving our cell-labeling experiments to the electron-microscopic level, so that we can identify the exact location and morphology of cycling cell populations. Finally, we have begun experiments to determine whether or not cyclopamine (antagonist of Hh signaling) and purmorphamine (agonist of Hh signaling) have effects on cell-cycling. *Isodiametra pulchra* was chosen as a second organism for these studies based on its evolutionarily primitive position and the availability of a transcriptome. So far, my lab has identified and sequenced orthologues of key components of the Hh pathway, tested the effects of cyclopamine and purmorphamine on cycling cells and on the organism's epidermal phenotype, and designed and tested real-time-PCR primers for some of these components. Our experience so far has been that each of these organisms provides a valuable platform for undergraduate research and education in cellular and developmental biology.

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### **Intracellular signaling pathways of LPA and S1P mediated chick retinal growth cone collapse**

One of the most complex systems is the nervous system. Furthermore, damage to the central nervous system usually is permanent, as very little regeneration is seen. However, neural growth is seen abundantly during development. Thus, we are studying the development of the nervous system focusing on the problem of axon guidance. In the visual system, the output neurons of the eye, the retinal ganglion cells, send their axons in a stereotypical pattern to targets in the brain, making defined axon guidance choices at set points along the path, including producing a topographic projection at the target. Although a number of axon guidance cues have been identified, it is clear that not all the players are known. We are investigating whether signaling lysophospholipids, especially LPA and S1P, could act as axon guidance cues. Using an in vitro growth cone collapse assay, we have shown that both LPA and S1P cause a dose-dependent, rapid growth cone collapse of chick retinal neurons, similar to other known inhibitory axon guidance cues. As LPA and S1P receptors are known to be GPCRs, we have analyzed the intracellular signaling pathways required for this retinal growth cone collapse through pharmacological inhibitors. Inhibition of rho kinase, downstream of G12/13, prevents LPA and S1P mediated growth cone collapse, indicating the requirement of this pathway. Blocking the Gq pathway, either by calcium chelation or inhibiting PLC, however, had no effect on growth cone collapse, suggesting this pathway is not involved in growth cone collapse. Interestingly, blocking Gi by pertussis toxin led to incomplete inhibition of LPA and S1P mediated growth cone collapse, suggesting a partial involvement of this pathway. Furthermore, in comparison with the *Xenopus* system, inhibition of p38 kinase blocked chick retinal growth cone collapse induced by LPA, similar to *Xenopus*, but did not block chick retinal growth cone collapse induced by S1P. Thus, we have defined that growth cone collapse of

chick retinal neurites by LPA and S1P is mediated by intracellular signaling through G12/13-rho-ROCK pathway as well as partially through the Gi pathway, with an interesting requirement of p38 kinase for LPA but not S1P induced growth cone collapse.

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**Identification and position of S- phase cells associated with the epidermis of *Stenostomum virginianum* (Catenulida) and *Aeolosoma headleyi* (Annelida)**

Daniel Stanton & Julian Smith III, Winthrop University

While the conventional neoblast stem cell system has been extensively studied in Triclad flatworms, less is known about this system in primitive catenulid flatworms. Even less is known concerning cell replacement in primitive Annelids. Accordingly we have instituted morphological studies of the stem-cell system in two organisms that reproduce asexually by paratomic fission (*Stenostomum virginianum* and in *Aeolosoma headleyi*). Previous data on M-phase cells from our lab have shown that dividing cells occur in all the three germ layers (epidermis, mesoderm, and gastrodermis) in these organisms. However, these data leave open questions: 1) in *S. virginianum*, it is not clear whether M-phase cells observed in the epidermis are a transit-amplifying population that is renewed from subepidermal stem cells, or if the epidermis contains a dedicated stem-cell population; 2) In *A. headleyi*, stem cells of the epidermis are thought to be basally-located cells, a hypothesis currently supported only by analogy to the condition in vertebrates. Although S-phase cells are conveniently labeled with 5-ethyl-2-deoxyuridine (EdU), our experience with a wide variety of vermiform invertebrates shows that EdU often fails to label organisms (such as *S. virginianum*) known to have stem-cell populations. Additionally, the EdU technique does not readily lend itself to electron-microscopic cytochemistry. Here, we report the modification and use of the older 5-bromo-2-deoxyuridine (BrdU) labeling method for addressing of the location of S-phase stem-cell populations in th

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**Expression and Purification of *Xanthomonas euvesicatoria* Avirulence Protein AvrBs1.1**

