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## ABSTRACTS

*Abstracts are listed alphabetically by the last name of the first author listed.*

### REPLICATING DNA DIFFERENTLY

Sarah M. Anderson and Trey Maddox (Advisor)

*Department of Chemistry*

*Bethel University, St. Paul, MN*

Since the discovery of DNA replication, scientists have worked to develop methodologies that nonenzymatically mimic its template-directed polymerization. One method that has proved successful is reductive amination on a DNA template. This is a two-step polymerization reaction involving equilibrium imine formation on the DNA template followed by reduction of the imine to an amine. The polymerization of amine nucleoside polymers by this process proceeds with step-growth kinetics to give chain-length and sequence-specific products. We are working to complete a new synthesis of the modified thymidine nucleoside needed for this DNA-directed polymerization, which additionally gives us access to the three other nucleoside bases. These monomers incorporate a 3'-aldehyde and a 5'-amine functionality. Our goal is to optimize this synthetic route in order to synthesize all four modified nucleosides and use the completed monomers to carry out reductive amination in order to further study the polymerization process.

### THE EXPRESSION OF SEMAPHORINS 4A, 4D, AND 7A IN EARLY HEMATOPOIETIC T PROGENITORS IN THE BONE MARROW OF C57BL/6 MICE

Leonor Año<sup>1</sup>, Grace E. Linder<sup>1</sup>, Udochukwu Obodo<sup>1</sup>, David J. Matthes<sup>2</sup>, and Devavani Chatterjea<sup>1</sup> (Advisor)

<sup>1</sup>*Biology Department*

*Macalester College, Saint Paul, MN*

<sup>2</sup>*Genetics, Cell Biology and Development*

*University of Minnesota, Minneapolis, MN*

Semaphorins are a group of transmembrane, secreted, and GPI-linked proteins that have been thoroughly studied in the nervous system where they function as "traffic" enzymes in guiding axonal growth. Although most exhaustively characterized in this realm, they are also expressed in several other systems. We have characterized the expression and localization of T cell semaphorins 4A, 4D, and 7A in developing thymocytes.

We show here that hematopoietic T cell progenitor cells (CD90<sup>+</sup>) enriched from the bone marrow have high levels of sema4A and 4D expression, but no sema7A. PlexinD1 and C1 (the receptors for sema4A and 7A) were also expressed in this subset, but there was no PlexinB1 or CD72 (the receptors for sema4A and 4D, respectively). This provides further evidence of the

dynamic expression and regulation of sema4A, 4D, and 7A in T cell development.

### FROM GFP TO pFLAG: CONFIRMATION OF INTRACELLULAR LOCATION OF KIAA1946

Jessica Apple, Anita Becker, and Geoffrey M. Goellner (Advisor)

*Department of Biological Sciences*

*Minnesota State University, Mankato, MN*

Although significant advances have been made in deciphering the human proteome, there are still close to 8,000 genes whose functions are still completely unknown. In this study, we have attempted to determine the cellular location of one such novel gene product—KIAA1946. Indeed, very little is known regarding KIAA1946's normal function in cells. However, we do know that it is likely expressed in the nervous system, and it has a polyglutamine region in its primary amino acid sequence—two interesting features because other polyglutamine proteins have been linked to severe neurodegenerative diseases such as Huntington's chorea. Previous studies (using green fluorescent protein—GFP) in our laboratory have shown that KIAA1946 likely localizes to cytoplasmic vesicles.

In the current study, we have attempted to confirm these initial data by using standard molecular biology tools (such as restriction digestion and ligation) to subclone KIAA1946 into a vector containing the FLAG epitope (commercial antibodies are available against FLAG). We checked for proper cloning of our FLAG-KIAA1946 fusion protein using restriction digestion coupled to agarose gel electrophoresis—and also officially confirmed our clone integrity using DNA sequencing. Next, we transfected our new FLAG-KIAA1946 clone into tissue culture animal cells, and allowed several days for the protein to localize appropriately within the cells. Finally, we employed immunofluorescence microscopy using antibodies against the FLAG moiety to "visualize" the location of our fusion protein. Data regarding KIAA1946's location within cells will provide us invaluable insight regarding the normal cellular function of this novel polyglutamine protein.

### CHARACTERIZATION OF C0293: A SMALL, NON-CODING RNA FROM *Escherichia coli*

Karen Bieraugel, Seth Huiras, and Janice Pellino (Advisor)

*Department of Chemistry*

*St. Olaf College, Northfield, MN*

Small, non-coding RNAs (sRNAs) are thought to be helpful in gene expression in prokaryotes, but only a small handful have been extensively researched. *Escherichia coli* (*E. coli*) is a model organism for *in vitro* studies using biochemistry methods for investigating sRNAs.

The sRNA strand C0293 was studied regarding its interactions with proteins. Multi-dimensional Protein Identification Technology (MudPIT), an analysis of affinity-selected proteins, identified numerous interacting proteins. The most abundant was heat-unstable nucleoid protein (HU), a transcriptional regulator that represses the gal operon and therefore prevents expression of enzymes required for galactose metabolism. We hypothesized that during times of minimal nutrient availability C0293 is expressed, binds to the HU protein, and thus activates the HU protein to repress the gal operon and prevents the bacterium from wasting limited energy. Further studies using gel mobility shift assays suggested the presence of the protein Hfq in an sRNA-protein complex. The creation of a bacterial cell line that overexpresses the C0293 sRNA may prove helpful in determining whether this cell line can properly metabolize galactose, although attempts at overexpression have been unsuccessful so far.

#### **QUANTITATIVE ANALYSIS OF THE EFFECTS OF ANDROGEN HORMONES ON ZEB-1 EXPRESSION**

David Benjamin and Bynthia Anose (Advisor)  
*Department of Chemistry*  
*Bethel University, St. Paul, MN*

Currently, no diagnostic tool is available to distinguish between localized and metastatic prostate cancer. However, it has recently been discovered that Zinc Finger E-Box Binding Homeobox-1 (ZEB-1) could possibly be used as a biomarker for prostate cancer metastasis. Previous research has shown that increased expression of ZEB-1, a transcription factor, correlates with cancer progression.

To gain further knowledge about this potential diagnostic gene, human prostate cancer cells from the cell line 22Rv1 were treated with androgen hormones to determine their effect on the expression of ZEB-1. After treatment, RNA was isolated and reverse transcribed into cDNA for its use in real time-polymerase chain reaction (RT-PCR). Quantification and quality control of the harvested RNA were also assessed using ultraviolet spectroscopy.

#### **IDENTIFICATION OF THE LIMITING RESOURCE IN AMERICAN DESERTS THROUGH ANALYSIS OF CREOSOTE (*Larrea Tridentata*) GROWTH IN WASH AND NON-WASH AREAS**

Jennifer Berger, Shoshana Blank, Annie Brownlee, Dana Fjare, Paula Miller, Elizabeth Stiles, and Steve Freedberg (Advisor)  
*Department of Biology*  
*St. Olaf College, Northfield, MN*

Creosote bush (*Larrea tridentata*) is a dominant species in both the Sonoran and Mojave deserts in the Southwestern United States. Although previous research has found that productivity and spacing of creosote bush differ between these two desert ecosystems, it is difficult to confirm that moisture availability is responsible for these differences because many other biotic and abiotic factors differ between the sites. Naturally formed washes allow a unique opportunity to tease apart the relative importance of water and nutrient availability for plant distribution, because washes should lead to higher water and/or nutrient availability while controlling for other factors that may impact plant growth and spacing.

We hypothesized that creosote bushes at our study sites in Organ Pipe Cactus National Monument and Death Valley National Park would have higher productivity in wash areas because creosote shows increased productivity with supplemental water and nitrogen (2). Carbon-Nitrogen ratios have also been found to be higher in more humid soils, so we hypothesized that the C/N would be higher in plants near the wash since they are exposed to more water (3). Primary production in Sonoran Desert streams may also be limited by nitrogen availability, and water flow interface in desert washes makes nutrients available to plants by bringing nutrients to the surface (1). We examined the nitrate concentrations in wash and non-wash soils in order to examine if there was any difference in the availability of nutrients and whether these values corresponded to differences in productivity of *Larrea tridentata*. Nitrate concentrations were significantly higher in wash areas than in non-wash areas in both Sonoran Desert study sites ( $p=0.044$ ,  $p<0.001$ , respectively). However, we also found significantly higher C:N ratios ( $p<0.001$ ) in wash areas, indicating that nitrogen is not a limiting resource. Interestingly, although higher water and nutrient availability are expected to correspond to more overall growth, no significant differences in plant size or productivity were found between wash and non-wash plants. Our results support the conclusion that water, and not nutrient availability, is the limiting resource affecting *Larrea* spacing, a finding that may have implications for the ecosystem's response to global climate change.

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### **BIOINFORMATICS ANALYSIS OF HIV'S REGULATION OF NUCLEAR FACTOR KAPPA B (NF-KB).**

Ann Bergstrand, Dawn Comstock, Ryan Schmidtman, Jamison Utzig, and Dr. Sanjive Qazi (Advisor)

*Department of Biology*

*Gustavus Adolphus College, Northfield, MN*

Current strategies for HIV therapy involve targeting of specific viral proteins and blocking their function. However, new drug resistant viral strains are constantly emerging and the general unavailability of broad spectrum anti-virals render this approach very challenging. Newer approaches that utilize the genome-wide responses are beginning to identify critical host genes for viral replication through characterization of HIV protein interaction with host proteins. One such pathway taken over by HIV is also host cell inflammatory pathway that uses Nuclear Factor Kappa B (NF-KB) as the principal transcription factor for viral replication to serve as a major target site for drug development. We performed a bioinformatic analysis and interrogated a number of genome-wide studies that screened for NF-KB binding and those that have identified host proteins required for viral replication. We identified potential new target sites utilizing host proteins critical for viral replication. The Kegg pathway compendium was screened to gain a more detailed understanding of how NF-KB promotes secondary diseases such as cancer.

### **LONG RANGE WAVE PROPAGATION THROUGH RANDOM MEDIA**

Elizabeth Bernhardt, Dr. Steven Tomsovic, Dr. Katherine Hegewisch, Dr. Erick Agrimson (Advisor)

*Department of Physics*

*Washington State University, Pullman, WA*

We present an exploration of the stability of long-range wave propagation through random media. As a ray travels through a medium, it encounters variations which cause slight changes in velocity. Sound in the ocean, for example, propagates through varying temperature, salination, and pressure. Over long ranges, these variations cause the ray to become chaotic. In order to determine the behavior of the ray, the evolution of its stability matrix must be studied. Through creation of an adaptive step-size Runge-Kutta differential equations solver, we examined the stability matrices of several hundred rays to determine the transition with increasing

propagation time between stable propagation and chaos. Our findings suggest that the timing of the transition is dependent on the initial kinetic energy in proportion to the average maxima and minima of the randomized plane potential. We would like to understand if rays with larger initial momentum tend to chaos as fast as rays with lower initial momentum.

### **ELECTRIC FIELD-INDUCED REVERSIBLE pH MICROARRAYS**

Kevin H. Bettendorf, Pradeep Ramiah Rajasekaran, Andres Weber, Justin Wolff, and Punit Kohli (Advisor)

*Department of Biochemistry*

*St. John's University, Collegeville, MN*

Microarrays can be used to test many different scenarios in biomedical, materials, and physical sciences without utilizing large quantities of sample or an extensive amount of time. Manipulating salt concentration, amount of reagent, type of reactants as well as other variables are all employed in microarray systems. Precise control over the pH in each well or spot is also an important and useful tool for microarray systems.

In our project, instead of varying concentrations of buffer solutions to control pH, hydrolysis of water was used. This was accomplished by controlling the polarity of the electrodes in the wells. The wells were fabricated by drilling pores of diameter ranging from 450  $\mu\text{m}$  to 1.5 mm into 3-mm-wide Teflon sheets. A medium of 3% agarose gel in water was used to fill the pores. Agarose was used because of its strong biocompatibility and ability to retain large amounts of water. Hydrolysis in agarose was performed using microelectrodes. Control over pH was easily attained by varying average current and time of hydrolysis. The pH values (ranging from 2 to 11) can be reversed to a desired value by controlling polarity of the electrodes, salt concentration, and applied charge into the solution. By utilizing hydrolysis to create pH gradient, we were able to return the pH to the original value or create an entirely different pH. The precise control over pH gained by the use of this process may be implemented in various biological areas.

### **CHRONIC CONSUMPTION OF ASPARTAME (IN THE FORM OF EQUAL SUGAR) AND ITS EFFECTS ON THE BASAL METABOLIC RATE, HEART RATE, URINARY PH, AND URINARY CALCIUM LEVELS OF *Mus musculus***

Andrea Bochna and Teresa DeGolier (Advisor)

*Department of Biological Sciences*

*Bethel University, St. Paul, MN*

Aspartame is a common artificial sweetener found in many drinks, gums, and foods. Although it is approved by the FDA, the safety of its use is under question due to recent research suggesting potential

interactions with cancer, heart rate, and urinary calcium excretion.

This research project investigated the effect of chronic consumption of aspartame on common lab mice (*Mus musculus*). Twelve treatment mice were given 9g/100 ml water of aspartame (in the form of Equal sugar) for 10 weeks, while the 12 control mice received plain water. All mice had free access to animal chow. No statistical difference was found in the basal metabolic rate between control mice and treatment mice. Urinary hydrogen ion excretion was statistically greater in treatment mice. This is likely due to the effective elimination of aspartame's metabolism into aspartic acid. Urinary calcium excretion was statistically higher in the control mice, but not statistically different when expressed as a 24-hour measurement. These results may suggest that chronic consumption of Equal (at a moderate dose) does not interfere with these physiological parameters.

### SYNTHESIS OF NOVEL ANTIBIOTICS

Salome Boroda and Thomas Ippoliti (Advisor)

*Chemistry Department*

*University of St. Thomas, Saint Paul, MN*

Molecular topology is a radically different way of designing drugs using mathematical models and properties of existing compounds. Recently, *Forward Engineering™*, a program developed by Medsyn Technologies, designed a new compound (**3**). It is predicted to have an 85% likelihood of being a novel biologically active antibiotic against gram-positive bacteria. The synthesis of **3** is a convergent synthesis in which two other compounds, an amino epoxide (**1**) and an alcohol (**2**) must first be synthesized and then combined in the final step. Compound **1** and **2** have been synthesized. Compound **2** was made using two different routes. The final step was performed but resulted in a compound that was not the desired product. The mechanism for the formation of this new compound will be presented.

### DETERMINING THE PATERNITY OF MOUNTAIN ASH (GENUS *Sorbus*) IN THE BLUFFS AROUND WINONA, MN

Brianna Braun, Dr. Phil Cochran, Dr. Debra Martin (Advisor)

*Department of Biology*

*Saint Mary's University of Minnesota, Minneapolis, MN*

Three species of mountain ash trees are found in the Midwest, but only two of these species are native. Trees that are classified as the exotic species have been found in the bluffs surrounding Winona, Minnesota. Since these trees are more ornamental and thus should not be

found in the wild, it was determined how these trees are related to each other, and which was the main source tree.

We examined the trees on the bluff and determined if they were closely related to each other, the trees at the bases of the bluffs in residential areas, or the trees much farther away such as the Saint Mary's University Campus. To determine the main source tree, Amplified Fragment Length Polymorphism (AFLP) was used and the DNA profile of each tree sample was compared. Results will be presented.

### ISOMORPHOUS SUBSTITUTION OF RARE EARTH ELEMENTS FOR CALCIUM IN VANADATE APATITE

Young Hee Cho, Susan Gerbensky, and Lyudmyla Ardanova (Advisor)

*Chemistry and Geology Department*

*Minnesota State University, Mankato, MN*

Compounds with the apatite structure have the general composition  $M_5(EO_4)_3X$ , where M are univalent to trivalent cations (Ca, Sr, Ba, Cd, Eu, Y, La, Na, K, and others); E are tetravalent to hexavalent cations P, V, As, Si, Ge, S, Cr, and others; and X represents anions OH, F, Cl, Br, I, O. Hydroxyapatite  $Ca_5(PO_4)_3OH$  is particularly interesting among them because of its chemical similarity to the principal inorganic constituent of bone tissue. Due to its absolute biocompatibility with living tissues, calcium hydroxyapatite ceramics are widely used as biomaterials in medicine (stomatology, maxillofacial surgery, traumatology, orthopedy). Unlike hydroxophosphates, the hydroxovanadates with the apatite structures remained insufficiently studied. The main promise of hydroxovanadate apatites is their potential applications as efficient luminescent and laser materials and catalysts.

We investigated the possibility for heterovalent isomorphous substitutions in hydroxovanadate apatite  $Ca_5(VO_4)_3OH$  under the scheme:  $Ca^{2+} + OH^- \rightarrow M^{3+} + O^{2-}$ , where  $M^{3+}$  is rare earth ion. Isomorphous substitutions in systems  $Ca_5-xM_x(VO_4)_3(OH)_{1-x}O_x$ , where  $M = Sm, Eu, Y$ , were studied by X-ray powder diffraction analysis and IR spectra. Samples were prepared by nitric-tartaric solutions method and calcined at final temperature of 750°C. Solid solutions formed in the systems  $Ca_5-xSm_x(VO_4)_3(OH)_{1-x}O_x$ ,  $Ca_5-xEu_x(VO_4)_3(OH)_{1-x}O_x$ , and  $Ca_5-xY_x(VO_4)_3(OH)_{1-x}O_x$  have substitution limits  $0 < x < 0.35$ ,  $0 < x < 0.35$ , and  $0 < x < 0.06$  correspondently. The apatite solid solutions coexist with calcium orthovanadate phase  $Ca_3(VO_4)_2$  and unknown X phase in heterogeneous regions of the systems. The unit cell parameters are decreased monotonically within homogeneous region of the systems corresponding to calcium-rare earth element ionic radii difference.

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**MODELING THE ACCUMULATION OF DELETERIOUS MUTATIONS IN ENVIRONMENTAL AND GENETIC SEX-DETERMINING SYSTEMS**

Spencer Debenport and Steve Freedberg (Advisor)  
*Department of Biology*  
*St. Olaf College, Northfield, MN*

The sex-determining system that characterizes a species is predicted to have large impacts on its sex ratio over time. In theory, this temporal sex ratio variation should result in population bottlenecks, and in turn, increased levels of harmful mutation accumulation.

In this study, we examined the effects of environmental and genetic sex determination on allele frequencies and selection against deleterious mutations in both semelparitous and iteroparitous populations. We created a model which examines selection against a mildly deleterious allele in dioecious systems with a focus on the percent of times it went to fixation. The deleterious allele was significantly more likely to go to fixation under semelparity in ESD species as compared with GSD species with a sample size of 2,000 runs. Under iteroparity, the deleterious allele went to fixation close to an equal number of times in each system. The results suggest that environmental sex determination should result in increased mutation accumulation only in short-lived species. An analysis of genetic data from reptiles supports the finding that long-lived organisms do not suffer greater mutation accumulation under environmental sex determination.

**COVENANTS NOT TO COMPETE: LEGAL ISSUES RELATED TO PROFESSIONS**

Dwight Denman and James K. Kennedy  
*Department of Accounting and Business Law*  
*Northern State University, Aberdeen, SD*

Covenants not to compete (CNC) is an important topic in business law. Essentially, a CNC is an agreement between employer and employee, which applies particularly to former employees, where the latter agrees not to offer for service similar duties previously performed for the former employer. The purpose of a CNC is to prevent the former employee from unfairly competing and/or gaining an unfair advantage over his/her former employer by using specific job acquired expertise, methods, and contacts to gain and/or possibly taken away customers/clients from the former employer. CNC is governed by contract law and is an agreement supported by consideration. Previous court cases, to be cited, have addressed the reasonableness of CNC provisions in both protecting the interests of former employers while allowing former employees the right to earn a living. Reasonableness provisions granted to former employees relate typically to time limitation and geographic boundary, where the former employee is

restricted from performing similar services for a reasonable period and within a reasonable distance from the former employer. This presentation will specifically examine CNC cases and precedents related to professions, where former employees have tested reasonableness provisions as to time limit and geography.

**THE SUCCESS RATE OF ONLINE ACCOUNTING STUDENTS: ONE SCHOOL'S PERSPECTIVE**

Dwight E. Denman and James K. Kennedy  
*Department of Accounting and Business Law*  
*Northern State University, Aberdeen SD*

Like many other universities and colleges, Northern State University is seeing to broaden its student bases by offering courses online. In Northern's School of Business, the Accounting Department faculty voiced concerns over whether the delivery of accounting courses online was an effective way of teaching the subject. Despite these concerns, Northern's administration has pushed for the development of online accounting courses, with a goal of having enough accounting courses offered online so that a 5-year accounting degree can be available through online courses by 2012.

In response to Northern's emphasis toward online course development, Northern offered its first online accounting course in the Fall, 2009 semester-Principals of Accounting I. This paper seeks to compare the success rates of its online students versus those students in its traditional face-to-face Principles of Accounting classes. The possible factors for its success or failure based on responses from the students, and what modifications, if any, which may be made to the online course to accommodate future students.

**DETECTION OF GAS PHASE EXTRACTS USING EESI**

Sara Diener and Anthony Borgerding (Advisor)  
*Department of Chemistry*  
*University of St. Thomas, Saint Paul, MN*

Gas phase microdialysis probes provide two major advantages when continually extracting volatile analytes from solution. They are fast (steady state <5 sec) and small (200Um X 3Um). The use of these probes has been combined with EESI to analyze gas-phase extraction streams. Traditional mass spectrometry ionization techniques do not work when detecting gas-phase extraction stream if water is present. Recent work has shown that extractive electrospray ionization, EESI, may be a good solution. The electrospray ionization source on a triple-quadrupole mass spectrometer was modified to allow ionization of a separate extraction stream. Ketones and amines were studied using this technique. We were able to detect butanone and pentanone at concentrations

as low as 10 micromolar, acetone at 100 micromolar, and sec-butylamine at 10 millimolar.

### GENE EXPRESSION CONNECTIONS BETWEEN HIV-1 VIRUS INFECTION AND NEURODEGENERATIVE DISEASES

James Ebeling, Nicole L. Soiseth, Kim Rostvold, and Sanjive Qazi (Advisor)  
Department of Biology  
Gustavus Adolphus College, St. Peter, MN

HIV-1 profoundly affects the brain very soon after peripheral infection to induce a range of neurological disorders. We show that some of the molecular signatures for these pathologies occur within 48 hours of infection using a high-throughput gene chip assay. Our study involved interrogating more than 12600 gene transcripts following infection with five distinct drug-resistant HIV-1 strains in CD4+ MT2 T-lymphoblastoid cells. Two-way cluster analysis of 397 genes whose expression was significantly different across viral treatment groups showed two groups of viral responses: HIV-1<sub>A17</sub>/HIV-1<sub>A17V</sub> and HIV-1<sub>RT-MDR</sub>/HIV-1<sub>HTLVIIIIB</sub>/HIV-1<sub>92BR019</sub>.

We employed a pathway analysis using the Kegg compendium and showed two sets of distinct responses. Differentially expressed genes were highly enriched in neural pathways such as Alzheimer's Disease and Huntington's Disease. In this study, we used bioinformatics tools such as Gene Cards to investigate specific gene targeting sites linking neurodegenerative diseases and HIV-1 infection to further support this gene expression signaling connection.

### OH OXIDATION OF ISOPRENE VIA PHOTOLYSIS OF HONO AND PT-MS DETECTION: A PRODUCT STUDY

Katherine E. Edelen and David R. Hanson (Advisor)  
Department of Chemistry  
Augsburg College, Minneapolis, MN

Photolytic HONO oxidation of isoprene was studied with a proton transfer-mass spectrometer, PT-MS, detector. NO levels typical of the atmosphere were present. The flow reactor is described and PT-MS detection of the end products are discussed, including ion break-up processes of the products, and overall sensitivities are estimated.

The parent plus proton ( $M\cdot H^+$ ) ion for methyl vinyl ketone (MVK) and methacrolein (MACR) was the most prominent product ion observed, and the yield of these species was about 65%. The C5-hydroxycarbonyl products were about a 9% yield, wherein the ion underwent dehydration,  $M\cdot H^+ - H_2O$ . By contrast, the  $M\cdot H^+$  ion for the C4-hydroxycarbonyl product apparently does not undergo complete dehydration; signal at 87 u comprised a ~5% yield. The  $M\cdot H^+$  ion for the

hydroxynitrates, 148 u, was disrupted by break-up processes (loss of HONO and  $HNO_3$ ) that lead to 101 u and 85 u (respectively) and the sum of these ions gives a yield of ~4%. A unified oxidation scheme is presented and the flow reactor was modeled with the OH + isoprene + NO kinetics to estimate reactant levels and the extent of secondary reactions.

### THE INVOLVEMENT OF MAST CELLS AND MAST CELL-DERIVED MEDIATORS IN INFLAMMATORY HYPERNOCEPTION IN MICE

Camilla Engblom, Carolina Mora Solano, Paul Maitland-McKinley, Juliann Allen, and Devavani Chatterjea  
Department of Biology  
Macalester College, St. Paul, MN

Pain is a cardinal component of inflammation and a significant public health issue. There is evidence that sentinel mast cells in the tissues can act as possible regulators of certain pain responses. Degranulation of mast cells resulted in increased expression of pErk in nociceptive neurons in a rat model of migraine-like stimulation. Moreover, mechanical pelvic pain in a model of interstitial cystitis was abrogated in mast cell-deficient mice. Cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-15, and CXCL1/IL-8, as well as neutrophil influx, have also been implicated in the potentiation of peripheral inflammatory pain in models of thermal and mechanical hindpaw hypernociception in rodents. Taken together, these findings indicate that mast cells and mast cell-derived mediators could be important players in pain.

We show that c48/80-mediated degranulation of mast cells causes pain as well as significant edema and upregulation of local IL-1 $\beta$ , TNF- $\alpha$ , and IL-15 levels in the hindpaws of mice in a model of thermal pain. Mast cell-deficient Wsh mice show abrogation of this pain response. IgE/Ag-mediated degranulation of mast cells in the paws of WT mice also causes pain and edema and it is accompanied by detectable upregulation of IL-1 $\beta$  and TNF- $\alpha$ , but not IL-15, in the paws. Our findings demonstrate that mast cells and mast cell-derived mediators may be necessary for the initiation of inflammatory pain.

### THE CONTRACTILE EFFECTS OF *Caulophyllum thalictroides* (BLUE COHOSH) ON *Rattus norvegicus* AORTIC AND INTESTINAL SMOOTH MUSCLE *in vitro*

Andrew Evelsizer and Teresa DeGolier (Advisor)  
Department of Biological Sciences  
Bethel University, St. Paul, MN

The following research was conducted to explore the effect of an herbal supplement, blue cohosh, on aortic and intestinal smooth muscle *in vitro*. It is known

experimentally and experientially by midwives that blue cohosh is a powerful contractor of the uterus and is thus used as a replacement for pituitary oxytocin during labor. Its effect had not been quantified, however, on several other areas of smooth muscle including blood vessels that would have implications on blood pressure

It was observed that blue cohosh had no contractile or vasodilatory response on aortic smooth muscle. After concluding that the distal colon was most receptive to blue cohosh, the remaining focus of the investigation was spent quantifying a dose response as well as testing various receptor blockers in an attempt to determine the binding mechanism of blue cohosh. It was found that there is an increasing dose response to contraction of the colon; however, none of the blockers (nicotinic cholinergic) were found to inhibit the effect of blue cohosh on colon tissue *in vitro*. Implications of these results suggest preventative steps to be taken to avoid unwanted high-dose responses to blue cohosh, which may initiate intestinal motility during labor.

#### INVESTIGATION OF THE LIGHT DEPENDENCE OF STATE TRANSITIONS IN PINE IN SUMMER COMPARED WITH WINTER

Angela Ferry and Amy Verhoeven (Advisor)

*Department of Biology*

*University of St. Thomas, St. Paul, MN*

Photosynthetic organisms must cope with changing light environments. One mechanism that helps them do this is state transitions, where light-harvesting proteins (LHC) shift between photosystem I (state I), and photosystem II (state II) via reversible phosphorylation of the LHC. Several studies indicate that plants shift from state II (where LHC is not phosphorylated) in darkness, to state I (where LHC is phosphorylated) in low light, and back to state II in high light. A study in conifers, however, showed high levels of LHC phosphorylation in high light.

The major goal of this research was to examine the light dependence of state transition in conifers, compared with angiosperms. Also, we examined the light dependence of LHC phosphorylation in conifers during winter stress. To do this, thylakoids were isolated from different light levels (darkness, low light, and high light) during the summer (from both eastern white pine (*Pinus strobus* L.) and Hosta "August Moon") and from pine during the winter. Thylakoid proteins were run out on SDS-PAGE and western blotting was performed using an anti-phosphothreonine antibody. The phosphorylated proteins were then detected using chemiluminescence. Our results have shown a high phosphorylation at low light in pine and Hosta during the summer months, which is consistent with previous studies. Also there appears to be no phosphorylation of LHC in winter, which is inconsistent with previous results. Our results will be discussed within the context of light regulation in conifers in summer compared with winter.

#### SOLID-STATE HYDROGEN BONDING AND MOLECULAR PACKING: TWO "BRIDGE-FLIPPED" ISOMERIC CARBOXYLIC ACIDS

Sarah M. Fink and William H. Ojala (Advisor)

*Department of Chemistry*

*University of St. Thomas, St. Paul, MN*

We designate as "bridge-flipped isomers" those organic molecules differing only in the orientation of a bridge of atoms connecting two major portions of the molecule. Families of organic compounds in which examples are found include the benzylideneanilines, in which the isomerism is Ar-CH=N-Ar' vs. Ar-N=CH-Ar', and the phenylhydrazones, in which the isomerism is Ar-CH=N-NH-Ar' vs. Ar-NH-N=CH-Ar' (Ar = aryl). Because isomers of this type assuming the same molecular packing arrangement in their respective crystals (and thereby having the same *crystal structure*) might be especially capable of co-crystallization to form new and useful solid crystalline materials, we are conducting a solid-state study of factors that either facilitate or hinder this *isostructuralism*.

Similarity in solid-state H-bonding motifs between bridge-flipped isomers might facilitate their assuming similar solid-state molecular packing arrangements, so we have determined (and describe here) the crystal structure of a carboxyl-substituted benzylideneaniline, 4-[(phenylimino)methyl]benzoic acid (I) to examine the role played by conventional solid-state H-bonding and by additional influences, including weaker solid-state interactions and conformational differences, in causing it to assume a crystal structure different from that of its isomer, 4-[(phenylmethylene)amino]benzoic acid (II). Our structure determination for (I) comes more than 40 years after initial cell data were first reported for this compound. Although our analysis shows that both (I) and (II) form H-bonded dimers in the solid state, this interaction is strictly and crystallographically centrosymmetric in (II) but not in (I), suggesting that conformational differences between molecules of (I) and (II) differentiate their crystal structures from each other.

#### EXPRESSION AND CHARACTERIZATION OF A FLAVIN HYDROXYLASE INVOLVED IN SIDEROPHORE BIOYNTHESES IN *Aspergillus nidulans*

*Aspergillus nidulans*

Christina M. Fitzsimmons and Christopher T. Calderone (Advisor)

*Department of Biology*

*Macalester College, St. Paul, MN*

Nonribosomal peptides (NRP) are a class of secondary metabolites with interesting biological activities produced by a number of bacteria, fungi, and plants. To obtain necessary iron from their environment, bacteria and fungi utilize a class of NRP known as siderophores.

Our research aims to characterize the enzyme SidA, a flavin hydroxylase that catalyzes the conversion of ornithine to hydroxyornithine in the biosynthetic pathway of the siderophore triacetylfusarinine C (TAFC) in *Aspergillus nidulans*. Data show that NADPH is consumed in the presence of SidA, ornithine, and FAD. Utilizing a spectrophotometric method for measuring the production of hydroxyornithine, we have confirmed the hydroxylation of ornithine to hydroxyornithine in the presence of SidA. Utilizing this assay, preliminary kinetic data show the  $K_m$  at 22°C to be approximately 70.2  $\mu\text{M}$  and the  $k_{cat}$  to be  $3.44 \times 10^6 \text{ sec}^{-1}$ .

Future research hopes to measure the efficiency of the SidA-catalyzed reaction and determine its substrate scope. We hope that by understanding TAFC biosynthesis in *A. nidulans*, we can work to develop an inhibitor for TAFC biosynthesis in *Aspergillus fumigatus*, a closely related species that is potentially lethal in immunocompromised individuals.

#### **IMMUNOLOGICAL EFFECT OF GLYCEROL MONOLAURATE ON *Mus musculus***

John Fox and Jeanne Minnerath (Advisor)

*Department of Biology*

*Saint Mary's University of Minnesota, Minneapolis, MN*

Glycerol monolaurate (i.e. GML or Lauricidin) has been shown to exhibit potent antimicrobial activity. Research indicates that GML may influence cells of the immune system by modulating their proliferation and cytokine production.

The goal of this study was to further examine the effects of GML on the immune system, and the study focused on determining whether GML influenced antibody titers and antibody affinities in an animal model. Thirty-three male BALB/c mice were separated into three groups. One group was tube-fed a "low" dose of GML dissolved in DMSO/saline (0.1  $\mu\text{g}/\text{GML}$  per mouse). A second group was tube-fed a "high" dose of GML dissolved in DMSO/saline (1.0  $\mu\text{g}/\text{GML}$  per mouse). The third group was tube-fed DMSO/saline alone and served as a control.

The mice were tube-fed daily for eight weeks. After two weeks of GML treatment, all 33 mice were immunized with a protein antigen, ovalbumin, to elicit a humoral immune response. Four weeks after the initial immunization, a subset of each group was re-injected with ovalbumin (OVA) to elicit a secondary immune response. At days 19, 28, and 42 post-immunization, serum samples were collected from all 33 mice. Indirect and competitive Enzyme Linked Immunosorbent Assays (ELISAs) were conducted on the serum samples to determine the antibody titers and affinities in each serum sample.

#### **PHYSIOLOGICAL RESPONSES TO CRUDE EXTRACT OF *Noturus gyrinus* DORSAL AND PECTORAL SPINES**

Hilary Framke and Dr. Randy Krainock (Advisor)

*Department of Biology*

*Saint Mary's University of Minnesota, Minneapolis, MN*

*Noturus gyrinus*, referred to as the tadpole madtom, is a catfish of the Ictaluridae family. The tadpole madtom, native to Central and North America, possesses venomous pectoral and dorsal spines. Previous research has shown that injection of a spine extract into various fish species and the mammalian species *Mus musculus* (mouse) resulted in decreased mobility compared with control subjects.

Spine extracts were prepared and given topically or by intraperitoneal, intramuscular, or subcutaneous injections to *Rana pipiens* (northern leopard frog). When treated topically, no behavioral effects were observed. Intraperitoneal injections caused only abdomen skin erythema. Intramuscular injections produced visual dermal erythema and diminished motor activity in the injected leg, with a full recovery within 24 hours. Subcutaneous injections caused spasticity in both hind legs and a noticeable reduction in awareness and activity, and death within 8 hours. Gastrocnemius muscle and sciatic nerve preps of double-pithed frogs demonstrated no effect on force of muscle contraction following topical application of the extract to the muscle and the nerve. A significant decrease in gastrocnemius muscle force of contraction was only observed following a topical application of the extract to the heart. Topical application of the extract on the heart also produced a decrease in heart rate and force of contraction of the atria and ventricle. An injection of the extract into the ventricle resulted in a reversible cardiac arrest, whereas an injection into the atria resulted in death.