Laura Wilt, Tyler Couch and Dr. Jason Hurlbert

Phytopathogenic xanthomonads possess a type III secretion system (T3SS) that serves to secrete effector proteins that interfere with host immunity and disrupt normal cellular function. Avirulence proteins (Avr) are type III effector proteins that are recognized by specific R gene products in resistant hosts and the binding of R gene products by Avr proteins may elicit a hypersensitive resistance (HR) response in some hosts. In the absence of a corresponding R gene, Avr proteins may act as virulence factors contributing to the pathogenicity of the microorganism. A novel Avr protein, AvrBs1.1, from the pepper pathogen *Xanthomonas euvesicatoria* was recently identified to elicit a delayed hypersensitive

response on *Capsicum annuum* leaves. Amino acid sequence analysis of AvrBs1.1 has revealed the presence of a Dual-Specificity Protein Tyrosine Phosphatase domain in C-terminus of the protein, meaning that the protein has the potential to bind substrates with phosphorylated Ser, Thr or Tyr residues. Screening for novel Resistance genes has identified a single binding partner of AvrBs1.1, the R gene product Bs7. Our laboratory has constructed homology models of AvrBs1.1 in addition to its homologue AvrBs7 from *X. gardneri* in order to better explain differences in hypersensitive response timings manifest in *C. annuum* after exposure to *X. gardneri* and *X. euvesicatoria* cultures. While the computational models have provided some insight into potential differences in activation of Bs7 in *C. annuum*, a better understanding of the mechanism by which the R gene and Avr proteins interact will be gained by x-ray crystallographic studies of the protein complexes. Towards this end, we have begun crystallization trials of recombinant AvrBs1.1. The coding sequence of *X. euvesicatoria* AvrBs1.1 was cloned into pET21A (Ampr/C-terminal hexahistidine tag) which was used to transform *E. coli* Rosetta2 (DE3) cultures. The resulting transformants were used to inoculate 6L of Luria-Bertani medium which were grown at 37C to an optical density at 600nm of 0.6, were induced by the addition of IPTG and then grown for 18 hours post-induction. The cultures were harvested by centrifugation and the recombinant AvrBs1.1 was purified by a combination of metal chelating affinity and gel filtration chromatographic methods. Identity of the purified protein was confirmed by MALDI-TOF MS. Crystallization screening of the protein is currently being performed.

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#### **Initial Characterization of Expression of a Putative Member of the p53 Superfamily in *Daphnia pulex***

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Recently, the entire genome of the aquatic organism *Daphnia pulex* has been sequenced (1). The sequence has revealed the presence of a putative member, based on homology, of the p53 superfamily of proteins; in humans this superfamily includes p53, p63 and p73. The human protein displaying the closest homology to the *D. pulex* protein is p63. The two polypeptides show 40% amino acid identity and 56% amino acid similarity between the two DNA binding domains. Significantly, both the dimerization sites and the zinc binding sites are conserved between the two proteins. The p63 protein has been demonstrated to be important in induction of nucleotide excision repair following UV irradiation in human cell lines (2); the manner of induction of p63 in these cell lines is yet unclear. Using reverse transcriptase-PCR and mRNA isolated from control and UV-irradiated *D. pulex*, we provide evidence of the presence of this gene product at the transcript level. This transcript appears to not be significantly upregulated by UV irradiation, as it is seen in both non-irradiated and UV irradiated samples.

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**Mechanical tension promotes mast cell degranulation through an RGD-integrin dependent pathway**