#### **X-RAY DIFFRACTION CHARACTERIZATION OF RESIDUAL STRESSES IMPARTED ON Ti-6-Al-4V**

Sheila R. Gagnon and Kenneth Rohly (Advisor)

*Department of Chemistry*

*Bethel University, St. Paul, MN*

Ti-6-Al-4V is used commonly in several medical applications. Imparting a layer of compressive residual stress at the surface of crystalline materials such as this metal alloy has been shown to improve strength. Compressive residual stress may be impressed in materials by various methods, including manufacturing processes intended primarily to obtain the net shape of the component or explicit surface modification methods such as shot peening.

X-ray diffraction was used to quantify the residual stresses produced on Ti-6Al-4V at various stages in mechanical processing: post-machining, post-deburring, and post-anodization. Test strips similar to

Almen strips of Ti-6-Al-4V were also shot peened at various Almen intensities, and residual stresses were measured using X-ray diffraction. This was done to determine if there is a relationship between the peening intensity and residual stress that is produced.

### MEASURING OXIDATIVE STRESS BASED ON GLUTATHIONE

Juliana C. Galarraga, Leah M. Michaels, and Mary Hadley (Advisor)  
*Department of Chemistry*  
*Minnesota State University, Mankato, MN*

Necrotizing enterocolitis (NEC) is a disease characterized by necrosis of mucosal intestinal cellular tissues of premature babies, which may be fatal. Currently the causes of NEC are unknown.

In this experiment, it is hypothesized that a mouse model lacking surfactant protein A (SP-A) (null-type), a protein with antioxidant properties, will show an elevated concentration of glutathione (GSH) in the mucosal intestinal tissues when compared with wild-type mice that retain SP-A. GSH is a key antioxidant, found throughout the body, that protects cells from oxidative stressors which can lead to cellular damage. Intestinal mucosal tissues of both wild-type and null-type mice at one and two weeks following birth were assayed to compare their concentrations of GSSG to total glutathione, that is, GSH plus GSSG. No differences in total glutathione concentrations between null-type and wild-type were found. Significant differences in GSSG concentrations were found between the two groups.

### DETERMINATION OF ABO VARIANTS USING ASP/SSCP ANALYSIS

Jessica German and Theresa Salerno (Advisor)  
*Department of Chemistry and Geology*  
*Minnesota State University, Mankato, Mankato, MN*

The ABO blood system is the most commonly known of the blood type systems. It is composed of four blood phenotypes: A, B, AB, and O. Each of these phenotypes is made from two different genes. The different genes vary in just a few base pairs, and these differences are called single nucleotide polymorphisms (SNPs). Minor variants are not detected by normal blood typing, but they may be significant for the acceptance of blood transfusions or organ transplants.

This study uses allele-specific polymerase chain reaction (ASP) combined with single-stranded conformation polymorphism (SSCP) to create gel patterns that distinguish among variant alleles. ASP is a method of amplifying DNA of a chosen sequence in the region containing one or more SNPs. SSCP uses denaturation, followed by quick cooling to create different foldings caused by nucleotide changes. This combined method has

been used successfully to detect common alleles (A, B, O<sub>101</sub>, O<sub>201</sub>) and at least two less common variants. Sample genotypes were verified by the development of a quantitative PCR SNP genotyping method. Samples showing unique patterns will be sequenced to determine the SNPs of these less common variants. These SSCP methods will allow us to screen for the ABO blood type variants by quick and inexpensive methods.

### CONTRACTILE EFFECTS OF *Cimicifuga racemosa* (BLACK COHOSH) ON *Mus musculus* UTERINE TISSUE *in vitro*

Anna Hachfeld and Teresa DeGolier (Advisor)  
*Department of Biological Sciences*  
*Bethel University, St. Paul, Minnesota*

The purpose of this study was to investigate the effects of black cohosh on isolated strips of uterine tissue. The objectives were to observe and quantify the contractile effects, to determine whether or not contractions were dose-dependent, and to compare the effects with previous studies on similar herbal remedies such as blue cohosh, red raspberry leaf, and squaw vine. Data were collected from 24 female mice using a standard smooth muscle organ bath. Black cohosh, when applied in extract form to the uterine tissue, caused the spontaneous motility pattern to become more regular and statistically more frequent. Multiple applications did not induce a significant fatigue in frequency. Contractile amplitudes of the spontaneous motility patterns were also statistically greater following the application of black cohosh. All of the induced contractile responses had a shorter duration than similarly evoked responses from blue cohosh, red raspberry leaf, and squaw vine given similar concentrations. This may indicate that black cohosh has a unique mechanism of contraction, and further studies should consider whether increasing the frequency of uterine contractions is as effective as increasing the force of contractions when promoting labor.

### APPLICATION OF DESILYLATIVE CLICK CHEMISTRY TO THE SYNTHESIS OF NOVEL LIGHT-EMITTING COMPOUNDS

Hannah Haley and Ronald G. Brisbois (Advisor)  
*Department of Chemistry*  
*Macalester College, St. Paul, MN*

Foundational investigations of the [3+2] dipolar cycloaddition between alkynes and azides have been complemented and extended tremendously by a regiocontrolled, Cu<sup>I</sup>-catalyzed modification reported in 2002. Because of its simplicity, broad scope of application, high yields, and atom economy, Cu<sup>I</sup>-catalyzed alkyne/azide “click chemistry” has had significant impact in all subdisciplines of chemistry that depend on synthesis.

We have been intrigued by reports that fluorescence intensity can be enhanced, or activated outright, by 1,2,3-triazolyl derivatization of a known fluorophore or of a non-fluorescent, but conjugated, scaffold. Here, we continue efforts to extend the scope of “click-enhanced/activated” fluorescence, particularly to polycyclic aromatic hydrocarbons. The application of a monodesilylative click reaction mechanism is also reported.

#### **PROBING FOR GLUCOCORTICOID RECEPTORS IN THE HUNTINGTON DISEASE MOUSE MODEL**

Arif Hamid, Lori Zacharoff, William Engeland, and Janet Dubinsky

*Department of Neuroscience*

*University of Minnesota, Minneapolis, MN*

Huntington Disease (HD) is a neurodegenerative disease caused by expanded CAG repeats in the HTT gene. In addition to chorea and cognitive decline, changes in homeostatic and endocrine functions such as weight loss, low bone density, diabetes, and increased level of corticosteroids have also been reported. Corticosteroids are secreted by the adrenal cortex in response to ACTH secreted into the bloodstream by the anterior pituitary. Hypothalamic release of corticotropin-releasing hormone, which regulates ACTH release, can be inhibited by hippocampal projections. Abnormal corticosterone levels have been reported in the R6/2 mouse model of HD. This strain is genetically engineered to express exon 1 of the human HTT gene with ~150 CAG repeats.

We have observed that R6/2 mice had enlarged adrenal cortex. Also, in a 30-minute restraint stress, plasma corticosterone levels in the transgenic mice, elevated and persisted 60 minutes after replacement into home cage, indicating a slowed recovery from stress. High corticosterone levels sustained through the recovery phase indicate changes in feedback pathways. Thus, we examined GR receptor density in hippocampus, cortex, and hypothalamus utilizing immunohistological staining and quantitative confocal microscopy. Preliminary results indicate that R6/2 mice expressed fewer glucocorticoid receptors at 9 weeks of age than littermates, thus dampening the inhibitory feedback input into the HPA axis from hippocampal pyramidal cells. Therefore, peripheral changes in basal cellular corticosterone secretion in HD may be secondary to abnormalities in the central nervous system.

#### **EFFECTS OF CHROMOSOME NUMBER VARIATIONS ON GLOBAL GENE EXPRESSION**

Carolyn Harris and Irina Makarevitch

*Department of Biology*

*Hamline University, St. Paul, MN*

Despite the widespread interest in aneuploidy, the molecular mechanisms that lead to phenotypic alterations in aneuploid organisms are still poorly understood. Moreover, it is not clear what gene interactions are involved in coping with gene dosage imbalance caused by aneuploidy on the global genomic level. Plants are more tolerant to aneuploidy than animals and present a good model for research. Here, we investigated aneuploidy effects on gene expression in the maize aneuploid that carried an extra copy of a small arm of chromosome 5 and exhibited several phenotypic traits, such as stunted growth, late development, partial tassel sterility, and knots in the leaves. We were primarily interested in understanding whether the phenotypic syndromes of aneuploidy could be attributed to a small number of affected genes or a complex network of gene interactions is involved. We also wanted to know whether different plant organs respond differently to aneuploidy. We compared expression levels of approximately 15,000 maize genes in meristems and leaves of aneuploid and wild type seedlings. In addition, we chose 30 genes that showed largest variation in seedlings and meristem-enriched tissues in response to aneuploidy and characterized their expression in eight different tissues and during early plant development in wild type and aneuploid plants. Our data demonstrated that different sets of genes are affected in different tissues and that many genes respond to aneuploidy by changing their expression pattern and becoming ectopically expressed. Such drastic changes of expression patterns are likely to contribute to phenotypic syndromes seen in aneuploid organisms.

#### **SOIL ORGANIC CARBON STOCKS AND DISTRIBUTION IN MANITOBA'S WESTERN HUDSON BAY LOWLANDS**

Patrick D. Henneghan, Kendra Passow, and Charles Umbanhowar Jr. (Advisor)

*Department of Biology*

*St. Olaf College, Northfield, MN*

Global warming is expected to raise temperatures in the Arctic by 2.8-7.8° C with a mean prediction of 5° C by the end of the 21st century, possibly shifting the Arctic from a sink to a source of atmospheric CO<sub>2</sub>.

To better understand the distribution of carbon in the Arctic, we quantified soil organic carbon (SOC) at a low arctic site just above treeline in the western Hudson Bay lowlands of northern Manitoba where soil organic carbon averaged 9.7 ± 3.2 kg C m<sup>-2</sup>. Our samples of carbon from lowland sites such as peat bogs and sedge wetlands were limited to the active layer, which contained 18.9 kg C m<sup>-2</sup> and 17.9 ± 6.0 kg C m<sup>-2</sup>, respectively. This is far greater than upland sites such as: dry tundra 7.9 ± 9.6 kg C m<sup>-2</sup> and open woodland 3.2 ± 1.6 kg C m<sup>-2</sup>. In upland sites, 57% of the carbon was located in the upper

7.3 cm of the sediment with negligible carbon stores in the deepest sampled horizon. Carbon is distributed more evenly through lowland sites, suggesting that these areas contain additional SOC stored within the permafrost. This, as well as the abundance of lowland sites (58.5% ground cover), suggests that wetlands will be the largest source of carbon in the Hudson Bay Lowlands as warming occurs.

#### **THE INCIDENCE OF STREPTOZOTOCIN(STZ)-INDUCED AUTOIMMUNE DIABETES IN JANUS TYROSINE KINASE(JAK)3-DEFICIENT MICE**

Maisee Her, Alan Voegelé, and Marina Cetkovic-Cvrlje (Advisor)

*Department of Biological Sciences*

*St. Cloud State University, St. Cloud, MN*

Type 1 Diabetes Mellitus (T1D) is an autoimmune disease wherein T cells attack insulin-producing pancreatic  $\beta$  cells, resulting in insulin deficiency. Streptozotocin (STZ) is a chemical found to create changes on the surface of mouse  $\beta$  cells when administered in low doses over several days, thereby inducing immune attack by T cells. The Janus tyrosine kinase (JAK)3 protein is involved in the signaling pathways of T cells.

It has been shown that an absence or inhibition of JAK3 affects T cell function; therefore, we hypothesized that T1D development would be affected by nonfunctional T cells in mice that lack expression of JAK3 (JAK3<sup>-/-</sup> mice). In order to test our hypothesis, we studied development of low-dose STZ-induced diabetes (40 mg/kg/day, 5 days) in JAK3<sup>-/-</sup> C57BL/6J male mice. Three groups of 2-3-month-old mice were utilized in the experiment: STZ-treated JAK3<sup>-/-</sup> (n=5), STZ-treated JAK3<sup>+/+</sup> (n=5), and control, vehicle-treated JAK3<sup>+/+</sup> group (n=5). Glycemic levels (blood glucose levels  $\geq 220$  g/dl or more indicates diabetes), body weight measurements, and immunophenotyping of different populations of T cells were performed on days 7, 14, and 21 post first STZ injection. At day 21, diabetes was evident in 80% of the STZ-treated JAK3<sup>-/-</sup> compared with 40% of the JAK3<sup>+/+</sup> mice. The JAK3<sup>-/-</sup> group had an average glucose level of  $322.8 \pm 123.9$  g/dl and an average body weight of  $33.4 \pm 2.9$  g as compared with  $193.4 \pm 29.7$  g/dl and  $35.9 \pm 3.6$  g in the JAK3<sup>+/+</sup> group. As a result, our preliminary studies suggest that the absence of JAK3 aggravates the STZ-induced autoimmune diabetes development in a mouse model.

#### **THE EFFECTS OF ATRAZINE ON PACK CELL VOLUME, GENDER, AND DEVELOPMENT OF *Gallus gallus***

Michele Hermes, Keith Fahrfoth, Charlotte Feddersen, and Debra Martin (Advisor)

*Chemistry Department*

*Saint Mary's University of Minnesota, Minneapolis, MN*

The herbicide atrazine was tested on the development of *Gallus gallus* (chicken). Three groups were injected with their respective treatment at the beginning of incubation including 69 eggs for the control group (water), 33 eggs for the low levels of atrazine (0.06 $\mu$ g/egg), 70 eggs for the intermediate level of atrazine (0.6  $\mu$ g/egg), and 34 eggs for the high level of atrazine (6 $\mu$ g/egg). The eggs were hatched at day 17 of development and blood samples were obtained.

Analysis showed that pack cell volume measure decreased significantly with increased levels of atrazine. From analysis of pictures of the hatched samples, the effect of atrazine on the development of *G. gallus* showed a significant difference when we compared the high level atrazine group with the low level atrazine and control groups. Atrazine was shown to have no significance when we compared male-versus-female ratios with the physical identification of gender. Gender determination by PCR of DNA isolated from blood samples of the groups also showed no significant results of atrazine-treated chickens having discrepancies in sex phenotype compared with sex genotype.

#### **FLOW CYTOMETRIC ANALYSIS OF CYTOKINE PROFILES IN LOW-DOSE-STZ (LDSTZ)-INDUCED MODEL OF AUTOIMMUNE DIABETES**

Joseph Hobbs, Larita Nandlal, and Marina Cetkovic-Cvrlje (Advisor)

*Department of Biology*

*St. Cloud State University, St. Cloud, MN*

Type I diabetes (T1D) is an autoimmune disease wherein pancreatic beta cells that produce insulin are attacked and destroyed by their own immune cells. The immune cells that initiate/protect against the attack of pancreatic beta cells secrete different cytokines. It is believed that TNF-alpha, IL-6 (secreted by macrophages), IL-2, IFN-gamma (secreted by Th1 cells), and IL-17 (secreted by Th 17 cells) exhibit pro-inflammatory effect, in contrast with anti-inflammatory effects of IL-4 and IL-10 (secreted by Th2 and regulatory T cells). A streptozotocin (STZ)-induced T1D model can be induced in a particular mouse strain (C57Bl/6J) by consecutive injections of low doses of STZ over the period of five days.

In order to study an involvement of different immune cell types in immunopathogenesis of LDSTZ mouse T1D, we evaluated cytokine secretion of splenocytes, isolated from STZ-treated and control

(vehicle-treated) 3-4-month-old C57BL/6J male mice, on days 7, 14, and 21 post first STZ injection. The splenocytes were cultured for 48 hours and cytokine levels were detected in cell culture supernatants by flow cytometry (FACSCalibur) using the BD Cytometric Bead Array Mouse Th1/Th2/Th17 cytokine kit. This kit allows simultaneous detection of following cytokines: IL-2, IL-4, IL-6, IFN-gamma, TNF-alpha, IL-17a, and IL-10. Our preliminary results showed a decrease of IFN-gamma, IL-6, IL-2, and IL-17 in STZ-treated mice during the first 14 days post STZ injection. IL-10 secretion was found elevated on day 7, but diminished at later time points. These data suggest that secretion of pathogenic cytokines in spleen does not reflect ongoing destructive process in pancreas.

### ANTIOXIDANT ACTIVITY IN SUMMER VERSUS WINTER IN THREE SPECIES OF CONIFERS

Kimberly Hughes, Alex Kokula, and Amy Verhoeven  
(Advisor)

*Department of Biology*

*University of St. Thomas, Saint Paul, MN*

Evergreen plants that grow in seasonally cold climates deal with an imbalance of light absorption and utilization because much less photosynthetic activity occurs at low temperatures. In these conditions photoprotective mechanisms, such as the antioxidant systems in plants, are crucial. Previous work has shown that glutathione and the enzyme glutathione reductase (GR), components of the ascorbate-glutathione cycle, increased in winter stress in *Taxus cuspidata* needles. Other components of the ascorbate-glutathione cycle did not increase. This led to the hypothesis that GR and glutathione have a unique role in winter stress that is separate from their role in the ascorbate-glutathione cycle.

The present study aims to determine whether this change in antioxidant response during winter is common for various plant species or a species-specific trend. We examined the level of glutathione and the activity of GR and of the enzyme ascorbate peroxidase (APX) in winter and summer conditions in three conifer species: eastern white pine (*Pinus strobus* L.), balsam fir [*Abies balsamea* (L.) P. Mill], and white cedar (*Thuja occidentalis* L.). The enzyme results indicate that GR increased significantly in all three species during winter, while APX increased in pine and cedar but not in fir. Results from glutathione show significant increases in pine, while results from the other species are pending. The data suggest that different species have distinct strategies for antioxidant acclimation to cold stress: pine and cedar apparently up-regulate their ascorbate-glutathione cycle, while fir up-regulates GR but not APX, as seen in *Taxus cuspidata*.

### *Xenopus laevis* ABCA3 GENE SEQUENCE AND EXPRESSION IN LUNG DEVELOPMENT

Jeremiah Johnson and Brian Hyatt (Advisor)

*Department of Biological Sciences*

*Bethel University, St. Paul, MN*

Current research has linked the role of the ABCA3 gene to impacting the quality of lung surfactant. ABCA3, produced by alveolar type II cells, plays a vital role in transporting the main lipid component in surfactant across membranes and into lamellar bodies whereby they are brought to the alveolar sac. Mutations of ABCA3 have been linked to Respiratory Distress Syndrome, a severe cardiovascular disease recognized at birth. Despite the clear importance of ABCA3 in lung surfactant function, research is in its infancy in understanding the function of this gene.

This research report identified the ABCA3 gene in *Xenopus laevis*, the African clawed frog, based on high homology with necessary ABCA3 domains and other species ABCA3 genes. The gene was found to be 4,696 base pairs long with an open reading frame of 3,051 base pairs. Determining its expression in *Xenopus laevis* revealed ABCA3's presence at every stage of development and in every adult tissue sampled.

### INDUCTION OF AUTOIMMUNE DIABETES IN MICE BY STREPTOZOTOCIN

Birendra KC, Hwee Kiat Gong, and Marina Cetkovic-Cvrlje (Advisor)

*Department of Biology*

*St. Cloud State University, St. Cloud, MN*

Several experimental models are used to study autoimmune Type 1 Diabetes (T1D). Streptozotocin (STZ)-induced autoimmune T1D in C57BL/6 mice is one of them.

In order to characterize development of T1D in this model, we set up five experiments wherein 3-4-month-old C57BL/6J males were treated with either five low doses of STZ or vehicle control (5-6 mice/group/experiment). STZ was injected in a dose of 40 mg/kg/day, intraperitoneally, for five consecutive days. The body weight was recorded on days 1, 7, 14, 21, and 28 post first STZ injection. At the same time, blood glucose levels were measured from the samples obtained from the mouse tail vein. A mouse was considered as diabetic when the glucose level of 220 mg/dL was reached. Our data, presented as a summary of five experiments, showed that diabetes incidence increased from 0 to 94 % from day 1 to day 28 in STZ-treated mice. Glycemic level also increased from  $175.6 \pm 22.2$  to  $328.6 \pm 70.8$  during the same course of time. The body weight monitoring showed that the body weights of STZ-treated mice increased from  $25.1 \pm 1.5$  to  $29.3 \pm 1.1$  g, while control mice increased their body weights from  $24.4 \pm 1.6$  to  $30 \pm 1.2$  g. In conclusion, these data confirm that we

successfully induced autoimmune T1D by low-dose STZ administration in C57BL/6 mice.

**EFFECT OF LOWERED ALDOSTERONE LEVELS ON THE EXPRESSION OF THE GR, MR, NHE-1, AND NHE-3 IN RAT KIDNEY AND PLACENTAL TISSUES**

Ami Kim and Theresa Salerno (Advisor)  
*Department of Chemistry and Geology*  
*Minnesota State University, Mankato, Mankato, MN*

Hypertension is a major health concern among adults because it can lead to premature death due to cardiac arrest. Prenatal programming of adult hypertension has also been studied, but the causes are not fully understood. Aldosterone is a key hormone that binds to mineralocorticoid receptors (MR) and this elicits the production of protein responsible for blood volume and pressure. Because glucocorticoid hormones can bind to both glucocorticoid receptors (GR) and MR, excess glucocorticoids have also been shown to contribute to hypertension. Additionally, studies have shown that sodium transport by the Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE) can participate in a hypertensive response.

In this study, outer layers of adrenal glands of rats were cryo-destructed to lower aldosterone levels. The objective of our research was to look at the effect of lowered aldosterone levels on the expression of GR, MR, and NHE isoforms 1 and 3 in the kidneys and placentae of normo-tensive rats (WKY). RNA was isolated using RNeasy Plus Mini Kit (Qiagen) and quantified spectrophotometrically. Total RNAs were converted back to cDNA using reverse transcriptase and random primers. Primers and probes were designed for Quantitative PCR (qPCR) and have been used successfully to amplify target mRNAs. Results show no definitive differences between treated and untreated rat kidney and placental tissues.

**CYP1A1, IL4 INTRON 3, AND THEIR EFFECTS ON BREAST CANCER AGGRESSIVENESS**

Vanessa Kleckner and Bert Ely (Advisor)  
*Department of Biological Sciences*  
*University of South Carolina, Columbia, SC*

Breast cancer genotypes were determined for two genes, Interleukin-4 (IL4) Intron 3 and CYP1A1. This experiment sought to determine whether a particular genotype for these genes correlates with various characteristics of breast cancer. Using patient DNA samples frozen in the lab, Polymerase Chain Reaction (PCR) and restriction enzyme digests were performed on about 280 European American samples for each gene. Gel pictures allowed interpretation of each patient's genotype. Using a statistical program called Stata, the distribution of patient genotypes was analyzed for 20 categories of breast cancer characteristics to see if there was any correlation

with a particular genotype. No matching correlations were obtained for CYP1A1. IL4 Intron 3 was associated significantly with both live birth and invasive cancer in European Americans. However, a second set of patients will be needed to confirm these conclusions.

**THE FORMATION OF G-QUARTET STRUCTURES USING PRIMARY ALKYLAMMONIUM CATIONS**

Mikhail Klimstra and Thomas C. Marsh (Advisor)  
*Department of Chemistry*  
*University of St. Thomas, St Paul, MN*

Guanine-rich oligonucleotides (GROs) and chromosomal G-rich sequence repeats possess the ability to form quadruple helical structures (G-DNA) through guanine self-recognition. The structural motif that organizes and stabilizes G-DNA is a tetrad of Hoogsteen H-bonded guanine bases known as a G-quartet. A key feature of G-DNA structures is the coordination of metal cations (K<sup>+</sup>, Na<sup>+</sup>, and Sr<sup>2+</sup>) or small molecular cations (NH<sub>4</sub><sup>+</sup>) that reside between stacked G-quartets. The conformation and stability of G-DNA is strongly influenced by the coordinating cation and in the case of GROs can lead to the formation of supramolecular polymers of G-DNA.

Recently, two alkyl ammonium chains have been utilized to form higher order structure with a specific oligonucleotide sequence: Tet 1.7 (dGGGGTTGGGG). Analysis through gel electrophoresis indicated that 1-amino-2-butanol and 1-amino-2-ethanol facilitate formation of G-DNA and support the self-assembly into a supramolecular polymer known as a G-wire. This is the first report of any G-DNA structure formation mediated by a 1° alkyl ammonium cation. The initial results presented here suggest that longer chain derivatives of 1° alkyl ammonium cations may also support formation of G-DNA structures. This would allow for a new class of small molecules for interaction with G-DNA that have potential applications in nanotechnology and molecular probes for G-DNA.

**THE EFFECTS OF ALLOPREGNANOLONE ON THE ESCALATION OF COCAINE AND SUCROSE SELF-ADMINISTRATION IN FEMALE RATS**

Brandon B. Knight and Marilyn E. Carroll (Advisor)  
*Department of Psychiatry*  
*University of Minnesota, Minneapolis, MN*

Previous studies suggest that women are more vulnerable than men to the rewarding effects of cocaine. Research indicates that estrogen, a female hormone, is a contributing factor, as it facilitates the acquisition, escalation, and reinstatement of cocaine-seeking behavior in female rats. In comparison, progesterone (PROG) attenuates the acquisition, reinstatement, and escalation of

cocaine-seeking behavior in female rats. Recently, it was reported that the PROG metabolite, allopregnanolone (ALLO), displayed similar results and decreased the reinstatement of cocaine-seeking behavior in female rats. However, it is currently unknown if ALLO decreases the escalation of cocaine seeking during extended access to the drug.

The purpose of this study was to examine the effects of ALLO on the escalation of cocaine self-administration and to determine the specificity of these effects using a nondrug reward (sucrose). Female rats were treated with ALLO or vehicle (VEH) and were allowed to lever press for iv infusions of cocaine (0.4 mg/kg) under an extended-access procedure that produces escalated drug intake. In a separate condition, ALLO- and VEH-treated female rats orally self-administered a 10% sucrose solution under similar extended-access conditions. ALLO attenuated the escalation of cocaine self-administration, but did not significantly affect sucrose self-administration. The results of this study suggest that ALLO may be useful in treating binge-like patterns of cocaine intake in female cocaine users. Supported by NIDA grants R01 DA003240 and K05 DA015267 (MEC) and a UROP grant (BBK)

#### **VINYLOXYBENZENE AND TRANS-CYCLOOCTENE CONTAINING NON-NATURAL SUBSTRATES FOR PROTEIN FARNESYL TRANSFERASE**

Peter Koziol, Jeffery Walter, Christian Thorvaldson, Benjamin Monson, and James Wollack (Advisor)

*Department of Chemistry*

*Hamline University, St. Paul, MN*

Protein farnesyl transferase (PFTase) covalently attaches 15 carbon farnesyl moieties to a cysteine in a C-terminal four-amino acid recognition sequence. PFTase has also been shown to transfer non-natural isoprenoid analogs to recombinant proteins or peptides that end in the amino acid tag (CVIA).

Here we present two non-natural substrates for PFTase. The first analog contains a trans-cyclooctene functionality that can be used to attach proteins to other biological molecules and surfaces through an inverse electron demand Diels Alder reaction. The second substrate is an isoprenoid attached to a vinyloxybenzene moiety. This alkene-containing moiety has been shown to make proteins become fluorescent through photoinduced cycloaddition of a diaryl tetrazole. This fluorescent tag has the advantage of being smaller than many other fluorescent protein tags (such as GFP); hence, it decreases the likelihood of interference to a protein's biological function. The tag may also be useful for the development of a coupled assay for measuring protein prenylation rates.

#### **EFFECTS OF PRESCRIBED BURNING ON SOIL NUTRIENT CONTENT AND COMPOSITION DURING PRAIRIE RESTORATION AT BELWIN CONSERVANCY**

Elizabeth Kringen and Gina Mancini-Samuels (Advisor)

*Department of Chemistry*

*St. Catherine University, St. Paul, MN*

Prairies in Minnesota were once part of one of the largest ecosystems in North America, spanning across more than 18 million acres. Of the native prairies which existed less than two hundred years ago, less than one percent remains today. The near elimination of these ecosystems has inspired efforts to protect and restore these important ecosystems.

The focus of this study was to investigate the effects the restoration technique of prescribed burning has on prairie restoration at Belwin Conservancy in Afton, Minnesota. The impacts of this technique were studied by analyzing soil nutrient content and composition, particularly measuring soil pH, nitrogen, phosphorus, and potassium content following a prescribed burn at Belwin for two different prairies, comparing soil samples from burned and unburned plots. No statistical differences were found for burned to unburned soil nutrient content, although differences were determined between the samplings from July to August, suggesting the rapid uptake of nutrients following a burn and changes in soil nutrient dynamics over the growing season.

#### **A NOVEL METHOD FOR STIMULATING GROWTH IN ALGAL SYSTEMS**

Randall Krug and Arlin Gyberg (Advisor)

*Department of Chemistry*

*Augsburg College, Minneapolis, Mn*

Harpin proteins have shown stimulatory effects in plants and have thus recently gathered attention as possible agricultural fertilizers. While a variety of responses have been demonstrated in plant systems, no attention has yet to be directed to the effects on algal systems. With an increasing interest in algae as a source for biodiesel fuel, various methods for stimulating growth rates have been investigated. Harpins have the potential to increase growth rates in a cost-efficient and environmentally friendly manner. The following experiment will examine the effects of harpin proteins and related compounds on the growth curves of a Minnesota native algal mixture.

**GENETIC VARIATION BETWEEN AND WITHIN LOCAL POPULATIONS OF TIMBER RATTLESNAKES, *Crotalus horridus***

Angela M. Lager, Dr. Phil Cochran, Dr. Debra Martin (Advisor)

*Department of Biology*

*Saint Mary's University, Winona, MN*

Over the last few centuries timber rattlesnakes (*Crotalus horridus*) have experienced a decline in their populations and habitat across the eastern United States (Brown 1993). This decline is theoretically based upon the idea of intrapopulation genetic variation and patterns of intrapopulation and interpopulation gene flow (Lande and Barrowclough 1987). It has been found that *Crotalus horridus* using the same hibernacula were more closely related to each other than they were to randomly selected individuals from the same study site (Bushar et al. 1998).

The genetic variation between and within local populations of timber rattlesnakes was assessed from 64 adult shed skins that were collected over an eight-year period and from three bluffs: two adjacent bluffs in Winona County and one in Houston County, Minnesota. It was hypothesized that *Crotalus horridus* from the same ledge should display greater genetic similarity than *C. horridus* from different ledges on the same bluff, that *C. horridus* from different ledges on the same bluff should display greater genetic similarity than *C. horridus* from different bluffs, and that *C. horridus* from adjacent bluffs in Winona County should display greater genetic similarity to each other than to *C. horridus* from Houston County. Genetic differences were revealed through the use of microsatellite analysis and the Polymerase Chain Reaction with the following loci primers: 5A, 7-150, 7-144, 5-183, 7-87, 3-155. Results will be presented.

**INORGANIC/ORGANIC MATERIALS PREPARED FROM PSEUDO-A-TYPE KEGGIN POLYOXOMETALATE SANDWICH COMPLEXES BRIDGED BY ORGANIC LIGANDS.**

Sara Larson and Wade A. Neiwert (Advisor)

*Department of Chemistry*

*Bethel University, St. Paul, MN*

As the limit of top-down construction of materials approaches, the use of polyoxometalates (POMs) as building blocks in ground-up nanoscale construction has become a new focus because of their straightforward preparation, structural diversity, and potential tunability to specific applications. The preparation of polyoxometalate-based materials has been undertaken, using Keggin polyoxometalate sandwich anions as nanoscale building blocks, linked by various bridging organic ligands (e.g., 4,4'-bipyridine). Sandwich complexes of the general form  $[(M(H_2O)_2)_3(AsW_9O_{33})_2]^{12-}$  (M = various divalent first-row transition metals) have been used. The POMs are

linked via coordination of the organic ligand to the "sandwiched" metals (M). The facile preparation of these materials stems from the rational design of the easily prepared building blocks. The Keggin polyoxometalate sandwich anions and the resulting inorganic-organic hybrid materials following coordination of a bridging ligand were characterized by FT-IR and single-crystal X-ray crystallography.

**EFFECT OF TEMPERATURE ON POLYGLUTAMINE TRACT MUTATION RATE IN BACTERIA**

Han Lee and Geoffrey M. Goellner (Advisor)

*Department of Biological Sciences*

*Minnesota State University, Mankato, MN*

Huntington's disease (HD) is a severe genetic disorder with progressive neurodegenerative symptoms, ranging from emotional disturbance to uncontrolled movements and mental deterioration. Symptoms typically manifest in midlife, and life expectancy is between 10 and 25 years after disease onset. Interestingly, elongation of a contiguous tract of glutamines (glutamine; amino acid) in the huntingtin protein, is the causative mutation underlying HD. In the "normal" human population, the HD glutamine tract length typically ranges from 6 to 36 amino acids in length (i.e., it is polymorphic). In individuals afflicted with HD, DNA mutation causes the number of huntingtin polyglutamines to reach well beyond the normal range, and HD manifests. If the polyglutamine tract is >60, severe symptoms appear in young ages, and it is called juvenile HD. Currently, little is known regarding the conditions that favor or inhibit mutation rate of the HD gene.

This research project investigated whether temperature has any effect on polyglutamine mutation rate in *Escherichia coli* (DH5 $\alpha$ ). *E. coli* with three different lengths of HD polyglutamine (Q33, Q45, and Q56) were grown at different temperatures, 33°C, 35°C, 37°C, and 39°C, and the lengths of the polyglutamine were compared with controls.

**PEERING INTO THE PAST--LEVERAGING THE LANDSAT ARCHIVE TO ASSESS LAND COVER CHANGE IN THE BRAINERD LAKES AREA**

Greg C. Liknes, Kirk M. Stueve, and Paul A. Sowers (Advisor)

*Forest Inventory and Analysis, Northern Research Station  
USDA Forest Service, St. Paul, MN*

Trees provide valuable ecosystem services to the Brainerd Lakes area, Minnesota, purifying water that flows into lakes and enhancing the local tourism industry. Therefore, it is important to develop spatially explicit and accurate historical baseline information on forest cover in

order to monitor recent changes. Census statistics show the population of Crow Wing County, Minnesota, has increased by more than 12% since 2000, presumably exerting pressure on the forest resource. Yet sample-based estimates from the USDA Forest Service Forest Inventory and Analysis (FIA) program indicate no statistically significant change in forest area for the county during the same time frame. While FIA provides critical information for strategic-scale planning by collecting a suite of ground-based measurements, the network of plots is not designed to observe smaller-scale changes to forest land. Information derived from space-borne satellites can be used in concert with *in situ* forest inventory data to provide more spatially detailed information on both forest losses and gains across the landscape.

Recently, satellite imagery in the Landsat archive has been made freely available to the public. This presents an opportunity to assess changes in the landscape from the synoptic view of a satellite going back several decades. Biennial growing season images (i.e., leaf-on) from 1986 to 2009 were obtained from two Landsat scenes containing the Brainerd Lakes area in Crow Wing County. The Vegetation Change Tracker algorithm, developed and vetted by the North American Forest Dynamics Program, was used to map changes in forest cover over the last 24 years. Initial assessments of 150-m buffers surrounding major lakes and rivers indicate a 1.6% reduction in forest cover from 1986 to 1999 and an additional 3.6% reduction from 1999 to 2009. Further analysis will establish the context for the loss of forest cover in proximity to water bodies relative to forest gains and losses in the greater Brainerd Lakes area.