Mechanical forces applied to the extracellular (ECM) matrices of various tissues are essential in maintaining tissue homeostasis and physiology. Alterations in mechanical tension can result in a maladaptive response that includes induced cellular stress and activation of pathological signaling pathways. Mechanical forces applied to the extracellular matrix are largely transmitted to the cell via receptors of the integrin family. Specifically, RGD-specific integrins have been shown to act as mechanotransducers converting mechanical stresses into biological signals. Mast cells are immune cells that participate in several biological functions including wound healing, fibrosis, cell recruitment, asthma, inflammation and mediation of vascular permeability. In several pathological diseases where changes in the mechanical environment stimulate changes in the ECM, mast cell density and activation are increased significantly. The biological changes that occur to mast cells during mechanical tension have not been investigated. Our objective was to 1) examine the mechanosensitivity of the mast cell by subjecting it to cyclic mechanical load, and 2) determine the role of RGD-specific integrins in mast cell response to mechanical force. We applied 5% and 10% cyclic, uniaxial tension to three dimensional fibrin constructs seeded with RBL-2H3 cells (a mast cell line) and monitored the cellular response over a period of 24 hrs. Our results showed that at 5% and 10% cyclic mechanical load, RBL-2H3 degranulation, as measured by secretion of  $\beta$ -hexosaminidase, significantly increased (2.1 to 2.3 fold, respectively) in a load- and time-dependent manner when compared to the non-stretched controls ( $p < 0.003$ ). Furthermore, mechanical loading did not compromise cell viability or increase cell proliferation. To determine if RGD-dependent integrins mediated mast cell degranulation in response to mechanical tension, we inhibited cell-matrix interactions by treating the RBL-2H3 cells with Echistatin, a disintegrin that tightly binds RGD-specific integrins. Treatment with Echistatin attenuated load-induced degranulation (by ~50%,  $p < 0.0004$ ) without compromising cell viability. These results suggest a novel mechanism by which mechanical stretch induces mast cell activation via RGD-dependent mechanotransduction.

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**Repeated nicotine administration upregulates microRNA-221 and microRNA-483 in the prefrontal cortex of rats raised in an enriched environment**

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Tobacco use is the number one preventable cause of death in the world yet there still is a critical need to define the molecular mechanisms that underlie susceptibility to nicotine addiction. Approximately 20-25% of tobacco users become addicts, and we are becoming increasingly aware that environment plays a role in nicotine susceptibility. An ideal animal model to address this issue uses rats raised in an

enriched condition (EC) containing novel objects and cohorts, a standard condition (SC) containing only cohorts, and an impoverished condition (IC) containing neither. Exposure to an enriched condition has been shown to contribute to a potential neurobiological basis of resistance to addiction; however the molecular mechanisms of enrichment-induced neuroadaptations remain elusive. We have previously shown that EC rats have increased behavioral sensitivity to the locomotor effects of chronic nicotine. Correspondingly, in response to chronic nicotine EC rats also display increased phosphorylated levels of dopamine- and cAMP-regulated phosphoprotein-32 (DARPP-32) at the threonine-34 site as well as phosphorylated cAMP-response element binding protein (CREB), two markers essential for drug-induced neuroplasticity, in the prefrontal cortex (PFC). MicroRNAs (miRNAs) are short, non-coding RNAs known to regulate gene expression post-transcriptionally and more recently have been implicated in drug-induced neuroplasticity. Therefore, to determine if miRNAs influence neuroplasticity associated with environmental enrichment in response to chronic nicotine we subjected EC, IC, and SC rats to chronic administration of nicotine (0.35 mg/kg) subcutaneously and used microarrays to profile miRNA expression in the PFC. Real-time PCR confirmed that EC rats treated with chronic nicotine had robust increases in miRNA-221 and miRNA-483 in the PFC suggesting these miRNAs may impact environment-induced neuroplasticity in the PFC in response to chronic nicotine administration, and may also play a role in EC rats increased behavioral sensitivity to nicotine.

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Humans and wildlife are exposed to environmental pollutants that have been shown to interfere with the thyroid hormone system, which may affect brain development. In this study, we illustrate the use of magnetic resonance imaging (MRI) and volumetric analysis to assess the effects of thyroid hormone disruption on the brain. We exposed pregnant Sprague Dawley rat dams to 3 or 10 ppm propylthiouracil (PTU) from gestation day 7 (GD7) until postnatal day 25 (P25) to determine the effects of a goitrogen on white matter (WM), gray matter (GM), and hippocampal volumes in offspring. We sacrificed pups at P25 but allowed some individuals to live to P90. P25 pups exposed to 10 ppm PTU displayed lowered levels of triiodothyronine (T3) and thyroxine (T4); cerebral WM, GM, and total brain volumes were significantly lower than the volumes of control animals. P90 adults exposed to 10 ppm PTU displayed normal T3 levels but lowered T4 levels; WM, GM, total brain, and hippocampal volumes were significantly lower than the volumes of control adults. A significant reduction in WM:GM volume ratios was observed in both P25 and P90 rats exposed to 10 ppm PTU. Additionally, heterotopias of the corpus callosum were observed in rats exposed to 10 ppm PTU. These results suggest that MRI coupled with volumetric analysis is a powerful technique to assess the effects of thyroid hormone disruption on myelination, and thus has great potential as a research approach to study this problem in humans and wildlife.