#### **THE RESPONSE OF *Micropterus salmoides* TO VENOM PRODUCED BY *Noturus gyrinus***

Ashley Loos and Dr. Randy Krainock  
Department of Biology  
Saint Mary's University of MN, Winona, MN

*Noturus gyrinus* is a baitfish in the upper Mississippi River that has venomous spines in its dorsal and pectoral fins. The physical response of *Micropterus salmoides*, largemouth bass, when injected with the *N. gyrinus* venom was tested. Nine largemouth bass received an initial injection of *N. gyrinus* venom in the caudal peduncle while nine others received a saline injection as a control. Three weeks later, a second injection of venom was administered to five of the nine originally injected with venom and four of the original control group; the remaining nine received a saline injection as a control. Observations were made on color change and spatial disorientation of the bass. After an initial injection of venom, the largemouth bass exhibited discoloration in varying degrees across the body surface in addition to differing degrees of spatial disorientation. Those injected twice with venom exhibited increased discoloration as well as increased spatial disorientation immediately

following *N. gyrinus* venom injection. Bass injected first with saline then venom exhibited elevated signs of disorientation when compared with those injected first with venom, though similar discoloration was observed.

#### **A STUDY OF WHITE-TAILED DEER MOVEMENT AND POPULATION ON THE ST. OLAF NATURAL LANDS**

Caitlin Marine and John Giannini (Advisor)  
Department of Biology  
St. Olaf College, Northfield, MN

The presence and movement of white-tailed deer (*Odocoileus virginianus*) is important to study due to the indirect effects on people and vegetation.

This investigation began 5 years ago to describe and gain a better understanding of deer movement, populations, and bedding activity on the St. Olaf College, Minnesota, natural lands. The 2009 wintering population was estimated to be 20 white-tailed deer, which represents a doubling since 2005. Track assays were performed starting in January and continued until mid-March. The deer showed clear travel patterns covering the study area, which was directly and positively associated with wind chill. In January, a survey of the deer beds yielded more information about the areas in which the deer reside as well as providing relative numbers for groups. Direct sighting of 16 deer confirms that a significant population is using the natural lands as a winter yarding area. This winter yielded very different results as snow depth played a large role in movement. Future investigation will allow for estimations of populations and could contribute to population management goals.

#### **OPIOID RECEPTORS AND TUMOR BIOLOGY IN MURINE MODEL OF BREAST CANCER**

Christopher Martinez and Kalpna Gupta (Advisor)  
Department of Medicine, Hematology, Oncology and Transplantation Division  
University of Minnesota, Minneapolis, MN

Minimizing pain in cancer patients has been an ongoing effort by physicians and the current treatment has been opioids, such as morphine. However, it has been shown that morphine sulfate promotes angiogenesis and breast cancer in mice (Gupta et al. 2002). Research efforts have been made to come up with a different type of analgesic that will not sacrifice pain relief while not causing angiogenesis, and tumor growth.

Using the C3-Tag mouse model of breast cancer, we investigated the association of opioid receptors with cell proliferation and activation pathways for cell survival/apoptosis. Techniques used were Western Blotting of tumor lysates and Immunohistochemistry of tumor sections. We investigated  $\mu$ -opioid receptor expression, cell proliferation, and the pathways that

control cell survival and apoptosis in tumor cells. Larger tumors showed a higher expression of MOR, KOR, and DOR as compared with smaller tumors from C3-Tag mice with breast cancer. Increased expression of opioid receptors (OR) in larger tumors correlated with increased expression of PCNA, p-MAPK/ERK, and p-AKT. Together these data suggest that increased expression of ORs may lead to increased growth of larger tumors. We speculate that treatment with morphine may lead to increased growth of larger tumors that express higher MOR.

#### **BREEDING JAK3 DEFICIENT MICE AT SCSU**

David McCarty, Brice Johnson, and Marina Cetkovic-Cvrlje (Advisor)

*Biology Department*

*St. Cloud State University, St. Cloud, MN*

The JAK3 gene encodes a tyrosine-kinase enzyme that functions in signal transduction and is predominantly expressed by T lymphocytes. The JAK3 enzyme is critical for the activation of T lymphocytes in response to cytokines, and individuals deficient in the JAK3 gene will exhibit characteristics of Severe Combined Immunodeficiency Disease (SCID). Type 1 Diabetes Mellitus is an autoimmune disease in which the insulin-producing pancreatic beta cells are destroyed by the host's own activated T lymphocytes. It can then be expected that individuals deficient in the JAK3 gene should not develop autoimmune diabetes. JAK3 knockout mice are thus very useful when comparing data using drug-induced Type 1 Diabetic models and immunosuppressive drugs. It is, however, very difficult to sustain a JAK3 knockout mouse colony because two such mice will not breed successfully. Therefore, it is necessary to perform controlled breeding of knockout and wild type mice to obtain a knockout progeny of sufficient number for experimental testing. By selectively breeding male JAK3 knockout mice purchased from Jackson Laboratories with female C57BL/6J mice at St. Cloud State University (SCSU), it is anticipated that roughly 25% of all second generation (F<sub>2</sub>) offspring will be deficient for the JAK3 gene.

The genotypes of all offspring birthed were determined using PCR and gel electrophoresis assays—the protocols for which were researched and written at SCSU. The majority of all mice birthed were successfully genotyped and sufficient numbers of JAK3-deficient mice were found, although some data were inconclusive.

#### **A SEMI-AUTOMATED APPROACH TO MAPPING TREE COVER IN RURAL MINNESOTA**

Dacia M. Meneguzzo and Greg C. Liknes

*Forest Inventory and Analysis, Northern Research Station  
USDA Forest Service, St. Paul, MN*

The USDA Forest Service's Forest Inventory and Analysis (FIA) program records and reports a variety of forest attribute information, such as tree species composition and biomass, based on data collected from a network of field plots that meet FIA's forest land criteria. However, detailed information about spatial location or arrangement of the forest is not provided and small forest features are often excluded by definition. To overcome this issue, inventory data can be supplemented with spatially-explicit satellite-derived land cover products to map the distribution of forest, but the spatial resolution is often too coarse to discern sparse tree resources. As forest sustainability efforts begin to focus not only on large, contiguous tracts of forest but also on trees occurring in narrow bands, such as windbreaks or along rivers, more spatial detail is required to monitor these undercounted tree resources. Therefore, the goal of this study was to develop an approach that used high-resolution aerial imagery to efficiently map the location of tree cover, particularly in rural agricultural areas where narrow plantings of trees and small, scattered patches of forest are prevalent.

Digital aerial imagery from 2008 was acquired for Minnesota from the USDA's National Agriculture Imagery Program (NAIP). Imagery from this particular year contains both visible and near-infrared (NIR) wavelength information, which is valuable when mapping tree cover. The high reflectance of NIR energy by vegetation was utilized by specialized software to partition the imagery into segments and subsequently label each segment as tree-covered or not tree-covered. A Geographic Information System (GIS) was then used to create a detailed map and estimate of tree-covered area for Steele County, MN from the classified output.

#### **ON THE DEVELOPMENTAL ORIGIN OF HEPATIC SINUSOIDAL ENDOTHELIUM: CONFIRMATION OF ANGIOCIRCULATORY VASCULOGENESIS**

Anna C. Meyer and Glenn K. Sherer (Advisor)

*Department of Biology*

*University of St. Thomas, St. Paul, MN*

Developmental mechanisms reported to account for the origin of hepatic sinusoidal endothelium include (1) *in situ* differentiation of mesenchymal cells (Sherer 1991), (2) sprouting from preexisting vessels (Gouysse et al. 2002), (3) transdifferentiation of mesothelial cells (Pérez-Pomares et al. 2004), and (4) recruitment of angioblasts and/or vascular endothelial cells (VECs) from the circulation (Pardanaud and Eichmann 2006; Zhang et al. 2006).

The present study was designed to investigate whether the last of these mechanisms, here termed angiocirculatory vasculogenesis, plays a role in the development of hepatic sinusoidal endothelium. Chick (*Gallus domesticus*) livers were harvested from

chick/quail (*Coturnix coturnix japonica*) parabioses maintained either *in ovo* or in shell-less culture and were examined for evidence of quail VECs by immunostaining with QH1 antibody. Numerous positive-stained cells were found to contribute to the sinusoids of parabiotic chick livers. Nonparabiotic chick liver, serving as a negative control, lacked staining. These findings confirm that at least some of the cells that form these capillary-level vascular structures are, as previously reported, acquired from circulating blood.

**ANALYSIS OF THE CAG TRINUCLEOTIDE REPEAT IN THE NOVEL POLYGLUTAMINE PROTEIN KIAA1946—EXCLUSION OF THIS GENETIC LOCUS AS A CANDIDATE GENE FOR NEURODEGENERATIVE DISEASE**

Eric Miller, Travis Mrkvicka, and Geoffrey Goellner (Advisor)

*Department of Biology*

*Minnesota State University, Mankato, Mankato, MN*

In 1872, an American physician, George Huntington, first described a condition, later named Huntington's disease (HD), in which middle-aged adults experience a loss of control over bodily movement and function. In 1993, the causative mutation was found to be an expansion of a specific triplet repeat DNA sequence (CAG—which codes of amino acid glutamine) within the huntingtin gene. The exact number of glutamines in the huntingtin gene normally varies from person to person; thus, it is polymorphic. However, expansion beyond the normal polymorphic range causes HD. Subsequently, a number of other severe neurodegenerative diseases have also been found to be “polyglutamine” diseases. Recently, the human genome-sequencing project has revealed other uncharacterized proteins with long glutamine stretches in their primary amino acid sequence. One such orphan gene is called KIAA1946.

Using standard molecular biology techniques, such as PCR and DNA sequencing, we have investigated whether KIAA1946's polyglutamine region is also polymorphic within the normal human population—and therefore a candidate gene for neurodegenerative disease. Preliminary results suggest that the glutamine stretch in KIAA1946 is in fact quite stable. Indeed, the most prevalent allele matches identically to the published sequence: CAA(CAG)<sub>8</sub>(CAA)<sub>5</sub>CAGCAA. Our results suggest that KIAA1946 should not be considered a strong candidate gene for uncharacterized neurodegenerative disorders.

**NITROGEN HOMEOSTASIS IN THE INVASIVE ARGENTINE ANT, *Linepithema humile***

Katie A. Miller<sup>1,2</sup>, Thomas C. Marsh<sup>1</sup> (Advisor), and Adam D. Kay<sup>2</sup> (Advisor)

<sup>1</sup>*Department of Chemistry*

<sup>2</sup>*Department of Biology*

*University of St. Thomas, St. Paul, MN*

Homeostasis of macronutrients is vital to the success of many organisms including social insects such as ants. Insects, in general, respond physiologically to different macronutrient ratios, but most of the studies in this area have focused on nitrogen and phosphorus as limiting macronutrients.

This study investigates the effects of varying the ratio of nitrogen to carbon in the form of defined protein:carbohydrate diets provided to laboratory colonies of the Argentine ant, *Linepithema humile*. When carbon is a limiting macronutrient, colonies experience an increased mortality rate among workers, workers have lower lipid content, and the colony has decreased viability. To gain a better understanding of the underlying biochemical cause of the increased mortality rate, the levels of total nitrogen and the nitrogenous waste product, uric acid, were measured. It was hypothesized that the consumption of the excess nitrogen puts an increasing burden on the limiting pool of carbon due to increased uric acid production. Measurement of total nitrogen by flash combustion gas chromatography revealed that the total percent nitrogen increased as the ratio of protein to carbohydrate increased in a colony's diet. Quantification of uric acid content per colony showed a similar trend as nitrogen became more prevalent in the diet treatments.

**COMPARING AMPHIBIAN DIVERSITY IN ORGANIC AGRICULTURAL AND FOREST FRAGMENT HABITATS IN POCOCI DE LIMON, COSTA RICA**

David C. Mitchell, Gerardo Chaves C. (Advisor), and Kathleen L. Shea (Advisor)

*Department of Biology*

*St. Olaf College, Northfield, MN*

Amphibian diversity was investigated in organic agricultural habitat and an adjacent fragment of secondary forest in Pococí, Limón Province, Costa Rica. The use of agricultural and restored habitat by amphibians was assessed in seven agricultural study sites and one forest fragment. Timed visual encounter surveys were conducted daily in the morning and night during March and April 2009. Species and microhabitat were recorded for each amphibian observed. Simpson indexes were used to compare amphibian species diversity between the study sites. All species observed in the forest fragment were also observed in the agricultural zone, while six species were observed only in the agricultural zone. The diversity index was significantly greater for the agricultural zone

than for the forest fragment ( $t = 11.67$ ,  $p = 0.01$ ). The fruit plantation had the greatest species diversity of the agricultural sites. Microhabitats used by the greatest diversity of amphibians were the ground-cover vegetation in the fruit plantations and the sugar cane crops in the agricultural zone. Results suggest that an organic farm with diverse production of crops located adjacent to a forest fragment may contain conditions for greater amphibian diversity than a secondary forest alone. Organic agricultural habitat containing a variety of microhabitats can provide suitable conditions for amphibian conservation in agricultural landscapes.

### INTERACTIONS AMONG NEAREST NEIGHBORS IN MINNOW SHOALS

Charlie Murphy and Craig Longtine (Advisor)

*Department of Biology*

*North Hennepin Community College, Brooklyn Park, MN*

Fathead minnows (*Pimephales promelas*) and common shiners (*Luxilus cornutus*) generally increase shoal cohesion in reaction to injury-associated chemical alarm cues (CAC) released in response to injury. Less well understood are the behavioral responses of individual fish that lead to changes in shoal cohesion. Additionally, shoaling behavior is typically investigated in conspecific shoals, but heterospecific shoals are common in nature.

We examined the behavior of fathead minnows (FM) and common shiner (CS) in the presence and absence of CAC isolated from FM. Observations were made of solitary individuals, conspecific shoals of 12 individuals, and heterospecific shoals of 9 FM and 3 CS. Synchronized digital camcorders recorded the vertical and horizontal movement of focal individuals and shoals. Motion-tracking software and motion analysis tools were used to track the movement of several individuals simultaneously. A visual trace of the movement of focal individuals was captured as well as changes in acceleration, velocity, angular velocity, angle of orientation, distance to nearest neighbors, shoal cohesion, and shoal polarization. In the absence of CAC, all measures of motion were lower in solitary individuals than in focal individuals in shoals, and FM typically displayed greater motion and lower shoal cohesion than CS. In the presence of CAC, both species generally exhibited reduced motion whether solitary or in shoals. Shoal cohesion and polarization increased in the presence of CAC. In heterospecific shoals, CAC had a greater effect on the behavior of FM relative to CS.

### CYCLOMETALLATED PLATINUM (II) COMPLEXES INCORPORATING 2-(2'-BENZOTHIENYL) PYRIDINE

Anna L. Myhre, Kent R. Mann, and Daron E. Janzen (Advisor)

*Department of Chemistry*

*St. Catherine University, St. Paul, MN*

Organometallic square planar platinum (II) complexes of the form  $[\text{Pt}(\text{btp})(\text{CNR})_2](\text{BF}_4)$  (btp = 2-(2'-benzothienyl) pyridine, R = Xylyl, Naphthyl) were synthesized and characterized. Numerous characterization methods were used to study these materials including NMR, ATR-IR, electrospray mass spectroscopy, solid-state and solution emission, and solution UV-Vis spectroscopy. Significant shifts in the emission wavelength are observed depending on the preparation of the samples (solution, film, or powder) and also on the nature of the isocyanide ligand. Steric demands of the isocyanide ligands influence the possible intermolecular interactions and solid-state packing. These differences in intermolecular interactions are the likely origin of the solid-state emission wavelength shifts. A single-crystal X-ray structure obtained for  $[\text{Pt}(\text{btp})(\text{CNXylyl})_2](\text{BF}_4)$  revealed close F(btp)-F(btp), F(Xylyl)-F(Xylyl), and Pt-F(btp) interactions and a linear infinite chain-packing motif of cations.

### COCAINE-SEEKING BEHAVIOR IN ADOLESCENT VS. ADULT RATS

Sean Navin<sup>1</sup>, Justin Anker<sup>2</sup>, and Marilyn Carroll<sup>2</sup>

<sup>1</sup>*Department of Neuroscience*

*University of St. Thomas, St. Paul, MN*

<sup>2</sup>*Department of Psychiatry*

*University of Minnesota, Minneapolis, MN*

Previous studies, both human and animal, have shown that adolescence is a critical period for the initiation and subsequent abuse of drugs. Adolescents also exhibit impulsive behavior, which is a major vulnerability factor in developing drug abuse.

In the present research several measures of cocaine-seeking behavior were employed: a Go/No-go procedure (a measure of impaired inhibition) and a multi-component reinstatement procedure consisting of drug-, cue-, and stress-induced priming conditions. Additionally, a delay discounting task (a measure of impulsive choice) was used to examine impulsivity with food reinforcement. For the impulsivity measures, the results indicated that cocaine-seeking behavior was more prevalent in adolescents than adult rats under the Go/No-go procedure and that adolescents were more impulsive than adults in the delay discounting task for food reward. Furthermore, adolescents exceeded adults in responding after cocaine and yohimbine (stress-inducing) primes during the reinstatement procedure while adults responded more (vs. adolescents) after exposure to drug-associated cues. The

results of this research suggest that several aspects of drug-seeking behavior contribute to adolescents' vulnerability to drug abuse, and that impulsivity may be a major contributor.

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**REACTIVITY OF HETEROBIMETALLICS WITH (ALLYL)GROUP X METAL FRAGMENTS BRIDGED BY (2-(DIPHENYLPHOSPHINO)ETHYL)CYCLOPENTADIENYL GROUP VI METAL TRICARBONYL METALLOLIGANDS TOWARD ORGANIC RADICALS**

Michelle C. Neary, Aaron P. Heerboth, and Paul J. Fischer (Advisor)

*Department of Chemistry*

*Macalester College, St. Paul, MN*

Heterobimetallic complexes with metal-metal bonds supported by bridging donor-functionalized cyclopentadienyl ligands facilitate reactions at metal-metal bonds that would likely result in homolytic or heterolytic M-M' bond cleavage without the kinetic stabilization afforded by the bridging ligand.