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**Synthesis of Benzisoxazolo[2,3-a]pyridinium Tetrafluoroborates**

The benzofuropyridine subunit is found in many compounds of pharmaceutical relevance. Related to these compounds are the lesser known benzisoxazolo[2,3-a]pyridinium salts (1) of which, prior to this work, only two examples had been reported [1]. Abramovitch et al. showed that these compounds could be synthesized through an intramolecular ring closure of a diazonium salt derived from a 2-(2-aminoaryl)pyridine N-oxide [1] and a recent discovery by Fagnou et al. [2] hinted that the starting material required for this reaction could be synthesized by the direct arylation of pyridine N-oxide at the 2-position with an o-bromoacetanilide. We felt that the combination of these two processes could lead to a general synthesis of the ring system 1. In our laboratory, we have found that direct arylation of pyridine N-oxide with various 2-bromoacetanilides (2) using microwave heating gave the desired 2-(2-acetaminophenyl)pyridine N-oxides (3) in isolated yields from 45-76%. These compounds were then subjected to hydrolysis, diazotization, and cyclization to produce the benzoisoxazolo[2,3-a]pyridinium tetrafluoroborates (4) in isolated yields ranging from 71-86% [3]. This protocol has been extended to the direct arylation of quinoline N-oxide to give 2-(2-acetaminophenyl)quinoline N-oxides (5). Future work will involve the cyclization of 5 and, in collaboration with our colleague Dr. Takita Sumter, evaluation of the cytotoxic activity of these novel compounds.

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**Targets of somatic hypermutation within immunoglobulin genes in zebrafish**

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In mammals, somatic hypermutation (SHM) of immunoglobulin (Ig) genes is critical for the generation of high affinity antibodies and effective immune responses. Knowledge of sequence specific biases in the targeting of somatic mutations can be useful to studies aimed to understand antibody repertoires produced in response to infections, B cell neoplasms, or autoimmune disease. To evaluate potential nucleotide targets of somatic mutation in zebrafish, we constructed an enriched cDNA library of VJ\_C segments from which randomly selected clones were sequenced and analyzed. Mutations were most prevalent in V regions with a bias towards single base transitions and increased mutation in

complementarity determining regions. Overall, mutations were overrepresented at WRCH/DGYW motifs suggestive of activation-induced cytidine deaminase (AID) targeting common in mice and humans. In contrast to mammalian models, N and P addition was not observed and mutations at AID hotspots were largely restricted to palindromic WRCH/DGYW motifs. Mutability indexes for di- and trinucleotide combinations confirmed c/G targets within WRCH/DGYW motifs to be statistically significant mutational hotspots and showed trinucleotides ATC and ATG to be mutation coldspots. Additive mutations in VJ-C sequences revealed patterns of clonal expansion consistent with affinity maturation responses seen in higher vertebrates. Taken together, the data reveal specific nucleotide targets of SHM in zebrafish and suggest both AID and affinity maturation contributes to antibody diversification in this emerging immunological model.

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**The USC DNA Microarray Facility**

The USC DNA Microarray Facility (<http://www.sccp.sc.edu/research/mcf/index.aspx>) is located at the SC College of Pharmacy, Coker Life Science Building, Room 617, in the Department of Pharmaceutical and Biomedical Sciences at the University of South Carolina, Columbia, SC. The Facility is an active component of SC INBRE, supporting some of the activities of SC INBRE's Bioinformatics Core, and is also a member of the Network of IDeA-funded Core Laboratories (NICL). The facility is equipped to provide custom microarrays for academic and industrial clients. We have successfully spotted cDNAs, 50 to 70 base pair oligonucleotides and 18 to 22 base pair short primers. In addition to nucleic acids, the Biochip Arrayer (Perkin Elmer) can spot antibodies, proteins, peptides and bacterial cells. In addition to custom microarray design and spotting, we provide full microarray services on the Agilent Technologies platform and on any other dual-label microarrays. We also provide extensive on-site training, both informally (to clients) and formally, conducting classes, workshops and visits for students and faculty across the SC INBRE network. We conduct directly, or assist clients in the conduction of all phases of microarray analysis, from RNA isolation to quantitation/quality control on an Agilent Bioanalyzer, to labeling, hybridization, data collection and analysis. We assist customers with gene expression data analysis using limma/limmaGUI, GeneSifter and GeneSpring GX software. Additionally, we perform cluster analysis using Cluster and Treeview software both from Eisen laboratory. This poster presents some examples of the SC INBRE-funded projects that are currently being conducted at the USC Microarray Facility.