Recently reported reactions of neutral allylpalladium(II) complexes with free radicals resulting in reductive elimination of alkenes prompted us to synthesize heterobimetallics with (allyl)M fragments (M = Ni, Pd) bridged by (2-(diphenylphosphino)ethyl)cyclopentadienyl group VI metal tricarbonyl metalloligands. Syntheses of (allyl)M complexes containing chromium, molybdenum, and tungsten are reported. The formulations of these complexes as heterobimetallics containing group VI-group X metal-metal bonds supported by bridging (2-(diphenylphosphino)ethyl)cyclopentadienyl ligands were established in the solid-state by single crystal X-ray crystallography. Reaction of these compounds with phenylazotriphenylmethane (PAT) produced only triphenylmethylallyl; all phenyl radical was consumed as biphenyl. A heterobimetallic effect appeared in the percent conversions as quantified by GC-MS; the chromium compounds offered the highest conversion, followed by tungsten and molybdenum.

**SELECTION OF RESISTANT BACTERIA WITHIN AN ENVIRONMENTAL COMMUNITY IN RESPONSE TO LOW-LEVEL EXPOSURE TO TRICLOSAN**

Jo Jo A. Nemeč, Louis H. Sigtermans, Aaron D. Gronseth, Abby H. Schutt, and Kristine H. Wammer (Advisor)

*Department of Chemistry*

*University of St. Thomas, St. Paul, MN*

The emergence of bacterial strains resistant to antibacterial compounds is a growing concern worldwide. Because of the possibility of resistance genes transferring between bacterial species, the rise of resistance in environmental bacteria is of interest.

This study explored the effect of exposure to low levels of triclosan (a widely used antibacterial agent found in many consumer and personal health-care products) on the selection of resistant bacteria in environmental communities. The first main objective was to determine current resistance levels to triclosan among bacterial communities collected from several natural water sources that vary in their land use and human impact. Bacterial samples from the Mississippi River, California coast, and Lake Superior were grown on solid media spiked with varying triclosan concentrations. Resistance levels did not vary significantly when bacteria obtained from highly impacted areas were compared with those from more pristine areas. The main second objective was to observe the impact of low concentrations of triclosan on bacterial communities over time. Bacteria collected from a natural water source were grown in bioreactors known as chemostats, exposed to low concentrations of triclosan, and tested periodically. Resistance was monitored by growing samples of bacteria from the chemostats in media containing lethal levels of triclosan. The bacteria were also analyzed for community composition changes over time via Automated Ribosomal Intergenic Spacer Analysis (ARISA). Our results indicate that significant community shifts are likely to be observed only with exposure to triclosan concentrations well above those observed to date in natural waters.

**A COMPARISON OF TWO METHODS FOR ISOLATION OF CD4+ T CELLS: YIELD, PURITY, AND FUNCTION OF ISOLATED CD4+ CELLS**

Marin Olson, Andrew Van Bruggen, and Marina Cetkovic-Cvrlje (Advisor)

*Department of Biological Sciences*

*St. Cloud State University, St. Cloud, MN*

T cells are implied in immunopathogenesis of autoimmune diseases, such as autoimmune type 1 diabetes. There are three major types of T cells: T helper (Th), T cytotoxic (Tc), and T regulatory (Tregs). Each T cell type is defined by its unique immunophenotype (cell surface markers). The cells are characterized by expression of CD4, while Treg cells contain CD25 as well as CD4 markers. In order to study the role of particular T cells type in development of disease, it is prerequisite to separate those T cells from the entire cell population. Our current studies have been focused on finding the optimal method for isolation of CD4+ T cells. We have used the widely accepted Myltenyi Biotec (MB) method for positive magnetic selection of mouse CD4+ T cells. The preliminary data (n=8 separations) showed that the purity of CD4+ cells obtained by MB method from the mouse

splenocytes was only  $87\pm 7\%$ , which was lower than expected. We are currently testing a different, StemCell Technologies (SCT) method for positive magnetic isolation of CD4+ T cells. Both MB and SCT methods will be used in parallel to isolate CD4+ cells from the splenocytes of C57BL/6 mice. The yield of isolated cells will be evaluated by comparing the number of isolated versus the initial cell number. The purity of CD4+ population will be tested post isolation by MB and SCT method by staining with anti-CD4 antibody. Finally, the function of isolated cells will be tested in proliferation assay by culturing cells with addition of anti-CD3 plus anti-CD28 antibodies.

#### **DNA-BASED ANALYSIS OF DIET OF TIMBER RATTLESNAKES (*Crotalus horridus*)**

Cory Ostreet

*Department of Biology*

*Saint Mary's University, Winona, MN*

Timber rattlesnakes (*Crotalus horridus*) are geographically widespread in the eastern United States but have declined throughout much of their range and are considered a threatened species in Minnesota. There is little information on the diet of timber rattlesnakes in Minnesota. Although some information can be obtained by gross examination of gut contents through dissection or palpation, identification of prey can be difficult during the later stages of digestion.

The objective of this study was to extract 16S mtDNA from fecal samples of *C. horridus*. The fecal samples were obtained from nuisance snakes during handling by Winona County rattlesnake responders. The mtDNA was digested with the restriction endonuclease *HindIII* in order to cleave predator DNA. Universal PCR primers were designed to anneal to a fragment of the 16S mtDNA of 27 prey species. As a control, primers were designed to amplify a small region of the 16S mtDNA of purified mouse DNA. The PCR-amplified prey DNA was cloned, sequenced and compared to nucleotide sequences deposited in the GenBank nucleotide database using the BLAST algorithm.

Recovered sequences showed 90% identity to voles of the genus *Microtus*. This is consistent with the results of the dissection of five Winona County rattlesnakes that were killed by cars or other anthropogenic causes. By running their gut contents through a series of sieves to recover teeth and bone fragments, it was possible to show that four snakes contained the remains of eight voles (one snake was empty).

#### **THE EFFECTS OF THE GLUCOCORTICOID DEXAMETHASONE ON SURFACTANT PROTEIN C EXPRESSION IN *Xenopus laevis* LUNG DEVELOPMENT**

Nathaniel Paulson and Brian Hyatt (Advisor)

*Department of Biological Sciences*

*Bethel University, St. Paul, MN*

Dexamethasone is a synthetic glucocorticoid commonly used in a treatment for pregnant women with a high chance of having a premature birth. Dexamethasone treatment has been shown to increase the maturation process of the infant's lungs and one result of that maturation is increased surfactant production. Dexamethasone's effects on lung development have been studied in many species, however little research has been done on *Xenopus laevis*, and none examining the effect of dexamethasone on lung maturation.

In this study *Xenopus laevis* (African Clawed frog) tadpoles were treated with dexamethasone concentrations of  $2 \times 10^{-4}$  M and  $2 \times 10^{-5}$  M by dissolving it in their growth media. Dexamethasone's effect on lung development was determined by measuring levels of surfactant protein C expression as an indicator of lung maturation using real-time PCR. Analysis of surfactant protein C gene expression fold differences indicated they were not statistically significant. This result provides a reference point for future research methods and techniques testing the effects of dexamethasone of surfactant protein C expression.

#### **HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF INSULITIS LESION IN C57BL/6 MICE WITH AUTOIMMUNE DIABETES**

Sumeet Poudel, Sunny D. Joshi, and Marina Cetkovic-Cvrlje (Advisor)

*Department of Biology*

*St. Cloud State University, St. Cloud, MN*

Insulinitis is a characteristic histopathologic lesion of autoimmune type 1 diabetes (T1D). It is characterized by the accumulation of so-called mononuclear cells inside and outside of the pancreatic islets of Langerhans. It actually reflects destruction of pancreatic insulin-producing beta cells by the immune cells such as T cells and macrophages. Different stages of insulinitis reflect different levels of islets' destruction [from stage 0 (normal islet) to 4 (completely destroyed islet)]. Higher level of destruction and higher insulinitis level are expected in mice that are closer to becoming diabetic.

In this study, insulinitis lesions were followed in 3-4-month-old C57BL/6J male mice where autoimmune T1D was induced by administration of low doses of chemical streptozotocin (n=25 mice). The control mice, of the same strain, sex, and age, received vehicle only (n=25). The insulinitis stages of STZ-treated and control

mice were studied at several time points post injection of streptozocin (day 7, 14, 21, and 28). The mice were sacrificed, pancreata removed, fixed in formalin, embedded in paraffin, sectioned, and stained by hematoxylin and eosin stain (H&E). Insulinitis was graded (stages 0, 1, 2, 3, and 4, based on the level of mononuclear cell infiltration) in three different H&E-stained sections of the pancreas/mouse. In conjunction to this, an additional section of pancreas was immunostained with insulin antibodies in order to detect the amount of remaining insulin-positive beta cells. This study allowed us to gain knowledge about the histopathological changes in mice that progress from normal to diabetic stage in STZ-induced model of T1D.

### SYNTHESIS OF GIP-CVIA

Rachel Pricer, Dan Mullen, and Mark Distefano  
*Department of Chemistry*  
*University of Minnesota, Minneapolis, MN*

Glucose-dependent insulinotropic polypeptide (GIP) is a 42-amino acid polypeptide under research for diabetes treatment. GIP is produced by endocrine K cells to regulate glucose production by targeting beta cells to increase insulin secretion after nutrient intake. Two disadvantages for use of this polypeptide as a treatment include a lifetime of 3-5 minutes in the bloodstream due to proteolytic degradation by dipeptidylpeptidase-IV (DPP-IV) and clearance from the kidney tubules within 20 minutes of secretion. A PEG addition has been proven to lead to increased kidney tubule retention as well as a decrease in enzymatic degradation by DPP-IV. A new method to attach PEG groups to GIP is by the action of farnesyltransferase (FTase) using bioorthogonal chemistry. FTase is able to add prenyl groups to peptides if CVIA, a CAAX box, is present.

The proposed reaction is to add a prenyl group with a terminal azide and react this compound with an alkyne attached to PEG. Modifying the polypeptide this way allows selective enzymatic modification with a tag of only four residues. Synthesis of GIP-CVIA has been successfully completed to utilize for the proposed reactions. Solid-phase peptide synthesis with double coupling reactions was used for synthesis. The peptide was purified using high-performance liquid chromatography and analyzed with mass spectroscopy. The analysis showed the product contained GIP-CVIA as well as a deletion peptide (M-283). Presently, ms/ms sequencing analysis is being used to identify the deletion peptide and further confirm the GIP-CVIA synthesis.

### FACTORS AFFECTING THE EXPRESSION OF 9,13-HYDROPEROXIDE LYASE IN CUCUMBERS

Samee M. Ranginwala, Ashok S. KC, and James E. Rife (Advisor)  
*Department of Chemistry and Geology*  
*Minnesota State University, Mankato, Mankato, MN*

Cucumbers (*Cucumis sativus*) produce 6- and 9-carbon-long aldehydes, which not only are important flavor and fragrance compounds but may also play roles in the plant's defense against pathogens. These aldehydes are produced from hydroperoxides of polyunsaturated fatty acids. Cucumbers apparently have a unique 9,13-Hydroperoxide Lyase (9,13-HPLase) that can cleave hydroperoxides at either the 9 or 13 positions on the fatty acid chain (Matsui, et al. *Phytochemistry* 67, 649-657, 2006). Matsui's group reported that mechanical wounding enhanced 9,13-HPLase activity. The objective of the current project was to develop a Quantitative Polymerase Chain Reaction (q-PCR) method to further investigate the effect of wounding on the expression of 9,13-HPLase. Cucumber plants were grown, and control and experimental samples were obtained from cotyledons as well as from primary and secondary leaves. RNA was extracted from the samples and reverse transcription was used to make cDNA copies of the mRNA transcripts. The relative amounts of the cDNA were measured using q-PCR. No significant differences in expression of 9,13-HPLase mRNA was observed in cotyledons, primary leaves, and secondary leaves of control plants. Wounding of cotyledons may slightly enhance expression in the wounded tissue, but the effect is not systemic. Wounding of primary leaves causes a more significant enhancement, which might have a slight systemic effect.

### CONSEQUENCE OF REESTABLISHMENT OF A SWIFT RIVER CHANNEL FOLLOWING RESERVOIR DRAWDOWN AT THE BOARDMAN RIVER, TRAVERSE CITY, MI

Nathan Sather<sup>1,2</sup>, Dr. Dave Mahan (Advisor)<sup>2</sup>, Garrett Crow (Advisor)<sup>2</sup>, Hal Reed (Advisor)<sup>2</sup>, and Ken Petersen (Advisor)<sup>1</sup>

*Department of Biology*

<sup>1</sup>*Bethel University, St. Paul, MN*

<sup>2</sup>*Au Sable Institute of Environmental Studies, Mancelona, MI*

The Boardman River is a groundwater-fed stream that meanders through Michigan's Grand Traverse and Kalkaska Counties. The Boardman and Sabin dams are two of four dams that impede the Boardman's flow and due to safety concerns are in the process of removal. The Boardman Dam Reservoir was lowered 17 feet in 2007, resulting in the reestablishment of river channel upstream. Research indicates that dams negatively affect stream ecosystems by increased sedimentation and alteration of natural habitat.

The purpose of this study was to characterize the macroinvertebrate community and observe differences between an undisturbed riffle (Shumsky Site), a newly developed riffle at the head of the old reservoir (Lone Pine Site), and at a riffle below a dam (Sabin Dam Site). Additionally, samples were compared with those taken in a similar 2008 study to determine the amount of recovery in the reestablished river channel at the Lone Pine Site. Macroinvertebrates were identified to lowest possible taxonomic level and distinguished by morphospecies.

Each of the three sites was found to be taxonomically distinct in its macroinvertebrate community composition. A total of 409 individuals were identified. Shumsky had 35 observed morphospecies, Lone Pines had 25 morphospecies, and Sabin Dam had 43 morphospecies. A comparison of the recovering site with the undisturbed, natural site using Sørensen's Quotient of Similarity indicated that the Lone Pine Site has not fully recovered, but has shown a considerable increase in similarity (40%) when compared with 2008 analysis (17.8%). Additionally, an improvement in richness of indicator species was observed.

#### PERFORMANCE UNDER A GO/NO-GO TASK FOR IV COCAINE OR FOOD IN ADOLESCENT AND ADULT RATS

Amy T. Saykao, Sean Navin, Justin J. Anker (Advisor), and Marilyn E. Carroll (Advisor)

*Department of Psychiatry*

*University of Minnesota, Minneapolis, MN*

Several factors put adolescents at greater risk for substance abuse compared with adults. For example, adolescents have a greater sensitivity to the reinforcing effects of drugs, are less sensitive to their aversive effects, and are more impulsive than adults.

The purpose of this study was to compare adolescent and adult rats on the Go/No-go procedure for food and iv cocaine reinforcement. Adolescents (25-65 days) and adult (90 days) male Wistar rats were trained to lever press for iv infusions of cocaine (0.4 mg/kg) or food pellets under a Go/No-go task. The procedure was composed of three 45-minute Go periods in which active lever presses were reinforced with cocaine. After the first and second Go component were two signaled 15-minute No-go periods when responses did not result in cocaine reinforcement. The results of the study indicated that adolescent rats earned more iv cocaine infusions compared with adult rats during the Go period. Adolescents also responded more in the No-go component, indicating that impulsivity was greater in adolescents than in adults. During the Go period for food reinforcement, adult rats responded more and earned more food pellets than adolescent rats. However, there were no significant group differences in responding during the No-go period. These results suggest that adolescent rats self-

administer more cocaine than adults and are more impulsive for cocaine reward than adults.

#### SOY-PROCESSING PLANT EFFLUENT EXHIBITS ESTROGENIC ACTIVITY *in vitro* AND FEMINIZES MALE FISH

Sara Seidl, Evan Eid, Pat Zawadski, Ryan Lill, Dustin Potter, Adam Johannsen, John Yang, and Dalma Martinovic-Weigelt (Advisor)

*Department of Biology*

*University of St. Thomas, St. Paul, MN*

Industrial effluents can contain a variety of estrogenic chemicals, which have potential to disrupt fish reproduction and development. This study focused on the use of an *in vitro* bioassay with estrogen-dependent human breast cancer cells stably transfected with luciferase reporter gene construct under control of triplet estrogen response elements (T47D-Kbluc cells) to detect and quantify total estrogenicity of a soy-processing plant waste effluent. The results of the *in vitro* T47D-Kbluc assay were compared with the induction of estrogenic gene transcription in male fathead minnows (*Pimephales promelas*) following *in vivo* exposure to the same effluent.

A grab sample of effluent for the paired *in vitro* and *in vivo* studies was collected in November 2010. The sample was concentrated and fractionated with varying concentrations of methanol (25-100%) using solid phase extraction C18 columns. Estrogenicity of the effluent fractions 75-100% as determined in the T47D-Kbluc assay was about 100 17 $\beta$ -estradiol equivalents (ng/L). Male fathead minnows exposed to the most estrogenic fraction of the effluent (75% methanol) for 48 hours showed a significant increase in expression of hepatic vitellogenin relative to controls. The *in vitro* and *in vivo* results both support the conclusion that the effluent released by the soy-processing plant contains significant estrogenic activity.

#### SYNTHESIS OF NOVEL LUMINOL DERIVATIVES

Nicholas A. Serratore and J. T. Ippoliti (advisor)

*Department of Chemistry*

*University of St. Thomas, St. Paul, MN*

The goal of this research was to synthesize and characterize new luminol derivatives. The purpose of making these luminol derivatives was to replace the NH<sub>2</sub> group of luminol with different-sized nitrogen containing rings and characterize the effects on the chemiluminescence of the molecule. N-methylphthalimide was used as the starting material for the synthesis of multiple luminol derivatives. The process of creating these derivatives began by first monobrominating the highly deactivated arene ring of N-methylphthalimide using HgO as a catalyst. This

brominated compound was then coupled with different-sized rings that contained secondary amines by means of a Buchwald-Hartwig palladium-driven coupling. The compounds were then converted to the luminol derivatives using hydrazine and high heat. The compound was then isolated and reacted with hydrogen peroxide in the presence of an iron catalyst to give the chemiluminescent reaction. The intensity of the chemiluminescence for each derivative was compared with that of luminol.

### CRYSTAL STRUCTURE AND METAL-METAL BOND IN AN ACTINIDE-GROUP 14 COMPLEX

Carly Schulz

*Department of Chemistry*

*University of Minnesota, Minneapolis, MN*

Discovering the properties and electronic nature of new bimetallic compounds is computationally challenging but very useful in such aspects as chemical bonding, catalysis, and structure. Not much is known about metal-metal bonds and electronic structure of the f-elements of the periodic table. It is important to study actinides, lanthanides, and transition metals because the novel bonding natures aide in the design of more efficient catalytic cycles. Incorporating heavy metals from the bottom of the periodic table into new catalytic cycles will more efficiently remove nuclear waste.

Quantum chemical methods were employed to investigate many metal-metal bonded compounds. Among this set, a uranium-tin compound,  $[(^5\text{-C}_5\text{H}_5)_3\text{U}(\text{SnPh}_3)]$ , was focused on. Uranium, an actinide, has some unique orbital characteristics. Determining the electronic configuration and bonding nature of this element (atomic number 92) will aid in the determination of synthesis, properties, and reactivities of this and many similar compounds with bimetal bonds in f-element chemistry. The  $[(^5\text{-C}_5\text{H}_5)_3\text{U}(\text{SnPh}_3)]$  structure was optimized and compared with an experimental structure to assess the geometry optimization parameters used. Then, the nature and bond length of the U-Sn bond was analyzed. The properties of certain basis functions were also compared. It was predicted that the optimized geometry of the particular  $[(^5\text{-C}_5\text{H}_5)_3\text{U}(\text{SnPh}_3)]$  structure would be pseudo-tetrahedral at both the U and Sn centers. Filling in these gaps will open a great number of doors to create new metal-metal compounds resulting in endless applications.

### ANALYSIS OF THE ANTIBACTERIAL PROPERTIES OF TETRACYCLINE PHOTOPRODUCTS USING *Vibrio fischeri* BIOLUMINESCENCE AND GROWTH TESTS

Matthew T. Slattery, Amanda M. Stemig, and Kristine Wammer (Advisor)

*Department of Chemistry*

*University of St. Thomas, Saint Paul, MN*

The presence of antibiotics in the environment is of concern due to the potential for selection of resistant bacteria. Tetracycline is one of many antibiotics that have been detected at low concentrations in natural waters.

In this study the photoproducts generated by photodegradation of tetracycline under environmentally relevant conditions were examined to determine their potential environmental significance. Tetracycline is known to degrade by direct photolysis into at least seven different photoproducts under varying conditions. Water hardness and pH are two characteristics of a natural environment that can alter the decomposition pathway of tetracycline. Here, both growth inhibition and luminescence inhibition of the bacteria *Vibrio fischeri* were measured in the presence of varied concentrations of both photolyzed and unphotolyzed tetracycline to determine the potential antibacterial activity of its photoproducts in diverse waters and conditions. In all cases studied to date, we have determined that the photoproducts retain no significant antibacterial activity.

### RELATION BETWEEN GENE SIGNALING EVENTS IN HIV-1 INFECTION AND CANCER PROLIFERATION

Nicole L. Soiseth, Laura Secor, and Sanjive Qazi

(Advisor)

*Department of Biology*

*Gustavus Adolphus College, St. Peter, MN*

Cancer is a significant cause of mortality and morbidity in people infected with HIV-1, with up to 40% developing a malignancy during their lifetime. We analyzed gene expression obtained from Affymetrix genechips to measure the effect of five distinct drug-resistant HIV-1 strains in CD4+ MT2 T-lymphoblastoid cells treated for 24 and 48 hours. Two-way cluster analysis of 397 genes whose expression was significantly different across viral treatment groups showed two groups of viral responses: HIV-1<sub>A17</sub>/HIV-1<sub>A17V</sub> and HIV-1<sub>RT-MDR</sub>/HIV-1<sub>HTLVIIIIB</sub>/HIV-1<sub>92BR019</sub>.

We employed a pathway analysis using the Kegg compendium which showed two sets of distinct responses. Differentially expressed genes were highly enriched in known cancer pathways such as chronic myeloid leukemia. Certain gene sites within these pathways have been targeted by drugs such as Imatinib and 1-Naphthyl for an anti-cancer effect. We investigated the known anti-cancer drug targeting sites for implications in the connection of gene signaling events between HIV infection and cancer proliferation.

## HYDROGEN BONDING IN CARBOHYDRATE DERIVATIVES: CRYSTAL STRUCTURE OF A GLUCOSE CYANOPHENYLHYDRAZINE

Leah R. Streitman and William H. Ojala (Advisor)

*Department of Chemistry*

*University of St. Thomas, St. Paul, MN*

Our interest in solid-state hydrogen bonding has led us to conduct a crystallographic study using single-crystal X-ray diffraction of derivatives of monosaccharides, perhaps the ultimate in hydrogen-bonded solids. Reaction of a monosaccharide with a nitrogenous base can yield an open-chain derivative (a Schiff base) or a cyclic derivative (a glycosylamine). We are examining base/monosaccharide combinations to determine in each case which derivative is obtained as the crystalline product and to examine the hydrogen-bonding scheme assumed in the solid state.

Previous work has demonstrated that the particular derivative obtained varies with the monosaccharide used and the reaction conditions employed. Here we describe the crystal and molecular structure of the glycosylamine formed by reaction between D-glucose and 4-cyanophenylhydrazine. Given the strong and favorable arrangements of H-bonds found in the crystal structures of monosaccharides, the H-bonding role (if any) of the nitrile group, a relatively weak H-bond acceptor, was of interest. We have found that in the crystal structure of this derivative, the hydrophobic cyanophenyl moiety bridges hydrophilic glucopyranosyl regions of the crystal through a hydrogen bond between the nitrile group and the O-2 hydroxyl of a neighboring molecule. The nitrile group is thus not entirely isolated from the array of hydroxyl group H-bond donors and acceptors but is in fact an integral part of it.

## X-RAY CRYSTALLOGRAPHY AND SOLID-STATE LUMINESCENCE OF ORGANOMETALLIC PLATINUM(II) COMPLEXES

Sarah S Sullivan<sup>1</sup>, Daron E Janzen (advisor)<sup>1</sup>, and Kent R Mann<sup>2</sup>

<sup>1</sup>*Department of Chemistry and Biochemistry  
St. Catherine University, St. Paul, MN*

<sup>2</sup>*Department of Chemistry*

*University of Minnesota, Minneapolis, MN*

Molecular materials were synthesized for potential use as solid-state sources of luminescence. We have successfully synthesized and characterized organometallic square planar platinum (II) complexes of the form  $[\text{Pt}(\text{C}^{\wedge}\text{N})(\text{CNCH}_3)_2](\text{BF}_4)$  (where  $\text{C}^{\wedge}\text{N}$  = ppy or bq, ppy = 2-phenylpyridinate; bq = 7,8-benzoquinolate). Methods of characterization to verify the composition and connectivity of these materials included NMR, IR, mass spectroscopy, solid-state and solution emission, X-ray crystallography, and solution

UV-Vis spectroscopy. Data were compared with a series of previously synthesized complexes of the form  $[\text{Pt}(\text{bq})(\text{CNR})_2](\text{BF}_4)$  ( $\text{R} = i\text{-Pr, Xylyl, p-C}_6\text{H}_4\text{-C}_2\text{H}_5$ ). A single-crystal X-ray structure obtained for  $[\text{Pt}(\text{ppy})(\text{CNCH}_3)_2](\text{BF}_4)$  shows close intermolecular Pt-Pt interactions (3.47-3.54Å) and close Pi-Pi interactions (ppy-ppy and ppy-CNCH<sub>3</sub>). The choice of the isonitrile ligand and physical state of all the newly synthesized compounds (crystal, film, or powder) dramatically affects the emission wavelength depending on the intermolecular interactions present.

## A COMPARISON OF *in vitro* T CELL POTENTIAL IN NU/NU AND WT COMMITTED T CELL PROGENITORS

Zachary Schwager, Marcos Garcia-Ojeda, and Devavani Chatterjea (Advisor)

*Department of Biology*

*Macalester College, St. Paul, MN*

Notch signaling is a highly conserved transduction system known to influence cell specialization during embryonic and adult life. Recently, Notch signaling has been implicated in directing immature hematopoietic cells toward the T cell fate *in vivo*, and multipotent leukocyte precursors cultured with Notch ligand have been shown to produce only T cells.

Our experiment compares the ability of Committed T cell Progenitors (CTPs) from the bone marrow of wild type and T cell deficient nude mice ( $\text{FOXN1}^{-/-}$ ) to differentiate into CD3-positive T cells when exposed to Notch signals *in vitro*. CTPs represent the earliest bone marrow population displaying commitment toward the T cell lineage and defective, Notch-signal-insensitive CTP populations may account for the paucity of T lymphocytes in nude mice. Sustained, high levels of Notch signal in the cellular microenvironment were achieved through co-culture of precursor cells with OP9-DL1 cells, a bone-marrow-derived stromal cell line that ectopically expresses the Notch ligand Delta-like 1. Results indicate that WT CTPs are significantly more robust in their potential to produce T cells in the presence of DL1 ligand. Nude CTPs gave rise to relatively few CD3-positive cells. This result may indicate a Notch related, pre-thymic defect as the cause of T cell deficiency in nude mice.

## SEED VARIATION IN LARGE-LEAVED AVENS: EFFECTS ON FITNESS AND DISPERSAL

Robin Tellefson, Roshni Shah, and Craig Longtine (Advisor)

*Department of Biology*

*North Hennepin Community College, Brooklyn Park, MN*

Large-leaved avens (LLA), *Geum macrophyllum*, is a herbaceous perennial found in moist

woods and meadows. LLA produces spherical infructescences that contain several hundred fruits. Each mature fruit is a seed with an elongated style, forming a burr with a single hook that can adhere to animal hair to facilitate long-distance seed dispersal. Number of seeds and, potentially, size of seeds is a crucial aspect of plant fitness. Variation in burr morphology may influence seed dispersal.

In this study, we determined the number of infructescences per plant, as well as number of fruits (hereafter, seeds) per infructescence, and mass of infructescences and individual seeds in a natural population of LLA. About 90% of plants had three to five infructescences, but a few plants had up to eight infructescences. The mean number of seeds and mean seed mass in an infructescence was independent of plant size or number of infructescences. Number of seeds was proportional to the number of infructescences. The size of individual seeds may also affect plant fitness. At the population level, seed mass was normally distributed and varied 10-fold. Heavier seeds had a significantly higher germination rate and grew more quickly, but time to germination was independent of seed mass. In the population, large seeds were uncommon. Burr length was weakly correlated with seed length. The size of the burr's hook was independent of seed size or burr length. The characteristics of LLA seeds that increase the probability of attachment to animal fur will also be discussed.

#### **THE ROLE OF BISON IN PRAIRIE RESTORATION: A LOOK AT HOW THE PRESENCE OF BISON IMPACTS SOIL NUTRIENTS**

Alouisa Thames and Gina Mancini-Samuelson (Advisor)  
Department of Chemistry  
St. Catherine University, St. Paul, MN

It is important to fully understand the impact bison have on prairie ecosystems because bison play an important role in prairie health. The aim of this research was to better understand the bison's role in prairie restoration, in regard to their impact on soil nutrient content. This research was conducted at the Belwin Conservancy in Afton, Minnesota. The pH, phosphorous, nitrogen, and potassium content of soil samples from the Croixview site at Belwin Conservancy, where buffalo have been introduced, were compared with that of soil samples from a plot across from the Croixview area where no buffalo have been introduced. A soil composition analysis was also performed and buffalo wallows were also analyzed.

It was found that potassium levels within each plot vary, displaying a patchlike pattern of high/low potassium. The data also showed that nitrogen is the "limiting reagent" of prairies as all of the plots analyzed had very little nitrogen.

The wallows had higher phosphorous and potassium levels compared with the other plots sampled. These higher nutrient levels may be due to the compact nature of wallow soil. The higher nutrient concentrations in the wallows could also be due to their lack of vegetation as there was no plant life growing in the wallows to take up the nutrients from the soil.

#### **SURFACE ANALYSIS OF MP35N CARDIAC STENTS**

Brett Theisen and Kenneth Rohly (Advisor)  
Department of Chemistry  
Bethel University, St. Paul, MN

MP35N is a metal commonly used in implantable medical devices, from the coiled wires used in leads to rods and screws used for spinal correction to cardiac stents. MP35N is an alloy composed of 35% nickel, 35% cobalt, 20% chromium, and 10% molybdenum and, like stainless steel, is corrosion resistant and strong but has improved hardness. In addition to many others, annealing, passivation, and electropolishing are processing steps applied to MP35N to improve mechanical properties and surface finish. The focus of this work is to evaluate the effect of these processing techniques on MP35N surfaces as evaluated by X-ray photoelectron spectroscopy, scanning electron microscopy, and white-light interferometry.

#### **ANTIBIOTICS AND THEIR EFFECTS ON THE ELECTRON TRANSPORT SYSTEM OF *Brassica oleracea* var. *botrytis***

Phillip Thomas  
Department of Biology  
Saint Mary's University of Minnesota, Winona, MN

There are antibiotics designed to specifically target bacterial ribosomes. However, due to the endosymbiotic theory that mitochondria were originally prokaryotes, both mitochondria and bacteria have very similar ribosomes. Due to this, many antibiotics are unable to distinguish between bacterial and mitochondrial ribosomes and thus are known to inhibit protein synthesis in mitochondria. Very little research has been done on the effects of the electron transport system, a vital part of cell function, as a result of disruption of protein synthesis or even as a result of the antibiotics themselves. There are many proteins involved throughout the process of cellular respiration and many integral membrane proteins reside within the mitochondrial membranes. Therefore, if the protein synthesis is being disrupted, there is a possibility that the proper electron carriers and membrane proteins are not being produced. Both of which are necessary for cellular respiration to occur. There is also the possibility that the antibiotics themselves are disrupting more than just protein synthesis within the mitochondria. For this

project, the isolated mitochondria were bathed in Tetracycline, Ampicillin, or Kanamycin and electron transport function was measured by testing succinate dehydrogenase activity using DCIP and allowing for a color change to be observed. The results were analyzed using the student's t-test and it was determined that Kanamycin had a statistically significant effect on the ETS while Tetracycline only showed a statistical trend and Ampicillin had no statistically significant effect on the ETS.

### **REINSTATEMENT OF DRUG-, CUE-, AND STRESS-INDUCED COCAINE-SEEKING BEHAVIOR IN ADOLESCENT AND ADULT FEMALE RATS**

Rachael M. Turner, Marilyn E. Carroll (Advisor), and Justin J. Anker (Advisor)  
*Department of Psychiatry*  
*University of Minnesota, Minneapolis, MN*

Due to several factors including increases in sex hormones, adolescence is a critical period for vulnerability to drug abuse. However, the underlying mechanisms of substance abuse in adolescents are still unknown.

The purpose of this study was to compare drug-, cue-, and stress-induced relapse of cocaine-seeking behavior in female adolescent- and adult rats and to examine the role of ovarian hormones in reinstatement. Female adolescent (25 days of age) and adult (90 days of age) Wistar rats were trained to lever press for i.v. infusions of cocaine. Rats then entered a five-day maintenance phase consisting of two, two-hour daily sessions. Subsequently, a ten-day extinction phase commenced, during which all visual and auditory stimuli associated with the drug were disconnected. After extinction, a five-day reinstatement phase began during which rats were tested on cocaine-, cue-, and yohimbine (stress)-induced reinstatement of cocaine-seeking. Estrous cycle phases were not controlled for but were monitored by daily swabbing. Adolescents exhibited increased cocaine seeking during maintenance compared with adults. No significant differences between adolescents and adults were observed during extinction, reinstatement (cocaine-, cue-, and yohimbine-induced), or estrous cycle phases. These results suggest that female adolescents (compared with adults) have a heightened vulnerability to self-administer cocaine, and they may also be vulnerable to withdrawal and relapse.

### **PURIFICATION AND SIGNALING EFFECTS OF AN ANTI-INSULIN RECEPTOR ANTIBODY, $\alpha$ IR1, ON BREAST CANCER CELLS**

Ryan R. Uselman, Hua Zhang (Advisor), and Douglas Yee (Advisor)  
*Department of Medicine*  
*Department of Pharmacology*  
*University of Minnesota, Minneapolis, MN*

Recent work from our laboratory suggests that insulin receptor (IR) plays an important role in breast cancer cell proliferation and metastasis, making it a promising target for breast cancer treatment. About 20 years ago, a mouse monoclonal anti-IR antibody,  $\alpha$ IR1, was developed; however, its anti-cancer effect has not been well characterized.

In this study, the mouse hybridoma expressing  $\alpha$ IR1 was grown in various media and studied for the production of antibody. Supernatant of the media was analyzed by SDS-PAGE and Western blotted with an anti-mouse antibody. Optimal conditions to grow the hybridoma were determined by the highest levels of  $\alpha$ IR1 in the supernatant. After culture,  $\alpha$ IR1 was isolated from the supernatant using a Protein G column and resulted in 81% recovery of  $\alpha$ IR1 monoclonal antibody. In order to determine the effect of  $\alpha$ IR1 antibody on breast cancer cell signaling, MCF-7 breast cancer cells were treated with  $\alpha$ IR1 for 24 hours. Treatment with  $\alpha$ IR1 effectively downregulated the level of insulin receptor. When MCF-7 cells were treated with insulin, adaptor molecules such as insulin receptor substrate (IRS1/2) were phosphorylated, leading to the activation of several downstream signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway, and the Akt pathway. Pretreatment of cells with  $\alpha$ IR1 resulted in attenuated phosphorylation of IRS1/2, Akt, and MAPK. Therefore, our results suggest that  $\alpha$ IR1 effectively downregulates the level of IR, and inhibits insulin-mediated downstream signaling pathways. This antibody will be used in future studies to examine its effects on breast cancer cell proliferation and motility.

### **DNA-TEMPLATED POLYMERIZATION VIA MODIFIED MONONUCLEOSIDES**

Mary Velasco, Sarah Anderson, Kaitlyn Ohden, and Trey Maddox (Advisor)  
*Department of Chemistry*  
*Bethel University, Saint Paul, MN*

The purpose of this research is to study the process of DNA-templated polymerization in the absence of enzymes. Synthetic procedures have been developed for the synthesis of 3'-aldehyde modified mononucleosides, including thymidine, adenine, 5'-cytosine, and guanine. The modified thymidine mononucleoside has been used in DNA-templated polymerization wherein the length specificity and step-growth kinetics were studied and demonstrated

to have high fidelity. Although this synthesis and polymerization has been performed previously, the work presented here focuses on the optimization of a new synthetic route capable of synthesizing all four of the DNA nucleosides. Once optimized, the synthetic scheme will be applied to the other three nucleosides and studies will begin on the fidelity and step-growth kinetics of DNA-templated polymerization with the mononucleosides created via the new synthetic route.

#### **A NOVEL BIS-SULFIDE EN ROUTE TO THE SYNTHESIS OF 4,4-BIPYRAZOLYL**

Vladimir Vinnik and Dr. J. Thomas Ippoliti (Advisor)

*Department of Chemistry*

*University of St. Thomas, St. Paul, MN*

A new synthetic route was developed to produce 4,4-bipyrazolyl, a molecule potentially useful toward the research of metal-organic frameworks. These frameworks act as storage spaces for gasses such as methane and of particular interest, hydrogen. The prospect of hydrogen stored in metal-organic frameworks is exciting due to its real potential as a fuel source. The synthetic route starts with the reduction of a furan derived diester, Dimethyl-3,4-furandicarboxylate, to the diol, furan-3,4-diyldimethanol. The following step was a modified Swern oxidation of the diol to the dialdehyde, furan-3,4-dicarbalddehyde. The oxidation step was done using sulfur trioxide pyridine complex in dimethyl sulfoxide and triethylamine. It was in the optimization of the reaction conditions for the oxidation that an interesting discovery was made which lead to the identification of a novel bis-sulfide via NMR and crystallography. The reaction conditions appropriate for a high yield synthesis of the bis-sulfide are still being explored. The following steps en route toward 4,4-bipyrazolyl were the hydrolysis of the dialdehyde to the tetraldehyde, 1,1,2,2-Ethanetetracarboxaldehyde, which was subsequently reacted with hydrazine to form 4,4-bipyrazolyl.

#### **USE OF Q-PCR TO MONITOR EXPRESSION OF VEGETATIVE LIPOXYGENASE IN SOYBEANS**

Sarah Wageman and James E. Rife (Advisor)

*Department of Chemistry and Geology*

*Minnesota State University, Mankato, Mankato, MN*

Lipoxygenases (LOX) form hydroperoxides by adding oxygen to 1, 4-pentadiene systems in polyunsaturated fatty acids. Soybeans (*Glycine max*) have numerous LOX isoforms. At least three isoforms have been found in the seeds while at least seven isoforms have been reported in vegetative tissue. Several functions have been proposed for these enzymes, including production of defense molecules, lipid metabolism, and nitrogen storage. The focus of this project was to develop a Quantitative Polymerase Chain Reaction (q-PCR) method

that could be used to monitor mRNA levels of multiple vegetative lipoxygenases. This would provide a tool to measure variations in LOX expression in different tissues at different developmental stages under different environmental conditions. Primer sets have been successfully designed to selectively amplify LOX 5, 7, and 9 cDNAs. Total RNA was isolated from roots, stems, cotyledons, and leaves of plants. Reverse transcription was used to make cDNA copies of the mRNAs. Relative quantities of cDNAs in the samples were measured by q-PCR. Efficiency studies indicated that q-PCR could quantify these cDNAs over a 1,000-fold range. Melt curves signified that the primers successfully discriminated between the different LOX cDNAs. Distinct, reproducible patterns of expression of LOX 5, 7, and 9 were observed in cotyledons, leaves, stems, and roots of 10-day old plants.

#### **CHARACTERIZATION OF LRP10, QSOX1, AND ST14: MAPcL GENES THAT ARE HIGHLY EXPRESSED IN BREAST TUMORS**

Elizabeth Wanous and Kristi Eglund (Advisor)

*Sanford Medical Center*

*University of South Dakota, Vermillion, SD*

In the United States, one in eight women will be diagnosed with breast cancer during her lifetime. Alterations in gene expression can affect the development and progression of breast carcinomas.

The Eglund laboratory is actively pursuing the discovery of novel tumor-associated genes through the use of a membrane-associated polyribosomal cDNA library (MAPcL). The library was compiled from MAP RNA, which encodes membrane and secreted proteins, isolated from four breast cancer cell lines (MCF7, ZR-75-1, SK-BR-3 and MDA-MB-231), one normal breast cell line (hTERT-HME1), and one prostate cancer cell line (LNCaP). To remove ubiquitously expressed genes, the MAPcL was subtracted with brain, liver, kidney, lung, and muscle libraries. To determine what genes are represented in the MAPcL, 25,000 cDNA clones were sequenced. Because the MAPcL is enriched for genes that are highly expressed in cancer and have restricted expression in normal tissues, the encoded proteins are promising candidates as breast cancer diagnostic markers, immunotherapy targets, and prognostic indicators.

The genes LRP10, QSOX1, and ST14 are highly represented in the MAPcL. We investigated the expression of these three genes in the library cell lines, normal human tissues, normal breast tissue, and breast tumors from patients.

## THE SYNTHESIS OF A NOVEL OXAZOLIDINONE WITH A THIAZOLE GROUP

Carmen M. Weaver and J. T. Ippoliti (Advisor)

*Department of Chemistry*

*University of St Thomas, Saint Paul, MN*

Oxazolidinones are the most recent class of antibiotics that have shown to be effective against resistant gram-positive strains of bacteria. Linezolid, branded Zyvox, is currently the only commercially available oxazolidinone. We are interested in creating an oxazolidinone that will be effective against new strains of MRSA (methicillin-resistant *Staphylococcus aureus*) and also a broader spectrum of Gram-positive bacteria than Zyvox. Another new area of research has explored thiazoles, which also have been shown to have antimicrobial properties.

Previously, Dr. Tom Ippoliti and his students have synthesized novel compounds utilizing an oxazolidinone ring and thiazazole groups in replacement of the fluorobenzene of Zyvox. In continuation of the research regarding these two antibiotic components, the following nine-step synthesis results in a novel oxazolidinone derivative combined with a thiazole ring instead of a thiazazole ring, accompanied by an electron-donating phenyl methoxy group as a substituent. The effectiveness of the final product has been tested against methicillin-resistant *Staphylococcus aureus*, revealing that the novel oxazolidinone does not have antimicrobial properties at concentrations ranging from 0.5 ug/mL- 50 ug/mL.

## CHARACTERIZATION OF THE PROKARYOTE COMMUNITY OF *Sarracenia purpurea* PHYTOTELMATA BY 16S rDNA ANALYSIS

Robbyn Weaver<sup>1</sup>, Brandi Gossard<sup>2</sup>, and Paul P.

Melchior<sup>1,2</sup> (Advisor)

<sup>1</sup>*Department of Bioscience*

*Minnesota State University Moorhead, Brooklyn Park, MN*

<sup>2</sup>*Department of Biology*

*North Hennepin Community College, Brooklyn Park, MN*

*Sarracenia purpurea* L., the purple pitcher plant, is a native species found in North America bogs and fens. The modified leaves of this plant form pitchers that collect rainwater, which in turn capture arthropods as a supplemental source of nutrients. While there have been reports describing the bacterial and protozoan communities of this pitcher fluid (phytotelmata), little is known about the archaeal component of this unique ecosystem.

The diversity of archaeal populations has often been underestimated in ecosystem analyses. This is due in large part to the difficulty in culturing many archaeal species. However, microbiologists have begun to use molecular techniques to isolate and compare conserved

16S ribosomal RNA gene (rDNA) sequences to characterize these communities more accurately. Databases of rDNA sequences have become robust enough in the past decade to make this a preferred method for identifying both culturable and unculturable archaea, and for characterizing prokaryotic communities in poorly studied microbial ecosystems.

In an effort to better understand the role of prokaryote diversity in the bog environment, we used the development of a plasmid clone library and 16S rDNA gene-sequencing techniques to characterize the bacterial and archaeal communities of the *Sarracenia purpurea* phytotelmata from a Minnesota bog.

## GENETIC DIVERSITY OF EURASIAN *Phragmites australis* HAPLOTYPE M POPULATIONS IN THE MINNEAPOLIS/ST. PAUL METROPOLITAN AREA

Teresa Weber<sup>1,2</sup>, Trent Eckberg<sup>2</sup>, Brian Weber<sup>2</sup>, and Paul Melchior<sup>1,2</sup> (Advisor)

<sup>1</sup>*Department of Bioscience*

*Minnesota State University Moorhead, Brooklyn Park, MN*

<sup>2</sup>*Department of Biology*

*North Hennepin Community College, Brooklyn Park, MN*

*Phragmites australis* (hereafter *Phragmites*) is an aquatic grass species associated with wetlands and tidal marshes throughout North America. The invasion of native North American *Phragmites* populations by a physically similar Eurasian haplotype (M) was recently documented. Several small populations of this non-native haplotype have been detected by our group in the drainage system of several major highways within the Minneapolis/St. Paul metropolitan area.

In this study, populations of *Phragmites* in the drainage areas along major highways within the study area were identified and analyzed to determine where and to what extent haplotype M has become established within the metropolitan area. Native or Eurasian lineage was determined for each population using established AFLP techniques for chloroplast DNA markers. Furthermore, chromosomal microsatellite DNA profiles from all specimens collected from the populations identified as haplotype M were analyzed to determine whether the non-native stands were clonal or genetically unique from each other. This information is critical in assessing the nature of the cryptic invasion of aquatic habitats in Minnesota by this non-native *Phragmites*.

### HIPPOCAMPAL-SPECIFIC IRON DEFICIENCY INDUCES DEFICITS IN SENSORIMOTOR GATING

Robert Wickham<sup>1</sup>, Stephanie Fretham<sup>2</sup>, Michael Georgieff<sup>2,3</sup>, Jonathan Gewirtz<sup>2,4</sup>, Mu Sun<sup>5</sup>  
*College of Biological Sciences<sup>1</sup>, Graduate Program in Neuroscience<sup>2</sup>, Department of Pediatrics<sup>3</sup>, Department of Psychology<sup>4</sup>, Department of Genetics, Cell Biology, and Development<sup>5</sup>*  
*University of Minnesota, Minneapolis, MN,*

Iron is a vital micronutrient critical to proper neurodevelopment. Iron deficiency during critical stages of nervous system development can lead to dysfunction in neuronal energy metabolism as well as alteration in dendrite structure. The hippocampus is particularly sensitive to the effects of low iron. Previous investigations using a mouse model containing a hippocampal-specific knockout of the gene *Slc11a2* [divalent metal transport-1 (DMT-1)] demonstrated disruption of hippocampal development as well as spatial memory deficits. DMT-1 is a transport protein involved with facilitating the passage of iron into cells. In the present study, we expand on the repercussions of hippocampal development abnormalities. We showed that iron in the hippocampus also impairs sensorimotor gating, as tested by prepulse inhibition (PPI) of the acoustic startle reflex. Moreover, we demonstrated that lowered hippocampal iron causes a decrease in anxiety as demonstrated by both open field and elevated plus tests. The present evidence provides further support for the role of the *Slc11a2* gene in proper neurodevelopment of the hippocampus.

### GRAM STAINING OF *Staphylococcus aureus* USING METHANOL-FIXATION

Melissa Wolf and Jeanne Minnerath (Advisor)  
*Department of Biology*  
*Saint Mary's University of Minnesota, Minneapolis, MN*

Gram staining bacteria is a fundamental technique introduced in general biology and microbiology laboratory courses. Two common problems students encounter when Gram staining bacteria are (1) having a difficult time locating bacterial cells on the microscope slide and (2) over-decolorizing bacterial cells during the staining procedure such that gram-positive bacteria, which should appear purple in color, are pink instead.

In this study, the method of fixation (heat versus methanol) that is used to adhere bacteria to the slide prior to staining was examined to see whether it influences the staining results. It was found that significantly greater numbers of *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) cells adhered to slides following methanol fixation compared with slides that were heat-fixed. Additionally, methanol-fixed cells of *S.*

*aureus* were consistently stained the correct color (a dark purple) while the staining of heat-fixed cells was more variable with cells ranging in color from purple to pink. Overall, the results indicate that students are more likely to successfully visualize and Gram stain bacteria if the cells are fixed with methanol rather than heat.

### VOLTAMMETRY WITH FLUOROUS SOLVENTS: EXPANSION OF THE ELECTROCHEMICAL WINDOW

Di Wu, Eric J. Olson, and Philippe Bühlmann (Advisor)  
*Department of Chemistry*  
*University of Minnesota, Minneapolis, MN*

Our previous work showed the first example of electrochemistry with the purely fluoruous system perfluoro(methylcyclohexane)/100 mM tetrabutylammonium tetrakis[3,5-bis(perfluorohexyl)phenyl] borate (NBu<sub>4</sub>BArF104) [1]. We showed an oxidation limit of 1.9 V vs. ferrocene, the cause of which was not clear.

Our goal is to determine the cause of the seemingly low oxidation limit and widen the electrochemical window available to electrochemistry in fluoruous media. The voltammogram of perfluoro(methylcyclohexane)/ NBu<sub>4</sub>BArF104 shows peaks at the oxidative limit, suggesting that this limit arises from the oxidation of hydrogenated impurities. Upon removal of the hydrogenated impurities by refluxing over acidic permanganate, peaks in the voltammogram decreased greatly but the observed oxidation limit remained unchanged. To test whether the limit results from the branched structure of perfluoro(methylcyclohexane), perfluoro(*n*-hexane) was used as the solvent. No change in the oxidative limit was observed, indicating that the fluoruous solvent is not the source of the oxidation limit. To determine if water has an effect on the oxidation limit, water was added into a perfluoro(*n*-hexane)/NBu<sub>4</sub>BArF104 solution. The addition of water produced no change in the oxidation potential. Having eliminated all likely sources of oxidation from the solvent, we shifted our attention to the electrolyte, specifically tetrabutylammonium. Cesium BArF104 was prepared. However, the low solubility of CsBArF104 did not sufficiently lower the resistance of fluoruous solutions to allow determination of their oxidation potentials. Further development of fluorophilic electrolytes is ongoing.

[1] E. J. Olson, P. G. Boswell, B. L. Givot, L. J. Yao, and P. Bühlmann, *J. Electroanal. Chem.* 639(2010):154–160.

**ANALYSIS OF METALS IN PRAIRIE SOIL USING FLAME ATOMIC ABSORPTION**

Patricia A. Yates and Gina Mancini-Samuels (Advisor)  
*Department of Chemistry*  
*St. Catherine University, St. Paul, MN*

The Belwin Conservancy located in Afton and West Lake Township, Minnesota, is one of the largest privately owned nature preserves in the area. In 2008, bison were introduced to the prairies, as these animals are an integral part of prairie restoration. Bison roll in the prairie soil to keep cool in the summer, creating compact wallows that improve the overall health of the prairie.

This research consisted of examining the concentrations of copper, iron, manganese, and zinc in Belwin Conservancy prairie soil using flame atomic absorption. The metal concentrations were compared specifically between wallow and non-wallow samples of soil, as it was hypothesized that there would be a greater metal concentration in the wallow samples. It was suspected that the presence of metals in the wallow samples would be greater because previous research determined that the nitrogen content was higher in wallow-versus-non-wallow soil. The higher concentrations would be a result of the bison's compaction of the soil and the lack of vegetation in the wallow areas.

Two extraction methods of analysis were used in the experiment. Separate solutions of Ethylenediaminetetraacetic acid (EDTA) and Diethylenetriaminepentaacetic acid (DTPA) were prepared and analyzed to determine if there was a significant binding difference of the metals for these compounds. Overall, it was determined that EDTA was a stronger extraction method; however, the metal concentrations between the wallow and non-wallow samples were not significantly different.

**IMMUNOPHENOTYPING OF IMMUNE CELLS INVOLVED IN PATHOGENESIS OF MOUSE TYPE 1 DIABETES INDUCED BY STREPTOZOTOCIN**

Jong Hoe Yee, Yong Heng Lee, and Marina Cetkovic-Cvrlje (Advisor)  
*Department of Biology*  
*St. Cloud State University, St. Cloud, MN*

Type 1 diabetes (T1D) is an autoimmune disease induced by the body's own immune cells, T cells, that attack the insulin-producing pancreatic  $\beta$  cells. Three major types of T cells play a role in the autoimmune attack: T helper (Th), T regulatory (Tr), and T cytotoxic cells (Tc) that express CD4, both CD4 and CD25, and CD8 surface marker, respectively. All of the T cells express the surface marker called CD3. It is believed that Th cells play both pathogenic and protective roles in immunopathogenesis of T1D. Tc cells are considered as

pathogenic, while Tregs exhibit the protective functions during the disease development.

In this study we used mice with streptozotocin (STZ)-induced T1D experimental model in order to evaluate the involvement of different T cell types during development of disease. STZ-treated and control mice (n=6-11/group) were sacrificed on days 7, 14, and 28 post STZ injection, splenocytes immunophenotyped (stained by appropriate monoclonal antibodies against surface markers), and analyzed by flow cytometer (FACSCalibur). Our preliminary data showed a significant decrease of the CD3+, as well as CD4+ and CD8+ T cell percentages on day 14 in STZ-treated compared with the control group. Interestingly, no differences were observed on day 30 in percentages of CD3+, CD4+, and CD8+ cells. These data suggest that the most prominent differences in T cell populations of STZ-treated compared with control mice are observed on/around day 14 when the T cell attack is on its peak and >50% of STZ-treated mice become diabetic.

**NOTES**